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UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	Attorney Docket No. 42534-708.305
	First Named Inventor AmirAli TALASAZ
	Title METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
	Priority Mail Express® Label No.
APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450
1. <input checked="" type="checkbox"/> Fee Transmittal Form (PTO/SB/17 or equivalent)	ACCOMPANYING APPLICATION PAPERS
2. <input type="checkbox"/> Applicant asserts small entity status. See 37 CFR 1.27	10. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) Name of Assignee _____
3. <input type="checkbox"/> Applicant certifies micro entity status. See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.	11. <input checked="" type="checkbox"/> 37 CFR 3.73(c) Statement <input checked="" type="checkbox"/> Power of Attorney (when there is an assignee)
4. <input checked="" type="checkbox"/> Specification [Total Pages <u>91</u>] Both the claims and abstract must start on a new page. (See MPEP § 608.01(a) for information on the preferred arrangement)	12. <input type="checkbox"/> English Translation Document (if applicable)
5. <input checked="" type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets <u>11</u>]	13. <input type="checkbox"/> Information Disclosure Statement (PTO/SB/08 or PTO-1449) <input type="checkbox"/> Copies of citations attached
6. Inventor's Oath or Declaration [Total Pages <u>2</u>] (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))	14. <input type="checkbox"/> Preliminary Amendment
a. <input type="checkbox"/> Newly executed (original or copy)	15. <input type="checkbox"/> Return Receipt Postcard (MPEP § 503) (Should be specifically itemized)
b. <input checked="" type="checkbox"/> A copy from a prior application (37 CFR 1.63(d))	16. <input type="checkbox"/> Certified Copy of Priority Document(s) (if foreign priority is claimed)
7. <input checked="" type="checkbox"/> Application Data Sheet * See note below. See 37 CFR 1.76 (PTO/AIA/14 or equivalent)	17. <input type="checkbox"/> Nonpublication Request Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
8. CD-ROM or CD-R in duplicate, large table, or Computer Program (Appendix) <input type="checkbox"/> Landscape Table on CD	18. <input checked="" type="checkbox"/> Other: <u>Track One Request</u> _____ _____ _____
9. Nucleotide and/or Amino Acid Sequence Submission (if applicable, items a. – c. are required)	
a. <input type="checkbox"/> Computer Readable Form (CRF)	
b. <input type="checkbox"/> Specification Sequence Listing on:	
i. <input type="checkbox"/> CD-ROM or CD-R (2 copies); or	
ii. <input type="checkbox"/> Paper	
c. <input type="checkbox"/> Statements verifying identity of above copies	
*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 must be included in an Application Data Sheet (ADS). (2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).	
19. CORRESPONDENCE ADDRESS	
<input checked="" type="checkbox"/> The address associated with Customer Number: <u>115823</u> OR <input type="checkbox"/> Correspondence address below	
Name	
Address	
City	State
Country	Zip Code
Telephone	Email
Signature	/Timothy A. Hott/
Date	2019-12-13
Name (Print/Type)	Timothy A. Hott
Registration No. (Attorney/Agent)	67740

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.		

Secrecy Order 37 CFR 5.2:

Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor	1				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	AmirAli		TALASAZ		
Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service					
City	Atherton	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	605 Penobscot Drive				
Address 2					
City	Redwood City	State/Province	CA		
Postal Code	94063	Country i	US		

Inventor	2				Remove
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Helmy		ELTOUKHY		
Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service					
City	Atherton	State/Province	CA	Country of Residence	US

Mailing Address of Inventor:

Address 1	605 Penobscot Drive				
Address 2					
City	Redwood City	State/Province	CA		
Postal Code	94063	Country i	US		

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
 For further information see 37 CFR 1.33(a).

00003

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

An Address is being provided for the correspondence information of this application.

Customer Number	115823		
Email Address	patents@guardanthealth.com	Add Email	Remove Email
Email Address	patentdocket@wsgr.com	Add Email	Remove Email

Application Information:

Title of the Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		
Attorney Docket Number	42534-708.305	Small Entity Status Claimed	<input type="checkbox"/>
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	11	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

Request Early Publication (Fee required at time of Request 37 CFR 1.219)

Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer number will be used for the Representative Information during processing.

Please Select One:	<input checked="" type="radio"/> Customer Number	<input type="radio"/> US Patent Practitioner	<input type="radio"/> Limited Recognition (37 CFR 11.9)
Customer Number	115823		

00004

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending						Remove
Application Number	Continuity Type		Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)			
	Continuation of		16672267	2019-11-01			
Prior Application Status	Pending						Remove
Application Number	Continuity Type		Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)			
16672267	Continuation of		16601168	2019-10-14			
Prior Application Status	Pending						Remove
Application Number	Continuity Type		Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)			
16601168	Continuation of		15892178	2018-02-08			
Prior Application Status	Patented						Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)		
15892178	Continuation of	14861989	2015-09-22	9920366	2018-03-20		
Prior Application Status	Pending						Remove
Application Number	Continuity Type		Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)			
14861989	Continuation of		PCTUS2014072383	2014-12-24			
Prior Application Status	Expired						Remove
Application Number	Continuity Type		Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)			
PCTUS2014072383	Claims benefit of provisional		61948509	2014-03-05			
Prior Application Status	Expired						Remove
Application Number	Continuity Type		Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)			
PCTUS2014072383	Claims benefit of provisional		61921456	2013-12-28			
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.							Add

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)	Remove

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant	1	<input type="button" value="Remove"/>
<p>If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.</p>		
<input type="button" value="Clear"/>		
<input checked="" type="radio"/> Assignee	<input type="radio"/> Legal Representative under 35 U.S.C. 117	<input type="radio"/> Joint Inventor
Person to whom the inventor is obligated to assign.		Person who shows sufficient proprietary interest
If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:		
<div style="border: 1px solid black; height: 20px; width: 100%;"></div>		
Name of the Deceased or Legally Incapacitated Inventor: <input type="text"/>		
If the Applicant is an Organization check here. <input checked="" type="checkbox"/>		
Organization Name	GUARDANT HEALTH, INC.	
Mailing Address Information For Applicant:		
Address 1	505 Penobscot Drive	
Address 2		
City	Redwood City	State/Province CA
Country	US	Postal Code 94063
Phone Number		Fax Number
Email Address	patents@guardanthealth.com	
Additional Applicant Data may be generated within this form by selecting the Add button. <input type="button" value="Add"/>		

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Assignee	1
-----------------	---

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here.

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the **INITIAL** filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Timothy A. Hott/		Date (YYYY-MM-DD)	2019-12-13	
First Name	Timothy	Last Name	Hott	Registration Number	67740

Additional Signature may be generated within this form by selecting the Add button.

Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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**DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN
APPLICATION DATA SHEET (37 CFR 1.76)****Title of
Invention**

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

As the below named inventor, I hereby declare that:

This declaration
is directed to:

The attached application, or

United States application or PCT international application number 14/861,989filed on September 22, 2015

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001
by fine or imprisonment of not more than five (5) years, or both.**WARNING:**

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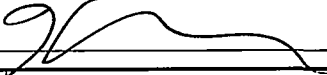
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DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
<p>As the below named inventor, I hereby declare that:</p> <p>This declaration is directed to: <input type="checkbox"/> The attached application, or <input checked="" type="checkbox"/> United States application or PCT international application number <u>14/861,989</u> filed on <u>September 22, 2015</u>.</p> <p>The above-identified application was made or authorized to be made by me.</p> <p>I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.</p> <p>I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.</p> <p style="text-align: center;">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p>	
<p>LEGAL NAME OF INVENTOR</p> <p>Inventor: <u>Helmy Eltokhy</u> Date (Optional): <u>11/10/15</u></p> <p>Signature: </p>	
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ABSTRACT OF THE DISCLOSURE

Disclosed herein in are methods and systems for determining genetic variants (e.g., copy number variation) in a polynucleotide sample. A method for determining copy number variations includes tagging double-stranded polynucleotides with duplex tags, sequencing polynucleotides from the sample and estimating total number of polynucleotides mapping to selected genetic loci. The estimate of total number of polynucleotides can involve estimating the number of double-stranded polynucleotides in the original sample for which no sequence reads are generated. This number can be generated using the number of polynucleotides for which reads for both complementary strands are detected and reads for which only one of the two complementary strands is detected.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for estimating a total number of double-stranded deoxyribonucleic acid (DNA) molecules in a sample, comprising:
 - (a) determining a quantitative measure of individual DNA molecules for which both strands are detected;
 - (b) determining a quantitative measure of individual DNA molecules for which only one strand is detected;
 - (c) using said quantitative measures determined in (a) and (b) to estimate a total number of double-stranded DNA molecules in the sample, wherein said total number comprises individual DNA molecules for which neither DNA strand is detected.
2. The method of Claim 1, further comprising inferring, from said quantitative measures determined in (a) and (b), a quantitative measure of individual DNA molecules for which neither strand is detected.
3. The method of Claim 1, further comprising determining a normalized quantitative measure of one or more genetic loci to determine copy number variation in said sample.
4. The method of Claim 1, wherein said sample comprises double-stranded polynucleotide molecules sourced substantially from cell-free nucleic acids.
5. The method of Claim 1, wherein determining said quantitative measure of individual DNA molecules comprises tagging said DNA molecules with a set of duplex tags, wherein each duplex tag differently tags complementary strands of a double-stranded DNA molecule in said sample to provide tagged strands.
6. The method of Claim 5, further comprising sequencing at least some of said tagged strands to produce a set of sequence reads.
7. The method of Claim 6, further comprising sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in said set, and (ii) each

unpaired read represents a first tagged strand having no second differently tagged complementary strand derived from a double-stranded polynucleotide molecule represented among said sequence reads in said set of sequence reads.

8. The method of Claim 7, further comprising determining quantitative measures of (i) said paired reads and (ii) said unpaired reads that map to each of one or more genetic loci to determine a quantitative measure of total double-stranded DNA molecules in said sample that map to each of said one or more genetic loci based on said quantitative measure of paired reads and unpaired reads mapping to each locus.

9. A method, comprising:

- (a) providing a sample comprising a set of double-stranded polynucleotide molecules, each double-stranded polynucleotide molecule including first and second complementary strands;
- (b) tagging said double-stranded polynucleotide molecules with a set of duplex tags, wherein each duplex tag differently tags said first and second complementary strands of a double-stranded polynucleotide molecule in said set;
- (c) sequencing at least some of said tagged strands to produce a set of sequence reads;
- (d) reducing and/or tracking redundancy in said set of sequence reads;
- (e) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in said set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among said sequence reads in said set of sequence reads;
- (f) determining quantitative measures of at least two of (i) said paired reads, (ii) said unpaired reads that map to each of one or more genetic loci, (iii) read depth of said paired reads and (iv) read depth of said unpaired reads; and
- (g) estimating with a programmed computer processor a quantitative measure of total double-stranded polynucleotide molecules in said set that map to each of said one or

more genetic loci based on said quantitative measures of said at least two of (i) said paired reads, (ii) said unpaired reads mapping to each locus, (iii) said read depth of said paired reads and (iv) said read depth of said unpaired reads.

10. The method of Claim 9, further comprising detecting copy number variation in said sample by determining a normalized total quantitative measure determined in (g) at each of said one or more genetic loci and determining copy number variation based on the normalized measure.

11. The method of Claim 9, wherein said sample comprises double-stranded polynucleotide molecules sourced substantially from cell-free nucleic acids.

12. The method of Claim 9, wherein said duplex tags are not sequencing adaptors.

13. The method of claim 9, wherein (f) comprises determining quantitative measures of said paired reads and said unpaired reads, and wherein in (g), said quantitative measure of total double-stranded polynucleotide molecules in said set that map to each of said one or more genetic loci is determined based on said quantitative measures of said paired reads and said unpaired reads.

14. The method of Claim 9, wherein reducing redundancy in said set of sequence reads comprises collapsing sequence reads produced from amplified products of an original polynucleotide molecule in said sample back to said original polynucleotide molecule.

15. The method of Claim 14, further comprising determining a consensus sequence for said original polynucleotide molecule.

16. The method of Claim 15, further comprising identifying polynucleotide molecules at one or more genetic loci comprising a sequence variant.

17. The method of Claim 15, further comprising determining a quantitative measure of paired reads that map to a locus, wherein both strands of said pair comprise a sequence variant.

18. The method of Claim 15, further comprising determining a quantitative measure of paired molecules in which only one member of said pair bears a sequence variant and/or determining a quantitative measure of unpaired molecules bearing a sequence variant.

19. A method for detecting copy number variation in deoxyribonucleic acid (DNA) molecules in a biological sample of a subject, comprising:

- (a) attaching adapters to ends of fragments generated from said DNA molecules in said biological sample of said subject, wherein said adapters tag a 5' end of a strand of an individual fragment among said fragments with a first tag and a 3' end of a complementary strand of said individual fragment with a second tag, thereby providing tagged fragment molecules;
- (b) sequencing at least a portion of each of said tagged fragment molecules to provide a plurality of sequencing reads;
- (c) mapping said plurality of sequencing reads to a first genetic locus and at least one second genetic locus in a reference genome, wherein said first tag and said second tag are indicative of which strand of said tagged fragment molecules each of said plurality of sequencing reads is derived;
- (d) using a programmed computer to determine a first total number of tagged fragment molecules for said first genetic locus and a second total number of tagged fragment molecules for said at least one second genetic locus, wherein each of said first total number and second total number is based on (i) a number of tagged fragments for which sequencing reads from both strands of said tagged fragment molecules are detected and (ii) a number of tagged fragment for which sequencing reads from only one strand of said tagged fragment molecules are detected, and wherein said first total

- number and said second total number comprises tagged fragment molecules for which neither strand was sequenced; and
- (e) comparing said first total number of tagged fragment molecules to said second total number of tagged fragment molecules to determine copy number variation in said DNA molecules.
20. The method of claim 19, further comprising detecting sequencing errors by comparing said sequencing reads derived from different strands of said tagged fragment molecules.
 21. The method of claim 19, further comprising amplifying said tagged fragment molecules prior to said sequencing and, subsequent to (b), collapsing said sequencing reads.
 22. The method of claim 21, further comprising detecting amplification errors by comparing said sequencing reads derived from different strands of said tagged fragment molecules.
 23. The method of claim 19, wherein said copy number variation is determined if said first total number is different from said second total number.
 24. The method of claim 19, wherein said biological sample is a cell-free biological sample.
 25. The method of claim 19, wherein said adapters comprise molecular barcodes.
 26. The method of claim 25, wherein said molecular barcodes are non-unique.
 27. The method of claim 26, wherein said molecular barcodes are non-unique and at least 50% of said tagged fragment molecules comprise a given molecular barcode that is shared by at least one other tagged fragment molecule.
 28. The method of claim 25, wherein said molecular barcodes are unique.
 29. The method of claim 25, further comprising amplifying said tagged fragment molecules prior to said sequencing and, subsequent to (b), collapsing said sequencing reads based at least in part on said molecular barcodes.
 30. The method of claim 19, wherein said copy-number variation in said DNA molecules is indicative of copy-number variation within genomes of cells in a tumor.

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

CROSS-REFERENCE

[0001] This application is a continuation of U.S. Application No. 16/672,267, filed November 1, 2019, which is a continuation of U.S. Application No. 16/601,168, filed October 14, 2019, which is a continuation of U.S. Application No. 15/892,178, filed February 8, 2018, which is a continuation of U.S. Application No. 14/861,989, filed September 22, 2015 (now U.S. Patent 9,920,366), which is a continuation application of International Application No. PCT/US2014/072383, filed December 24, 2014, which application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/921,456, filed December 28, 2013, and U.S. Provisional Application No. 61/948,509, filed March 5, 2014, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] The detection and quantification of polynucleotides is important for molecular biology and medical applications, such as diagnostics. Genetic testing is particularly useful for a number of diagnostic methods. For example, disorders that are caused by rare genetic alterations (*e.g.*, sequence variants) or changes in epigenetic markers, such as cancer and partial or complete aneuploidy, may be detected or more accurately characterized with DNA sequence information.

[0003] Early detection and monitoring of genetic diseases, such as cancer, is often useful and needed in the successful treatment or management of the disease. One approach may include the monitoring of a sample derived from cell-free nucleic acids, a population of polynucleotides that can be found in different types of bodily fluids. In some cases, disease may be characterized or detected based on detection of genetic aberrations, such as copy number variation and/or sequence variation of one or more nucleic acid sequences, or the development of other certain rare genetic alterations. Cell-free DNA (cfDNA) may contain genetic aberrations associated with a particular disease. With improvements in sequencing and techniques to manipulate nucleic acids, there is a need in the art for improved methods and systems for using cell-free DNA to detect and monitor disease.

[0004] In particular, many methods have been developed for accurate copy number variation estimation, especially for heterogeneous genomic samples, such as tumor-derived gDNA or for cfDNA for many applications (*e.g.*, prenatal, transplant, immune, metagenomics or cancer diagnostics). Most of these methods include sample preparation whereby the original nucleic acids are converted into a sequenceable library, followed by massively parallel sequencing, and finally bioinformatics to estimate copy number variation at one or more loci.

SUMMARY

[0005] Although many of these methods are able to reduce or combat the errors introduced by the sample preparation and sequencing processes for all molecules that are converted and sequenced, these methods are not able to infer the counts of molecules that were converted but not sequenced. Since this count of converted by unsequenced molecules can be highly variable from genomic region to region, these counts can dramatically and adversely affect the sensitivity that can be achieved.

[0006] To address this issue, input double-stranded deoxyribonucleic acid (DNA) can be converted by a process that tags both halves of the individual double-stranded molecule, in some cases differently. This can be performed using a variety of techniques, including ligation of hairpin, bubble, or forked adapters or other adaptors having double-stranded and single stranded segments (the unhybridized portion of a bubble, forked or hairpin adapter are deemed single-stranded herein). If tagged correctly, each original Watson and Crick (*i.e.*, strand) side of the input double-stranded DNA molecule can be differently tagged and identified by the sequencer and subsequent bioinformatics. For all molecules in a particular region, counts of molecules where both Watson and Crick sides were recovered (“Pairs”) versus those where only one half was recovered (“Singlets”) can be recorded. The number of unseen molecules can be estimated based on the number of Pairs and Singlets detected.

[0007] An aspect of the present disclosure provides a method for detecting and/or quantifying rare deoxyribonucleic acid (DNA) in a heterogeneous population of original DNA fragments, comprising tagging the original DNA fragments in a single reaction using a library of a plurality of different tags such that greater than 30% of the fragments are tagged at both ends, wherein each of the tags comprises a molecular barcode. The single reaction can be in a single

reaction vessel. Greater than 50% of the fragments can be tagged at both ends. The plurality of different tags can be no more than any of 100, 500, 1000, 10,000 or 100,000 different tags.

[0008] Another aspect provides a set of library adaptors that can be used to tag the molecules of interest (e.g., by ligation, hybridization, etc.). The set of library adaptors can comprise plurality of polynucleotide molecules with molecular barcodes, wherein the plurality of polynucleotide molecules are less than or equal to 80 nucleotide bases in length, wherein the molecular barcodes are at least 4 nucleotide bases in length, and wherein (a) the molecular barcodes are different from one another and have an edit distance of at least 1 between one another; (b) the molecular barcodes are located at least one nucleotide base away from a terminal end of their respective polynucleotide molecules; (c) optionally, at least one terminal base is identical in all of the polynucleotide molecules; and (d) none of the polynucleotide molecules contains a complete sequencer motif.

[0009] In some embodiments, the library adaptors (or adapters) are identical to one another but for the molecular barcodes. In some embodiments, each of the plurality of library adaptors comprises at least one double-stranded portion and at least one single-stranded portion (e.g., a non-complementary portion or an overhang). In some embodiments, the double-stranded portion has a molecular barcode selected from a collection of different molecular barcodes. In some embodiments, the given molecular barcode is a randomer. In some embodiments, each of the library adaptors further comprises a strand-identification barcode on the at least one single-stranded portion. In some embodiments, the strand-identification barcode includes at least 4 nucleotide bases. In some embodiments, the single-stranded portion has a partial sequencer motif. In some embodiments, the library adaptors do not include a complete sequencer motif.

[0010] In some embodiments, none of the library adaptors contains a sequence for hybridizing to a flow cell or forming a hairpin for sequencing.

[0011] In some embodiments, all of the library adaptors have a terminal end with nucleotide(s) that are the same. In some embodiments, the identical terminal nucleotide(s) are over two or more nucleotide bases in length.

[0012] In some embodiments, each of the library adapters is Y-shaped, bubble shaped or hairpin shaped. In some embodiments, none of the library adapters contains a sample identification motif. In some embodiments, each of the library adapters comprises a sequence

that is selectively hybridizable to a universal primer. In some embodiments, each of the library adapters comprises a molecular barcode that is at least 5, 6, 7, 8, 9 and 10 nucleotide bases in length. In some embodiments, each of the library adapters is from 10 nucleotide bases to 80 in length, or 30 to 70 nucleotide bases in length, or 40 to 60 nucleotide bases in length. In some embodiments, at least 1, 2, 3, or 4 terminal bases are identical in all of the library adapters. In some embodiments, at least 4 terminal bases are identical in all of the library adapters.

[0013] In some embodiments, the edit distance of the molecular barcodes of the library adapters is a Hamming distance. In some embodiments, the edit distance is at least 1, 2, 3, 4 or 5. In some embodiments, the edit distance is with respect to individual bases of the plurality of polynucleotide molecules. In some embodiments, the molecular barcodes are located at least 10 nucleotide base away from a terminal end of an adapter. In some embodiments, the plurality of library adapters includes at least 2, 4, 6, 8, 10, 20, 30, 40 or 50 different molecular barcodes, or from 2-100, 4-80, 6-60 or 8-40 different molecular barcodes. In any of the embodiments herein, there are more polynucleotides (e.g., cfDNA fragments) to be tagged than there are different molecular barcodes such that the tagging is not unique.

[0014] In some embodiments, the terminal end of an adaptor is configured for ligation (e.g., to a target nucleic acid molecule). In some embodiments, the terminal end of an adaptor is a blunt end.

[0015] In some embodiments, the adaptors are purified and isolated. In some embodiments, the library comprises one or more non-naturally occurring bases.

[0016] In some embodiments, the polynucleotide molecules comprise a primer sequence positioned 5' with respect to the molecular barcodes.

[0017] In some embodiments, the set of library adapters consists essentially of the plurality of polynucleotide molecules.

[0018] In another aspect, a method comprises (a) tagging a collection of polynucleotides with a plurality of polynucleotide molecules from a library of adaptors to create a collection of tagged polynucleotides; and (b) amplifying the collection of tagged polynucleotides in the presence of sequencing adaptors, wherein the sequencing adaptors have primers with nucleotide sequences that are selectively hybridizable to complementary sequences in the plurality of polynucleotide molecules. The library of adaptors may be as described above or elsewhere

herein. In some embodiments, each of the sequencer adaptors further comprises an index tag, which can be a sample identification motif.

[0019] Another aspect, provides a method for detecting and/or quantifying rare DNA in a heterogeneous population of original DNA fragments, wherein the rare DNA has a concentration that is less than 1%, the method comprising (a) tagging the original DNA fragments in a single reaction such that greater than 30% of the original DNA fragments are tagged at both ends with library adaptors that comprise molecular barcodes, thereby providing tagged DNA fragments; (b) performing high-fidelity amplification on the tagged DNA fragments; (c) optionally, selectively enriching a subset of the tagged DNA fragments; (d) sequencing one or both strands of the tagged, amplified and optionally selectively enriched DNA fragments to obtain sequence reads comprising nucleotide sequences of the molecular barcodes and at least a portion of the original DNA fragments; (e) from the sequence reads, determining consensus reads that are representative of single-strands of the original DNA fragments; and (f) quantifying the consensus reads to detect and/or quantify the rare DNA at a specificity that is greater than 99.9%.

[0020] In some embodiments, (e) comprises comparing sequence reads having the same or similar molecular barcodes and the same or similar end of fragment sequences. In some embodiments, the comparing further comprises performing a phylogenetic analysis on the sequence reads having the same or similar molecular barcodes. In some embodiments, the molecular barcodes include a barcode having an edit distance of up to 3. In some embodiments, the end of fragment sequence includes fragment sequences having an edit distance of up to 3.

[0021] In some embodiments, the method further comprises sorting sequence reads into paired reads and unpaired reads, and quantifying a number of paired reads and unpaired reads that map to each of one or more genetic loci.

[0022] In some embodiments, the tagging occurs by having an excess amount of library adaptors as compared to original DNA fragments. In some embodiments, the excess is at least a 5-fold excess. In some embodiments, the tagging comprises using a ligase. In some embodiments, the tagging comprises attachment to blunt ends.

[0023] In some embodiments, the method further comprises binning the sequence reads according to the molecular barcodes and sequence information from at least one end of each of the original DNA fragments to create bins of single stranded reads. In some embodiments, the

method further comprises, in each bin, determining a sequence of a given original DNA fragment among the original DNA fragments by analyzing sequence reads. In some embodiments, the method further comprises detecting and/or quantifying the rare DNA by comparing a number of times each base occurs at each position of a genome represented by the tagged, amplified, and optionally enriched DNA fragments.

[0024] In some embodiments, the library adaptors do not contain complete sequencer motifs. In some embodiments, the method further comprises selectively enriching a subset of the tagged DNA fragments. In some embodiments, the method further comprises, after enriching, amplifying the enriched tagged DNA fragments in the presence of sequencing adaptors comprising primers. In some embodiments, (a) provides tagged DNA fragments having from 2 to 1000 different combinations of molecular barcodes.

[0025] In some embodiments, the DNA fragments are tagged with polynucleotide molecules from a library of adaptors as described above or elsewhere herein.

[0026] In another aspect, a method for processing and/or analyzing a nucleic acid sample of a subject comprises (a) exposing polynucleotide fragments from the nucleic acid sample to a set of library adaptors to generate tagged polynucleotide fragments; and (b) subjecting the tagged polynucleotide fragments to nucleic acid amplification reactions under conditions that yield amplified polynucleotide fragments as amplification products of the tagged polynucleotide fragments. The set of library adaptors comprises a plurality of polynucleotide molecules with molecular barcodes, wherein the plurality of polynucleotide molecules are less than or equal to 80 nucleotide bases in length, wherein the molecular barcodes are at least 4 nucleotide bases in length, and wherein (1) the molecular barcodes are different from one another and have an edit distance of at least 1 between one another; (2) the molecular barcodes are located at least one nucleotide base away from a terminal end of their respective polynucleotide molecules; (3) optionally, at least one terminal base is identical in all of the polynucleotide molecules; and (4) none of the polynucleotide molecules contains a complete sequencer motif.

[0027] In some embodiments, the method further comprises determining nucleotide sequences of the amplified tagged polynucleotide fragments. In some embodiments, the nucleotide sequences of the amplified tagged polynucleotide fragments are determined without polymerase chain reaction (PCR). In some embodiments, the method further comprises

analyzing the nucleotide sequences with a programmed computer processor to identify one or more genetic variants in the nucleotide sample of the subject. In some embodiments, the one or more genetic variants are selected from the group consisting of base change(s), insertion(s), repeat(s), deletion(s), copy number variation(s) and transversion(s). In some embodiments, the one or more genetic variants include one or more tumor associated genetic alterations.

[0028] In some embodiments, the subject has or is suspected of having a disease. In some embodiments, the disease is cancer. In some embodiments, the method further comprises collecting the nucleic acid sample from the subject. In some embodiments, the nucleic acid sample is collected from a location selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, cerebral spinal fluid and tears of the subject. In some embodiments, the nucleic acid sample is a cell-free nucleic acid sample. In some embodiments, the nucleic acid sample is collected from no more than 100 nanograms (ng) of double-stranded polynucleotide molecules of the subject.

[0029] In some embodiments, the polynucleotide fragments comprise double-stranded polynucleotide molecules. In some embodiments, in (a), the plurality of polynucleotide molecules couple to the polynucleotide fragments via blunt end ligation, sticky end ligation, molecular inversion probes, PCR, ligation-based PCR, multiplex PCR, single stranded ligation, and single stranded circularization. In some embodiments, exposing the polynucleotide fragments of the nucleic acid sample to the plurality of polynucleotide molecules yields the tagged polynucleotide fragments with a conversion efficiency of at least 10%. In some embodiments, any of at least 5%, 6%, 7%, 8%, 9%, 10%, 20%, or 25% of the tagged polynucleotide fragments share a common polynucleotide molecule or sequence. In some embodiments, the method further comprises generating the polynucleotide fragments from the nucleic acid sample.

[0030] In some embodiments, the subjecting comprises amplifying the tagged polynucleotide fragments from sequences corresponding to genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4,

SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.

[0031] In another aspect, a method comprises (a) generating a plurality of sequence reads from a plurality of polynucleotide molecules, wherein the plurality of polynucleotide molecules cover genomic loci of a target genome, wherein the genomic loci correspond to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1; (b) grouping with a computer processor the plurality of sequence reads into families, wherein each family comprises sequence reads from one of the template polynucleotides; (c) for each of the families, merging sequence reads to generate a consensus sequence; (d) calling the consensus sequence at a given genomic locus among the genomic loci; and (e) detecting at the given genomic locus any of genetic variants among the calls, frequency of a genetic alteration among the calls, total number of calls, and total number of alterations among the calls.

[0032] In some embodiments, each family comprises sequence reads from only one of the template polynucleotides. In some embodiments, the given genomic locus comprises at least one nucleic acid base. In some embodiments, the given genomic locus comprises a plurality of nucleic acid bases. In some embodiments, the calling comprises calling at least one nucleic acid base at the given genomic locus. In some embodiments, the calling comprises calling a plurality of nucleic acid bases at the given genomic locus. In some embodiments, the calling comprises any one of phylogenetic analysis, voting, weighing, assigning a probability to each read at the locus in a family and calling the base with the highest probability.

[0033] In some embodiments, the method further comprises performing (d)-(e) at an additional genomic locus among the genomic loci. In some embodiments, the method further

comprises determining a variation in copy number at one of the given genomic locus and additional genomic locus based on counts at the given genomic locus and additional genomic locus.

[0034] In some embodiments, the grouping comprises classifying the plurality of sequence reads into families by identifying (i) different molecular barcodes coupled to the plurality of polynucleotide molecules and (ii) similarities between the plurality of sequence reads, wherein each family includes a plurality of nucleic acid sequences that are associated with a different combination of molecular barcodes and similar or identical sequence reads. Different molecular barcodes have different sequences.

[0035] In some embodiments, the consensus sequence is generated by evaluating a quantitative measure or a statistical significance level for each of the sequence reads. In some embodiments, the quantitative measure comprises use of a binomial distribution, exponential distribution, beta distribution, or empirical distribution. In some embodiments, the method further comprises mapping the consensus sequence to the target genome. In some embodiments, the plurality of genes includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50 or all of the plurality of genes selected from the group.

[0036] Another aspect of the present disclosure provides a method, comprising (a) providing template polynucleotide molecules and a set of library adaptors in a single reaction vessel, wherein the library adaptors are polynucleotide molecules that have different molecular barcodes (e.g., from 2 to 1,000 different molecular barcodes), and wherein none of the library adaptors contains a complete sequencer motif; (b) in the single reaction vessel, coupling the library adaptors to the template polynucleotide molecules at an efficiency of at least 10%, thereby tagging each template polynucleotide with a tagging combination that is among a plurality of different tagging combinations (e.g., 4 to 1,000,000 different tagging combinations), to produce tagged polynucleotide molecules; (c) subjecting the tagged polynucleotide molecules to an amplification reaction under conditions that yield amplified polynucleotide molecules as amplification products of the tagged polynucleotide molecules; and (d) sequencing the amplified polynucleotide molecules.

[0037] In some embodiments, the template polynucleotide molecules are blunt ended or sticky-ended. In some embodiments, the library adaptors are identical but for the molecular

barcodes. In some embodiments, each of the library adaptors has a double stranded portion and at least one single-stranded portion. In some embodiments, the double-stranded portion has a molecular barcode among the molecular barcodes. In some embodiments, each of the library adaptors further comprises a strand-identification barcode on the at least one single-stranded portion. In some embodiments, the single-stranded portion has a partial sequencer motif. In some embodiments, the library adaptors have a sequence of terminal nucleotides that are the same. In some embodiments, the template polynucleotide molecules are double-stranded. In some embodiments, the library adaptors couple to both ends of the template polynucleotide molecules.

[0038] In some embodiments, subjecting the tagged polynucleotide molecules to the amplification reaction comprises non-specifically amplifying the tagged polynucleotide molecules.

[0039] In some embodiments, the amplification reaction comprises use of a priming site to amplify each of the tagged polynucleotide molecules. In some embodiments, the priming site is a primer. In some embodiments, the primer is a universal primer. In some embodiments, the priming site is a nick.

[0040] In some embodiments, the method further comprises, prior to (e), (i) separating polynucleotide molecules comprising one or more given sequences from the amplified polynucleotide molecules, to produce enriched polynucleotide molecules; and (ii) amplifying the enriched polynucleotide molecules with sequencing adaptors.

[0041] In some embodiments, the efficiency is at least 30%, 40%, or 50%. In some embodiments, the method further comprises identifying genetic variants upon sequencing the amplified polynucleotide molecules. In some embodiments, the sequencing comprises (i) subjecting the amplified polynucleotide molecules to an additional amplification reaction under conditions that yield additional amplified polynucleotide molecules as amplification products of the amplified polynucleotide molecules, and (ii) sequencing the additional amplified polynucleotide molecules. In some embodiments, the additional amplification is performed in the presence of sequencing adaptors.

[0042] In some embodiments, (b) and (c) are performed without aliquoting the tagged polynucleotide molecules. In some embodiments, the tagging is non-unique tagging.

[0043] Another aspect, provides a system for analyzing a target nucleic acid molecule of a subject, comprising a communication interface that receives nucleic acid sequence reads for a plurality of polynucleotide molecules that cover genomic loci of a target genome; computer memory that stores the nucleic acid sequence reads for the plurality of polynucleotide molecules received by the communication interface; and a computer processor operatively coupled to the communication interface and the memory and programmed to (i) group the plurality of sequence reads into families, wherein each family comprises sequence reads from one of the template polynucleotides, (ii) for each of the families, merge sequence reads to generate a consensus sequence, (iii) call the consensus sequence at a given genomic locus among the genomic loci, and (iv) detect at the given genomic locus any of genetic variants among the calls, frequency of a genetic alteration among the calls, total number of calls; and total number of alterations among the calls, wherein the genomic loci correspond to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.

[0044] In another aspect, a set of oligonucleotide molecules that selectively hybridize to at least 5 genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.

[0045] In some embodiments, the oligonucleotide molecules are from 10-200 bases in length. In some embodiments, the oligonucleotide molecules selectively hybridize to exon

regions of the at least 5 genes. In some embodiments, the oligonucleotide molecules selectively hybridize to at least 30 exons in the at least 5 genes. In some embodiments, multiple oligonucleotide molecules selectively hybridize to each of the at least 30 exons. In some embodiments, the oligonucleotide molecules that hybridize to each exon have sequences that overlap with at least 1 other oligonucleotide molecule.

[0046] In another aspect, a kit comprises a first container containing a plurality of library adaptors each having a different molecular barcode; and a second container containing a plurality of sequencing adaptors, each sequencing adaptor comprising at least a portion of a sequencer motif and optionally a sample barcode. The library adaptors can be as described above or elsewhere herein.

[0047] In some embodiments, the sequencing adaptor comprises the sample barcode. In some embodiments, the library adaptors are blunt ended and Y-shaped, and are less than or equal to 80 nucleic acid bases in length. In some embodiments, the sequencing adaptor is up to 70 bases from end to end.

[0048] In another aspect, a method for detecting sequence variants in a cell free DNA sample, comprising detecting rare DNA at a concentration less than 1% with a specificity that is greater than 99.9%.

[0049] In another aspect, a method comprises detecting genetic variants in a sample comprising DNA with a detection limit of at least 1% and specificity greater than 99.9%. In some embodiments, the method further comprises converting cDNA (e.g. cfDNA) into adaptor tagged DNA with a conversion efficiency of at least 30%, 40%, or 50% and reducing sequencing noise (or distortion) by eliminating false positive sequence reads.

[0050] Another aspect provides a method, comprising (a) providing a sample comprising a set of double-stranded polynucleotide molecules, each double-stranded polynucleotide molecule including first and second complementary strands; (b) tagging the double-stranded polynucleotide molecules with a set of duplex tags, wherein each duplex tag differently tags the first and second complementary strands of a double-stranded polynucleotide molecule in the set; (c) sequencing at least some of the tagged strands to produce a set of sequence reads; (d) reducing and/or tracking redundancy in the set of sequence reads; (e) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads

generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads; (f) determining quantitative measures of (i) the paired reads and (ii) the unpaired reads that map to each of one or more genetic loci; and (g) estimating with a programmed computer processor a quantitative measure of total double-stranded polynucleotide molecules in the set that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads mapping to each locus.

[0051] In some embodiments, the method further comprises (h) detecting copy number variation in the sample by determining a normalized total quantitative measure determined in step (g) at each of the one or more genetic loci and determining copy number variation based on the normalized measure. In some embodiments, the sample comprises double-stranded polynucleotide molecules sourced substantially from cell-free nucleic acids. In some embodiments, the duplex tags are not sequencing adaptors.

[0052] In some embodiments, reducing redundancy in the set of sequence reads comprises collapsing sequence reads produced from amplified products of an original polynucleotide molecule in the sample back to the original polynucleotide molecule. In some embodiments, the method further comprises determining a consensus sequence for the original polynucleotide molecule. In some embodiments, the method further comprises identifying polynucleotide molecules at one or more genetic loci comprising a sequence variant. In some embodiments, the method further comprises determining a quantitative measure of paired reads that map to a locus, wherein both strands of the pair comprise a sequence variant. In some embodiments, the method further comprises determining a quantitative measure of paired molecules in which only one member of the pair bears a sequence variant and/or determining a quantitative measure of unpaired molecules bearing a sequence variant. In some embodiments, the sequence variant is selected from the group consisting of a single nucleotide variant, an indel, a transversion, a translocation, an inversion, a deletion, a chromosomal structure alteration, a gene fusion, a chromosome fusion, a gene truncation, a gene amplification, a gene duplication and a chromosomal lesion.

[0053] Another aspect provides a system comprising a computer readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method comprising (a) receiving into memory a set of sequence reads of polynucleotides tagged with duplex tags; (b) reducing and/or tracking redundancy in the set of sequence reads; (c) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads; (d) determining quantitative measures of (i) the paired reads and (ii) the unpaired reads that map to each of one or more genetic loci; and (e) estimating a quantitative measure of total double-stranded polynucleotide molecules in the set that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads mapping to each locus.

[0054] Another aspect provides a method, comprising (a) providing a sample comprising a set of double-stranded polynucleotide molecules, each double-stranded polynucleotide molecule including first and second complementary strands; (b) tagging the double-stranded polynucleotide molecules with a set of duplex tags, wherein each duplex tag differently tags the first and second complementary strands of a double-stranded polynucleotide molecule in the set; (c) sequencing at least some of the tagged strands to produce a set of sequence reads; (d) reducing and/or tracking redundancy in the set of sequence reads; (e) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads; and (f) determining quantitative measures of at least two of (i) the paired reads, (ii) the unpaired reads that map to each of one or more genetic loci, (iii) read depth of the paired reads and (iv) read depth of unpaired reads.

[0055] In some embodiments, (f) comprises determining quantitative measures of at least three of (i)-(iv). In some embodiments, (f) comprises determining quantitative measures of all of (i)-(iv). In some embodiments, the method further comprises (g) estimating with a programmed computer processor a quantitative measure of total double-stranded polynucleotide molecules in the set that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads and their read depths mapping to each locus.

[0056] In another aspect, a method comprises (a) tagging control parent polynucleotides with a first tag set to produce tagged control parent polynucleotides, wherein the first tag set comprises a plurality of tags, wherein each tag in the first tag set comprises a same control tag and an identifying tag, and wherein the tag set comprises a plurality of different identifying tags; (b) tagging test parent polynucleotides with a second tag set to produce tagged test parent polynucleotides, wherein the second tag set comprises a plurality of tags, wherein each tag in the second tag set comprises a same test tag that is distinguishable from the control tag and an identifying tag, and wherein the second tag set comprises a plurality of different identifying tags; (c) mixing tagged control parent polynucleotides with tagged test parent polynucleotides to form a pool; (d) amplifying tagged parent polynucleotides in the pool to form a pool of amplified, tagged polynucleotides; (e) sequencing amplified, tagged polynucleotides in the amplified pool to produce a plurality of sequence reads; (f) grouping sequence reads into families, each family comprising sequence reads generated from a same parent polynucleotide, which grouping is optionally based on information from an identifying tag and from start/end sequences of the parent polynucleotides, and, optionally, determining a consensus sequence for each of a plurality of parent polynucleotides from the plurality of sequence reads in a group; (g) classifying each family or consensus sequence as a control parent polynucleotide or as a test parent polynucleotide based on having a test tag or a control tag; (h) determining a quantitative measure of control parent polynucleotides and control test polynucleotides mapping to each of at least two genetic loci; and (i) determining copy number variation in the test parent polynucleotides at at least one locus based on relative quantity of test parent polynucleotides and control parent polynucleotides mapping to the at least one locus.

[0057] In another aspect, a method comprises (a) generating a plurality of sequence reads from a plurality of template polynucleotides, each polynucleotide mapped to a genomic locus;

(b) grouping the sequence reads into families, each family comprising sequence reads generated from one of the template polynucleotides; (c) calling a base (or sequence) at the genomic locus for each of the families; (d) detecting at the genomic locus any of genomic alterations among the calls, frequency of a genetic alteration among the calls, total number of calls and total number of alterations among the calls.

[0058] In some embodiments, calling comprises any of phylogenetic analysis, voting, weighing, assigning a probability to each read at the locus in a family, and calling the base with the highest probability. In some embodiments, the method is performed at two loci, comprising determining CNV at one of the loci based on counts at each of the loci.

[0059] Another aspect provides a method for determining a quantitative measure indicative of a number of individual double-stranded DNA fragments in a sample comprising (a) determining a quantitative measure of individual DNA molecules for which both strands are detected; (b) determining a quantitative measure of individual DNA molecules for which only one of the DNA strands are detected; (c) inferring from (a) and (b) above a quantitative measure of individual DNA molecules for which neither strand was detected; and (d) using (a)-(c) determining the quantitative measure indicative of a number of individual double-stranded DNA fragments in the sample.

[0060] In some embodiments, the method further comprises detecting copy number variation in the sample by determining a normalized quantitative measure determined in step (d) at each of one or more genetic loci and determining copy number variation based on the normalized measure. In some embodiments, the sample comprises double-stranded polynucleotide molecules sourced substantially from cell-free nucleic acids.

[0061] In some embodiments, determining the quantitative measure of individual DNA molecules comprises tagging the DNA molecules with a set of duplex tags, wherein each duplex tag differently tags complementary strands of a double-stranded DNA molecule in the sample to provide tagged strands. In some embodiments, the method further comprises sequencing at least some of the tagged strands to produce a set of sequence reads. In some embodiments, the method further comprises sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide

molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads. In some embodiments, the method further comprises determining quantitative measures of (i) the paired reads and (ii) the unpaired reads that map to each of one or more genetic loci to determine a quantitative measure of total double-stranded DNA molecules in the sample that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads mapping to each locus.

[0062] In another aspect, a method for reducing distortion in a sequencing assay, comprises (a) tagging control parent polynucleotides with a first tag set to produce tagged control parent polynucleotides; (b) tagging test parent polynucleotides with a second tag set to produce tagged test parent polynucleotides; (c) mixing tagged control parent polynucleotides with tagged test parent polynucleotides to form a pool; (d) determining quantities of tagged control parent polynucleotides and tagged test parent polynucleotides; and (e) using the quantities of tagged control parent polynucleotides to reduce distortion in the quantities of tagged test parent polynucleotides.

[0063] In some embodiments, the first tag set comprises a plurality of tags, wherein each tag in the first tag set comprises a same control tag and an identifying tag, and wherein the first tag set comprises a plurality of different identifying tags. In some embodiments, the second tag set comprises a plurality of tags, wherein each tag in the second tag set comprises a same test tag and an identifying tag, wherein the test tag is distinguishable from the control tag, and wherein the second tag set comprises a plurality of different identifying tags. In some embodiments, (d) comprises amplifying tagged parent polynucleotides in the pool to form a pool of amplified, tagged polynucleotides, and sequencing amplified, tagged polynucleotides in the amplified pool to produce a plurality of sequence reads. In some embodiments, the method further comprises grouping sequence reads into families, each family comprising sequence reads generated from a same parent polynucleotide, which grouping is optionally based on information from an identifying tag and from start/end sequences of the parent polynucleotides, and, optionally, determining a consensus sequence for each of a plurality of parent polynucleotides from the plurality of sequence reads in a group.

[0064] In some embodiments, (d) comprises determining copy number variation in the test parent polynucleotides at greater than or equal to one locus based on relative quantity of test parent polynucleotides and control parent polynucleotides mapping to the locus.

[0065] Another aspect provides a method comprising (a) ligating adaptors to double-stranded DNA polynucleotides, wherein ligating is performed in a single reaction vessel, and wherein the adaptors comprise molecular barcodes, to produce a tagged library comprising an insert from the double-stranded DNA polynucleotides, and having between 4 and 1 million different tags; (b) generating a plurality of sequence reads for each of the double-stranded DNA polynucleotides in the tagged library; (c) grouping sequence reads into families, each family comprising sequence reads generated from a single DNA polynucleotide among the double-stranded DNA polynucleotides, based on information in a tag and information at an end of the insert; and (d) calling bases at each position in the double-stranded DNA molecule based on bases at the position in members of a family. In some embodiments, (b) comprises amplifying each of the double-stranded DNA polynucleotide molecules in the tagged library to generate amplification products, and sequencing the amplification products. In some embodiments, the method further comprises sequencing the double-stranded DNA polynucleotide molecules a plurality of times. In some embodiments, (b) comprises sequencing the entire insert. In some embodiments, (c) further comprises collapsing sequence reads in each family to generate a consensus sequence. In some embodiments, (d) comprises calling a plurality of sequential bases from at least a subset of the sequence reads to identify single nucleotide variations (SNV) in the double-stranded DNA molecule.

[0066] Another aspect provides a method of detecting disease cell heterogeneity from a sample comprising polynucleotides from somatic cells and disease cells. The method comprises quantifying polynucleotides in the sample bearing a nucleotide sequence variant at each of a plurality of genetic loci; determining copy number variation (CNV) at each of the plurality of genetic loci, wherein the CNV indicates a genetic dose of a locus in the disease cell polynucleotides; determining with a programmed computer processor a relative measure of quantity of polynucleotides bearing a sequence variant at a locus per the genetic dose at the locus for each of a plurality of the loci; and comparing the relative measures at each of the plurality of loci, wherein different relative measures is indicative of tumor heterogeneity.

[0067] In another aspect, a method comprises subjecting a subject to one or more pulsed therapy cycles, each pulsed therapy cycle comprising (a) a first period during which a drug is administered at a first amount; and (b) a second period during which the drug is administered at a second, reduced amount, wherein (i) the first period is characterized by a tumor burden detected above a first clinical level; and (ii) the second period is characterized by a tumor burden detected below a second clinical level.

[0068] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0069] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0001] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “figure” and “FIG.” herein), of which:

[0070] **FIG. 1** is a flowchart representation of a method of the present disclosure for determining copy number variation (CNV);

[0071] **FIG. 2** depicts mapping of pairs and singlets to Locus A and Locus B in a genome;

[0072] **FIG. 3** shows a reference sequence encoding a genetic Locus A;

[0073] **FIGs. 4A-C** shows amplification, sequencing, redundancy reduction and pairing of complementary molecules;

[0074] FIG. 5 shows increased confidence in detecting sequence variants by pairing reads from Watson and Crick strands;

[0075] FIG. 6 shows a computer system that is programmed or otherwise configured to implement various methods of the present disclosure;

[0076] FIG. 7 is schematic representation of a system for analyzing a sample comprising nucleic acids from a user, including a sequencer; bioinformatic software and internet connection for report analysis by, for example, a hand held device or a desk top computer;

[0077] FIG. 8 is a flowchart representation of a method of this invention for determining CNV using pooled test and control pools; and

[0078] FIGs. 9A-9C schematically illustrate a method for tagging a polynucleotide molecule with a library adaptor and subsequently a sequencing adaptor.

DETAILED DESCRIPTION

[0079] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0080] The term “genetic variant,” as used herein, generally refers to an alteration, variant or polymorphism in a nucleic acid sample or genome of a subject. Such alteration, variant or polymorphism can be with respect to a reference genome, which may be a reference genome of the subject or other individual. Single nucleotide polymorphisms (SNPs) are a form of polymorphisms. In some examples, one or more polymorphisms comprise one or more single nucleotide variations (SNVs), insertions, deletions, repeats, small insertions, small deletions, small repeats, structural variant junctions, variable length tandem repeats, and/or flanking sequences,. Copy number variants (CNVs), transversions and other rearrangements are also forms of genetic variation. A genomic alternation may be a base change, insertion, deletion, repeat, copy number variation, or transversion.

[0081] The term “polynucleotide,” as used herein, generally refers to a molecule comprising one or more nucleic acid subunits. A polynucleotide can include one or more subunits selected from adenosine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), or variants thereof. A

nucleotide can include A, C, G, T or U, or variants thereof. A nucleotide can include any subunit that can be incorporated into a growing nucleic acid strand. Such subunit can be an A, C, G, T, or U, or any other subunit that is specific to one or more complementary A, C, G, T or U, or complementary to a purine (i.e., A or G, or variant thereof) or a pyrimidine (i.e., C, T or U, or variant thereof). A subunit can enable individual nucleic acid bases or groups of bases (e.g., AA, TA, AT, GC, CG, CT, TC, GT, TG, AC, CA, or uracil-counterparts thereof) to be resolved. In some examples, a polynucleotide is deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), or derivatives thereof. A polynucleotide can be single-stranded or double stranded.

[0082] The term “subject,” as used herein, generally refers to an animal, such as a mammalian species (e.g., human) or avian (e.g., bird) species, or other organism, such as a plant. More specifically, the subject can be a vertebrate, a mammal, a mouse, a primate, a simian or a human. Animals include, but are not limited to, farm animals, sport animals, and pets. A subject can be a healthy individual, an individual that has or is suspected of having a disease or a predisposition to the disease, or an individual that is in need of therapy or suspected of needing therapy. A subject can be a patient.

[0083] The term “genome” generally refers to an entirety of an organism’s hereditary information. A genome can be encoded either in DNA or in RNA. A genome can comprise coding regions that code for proteins as well as non-coding regions. A genome can include the sequence of all chromosomes together in an organism. For example, the human genome has a total of 46 chromosomes. The sequence of all of these together constitutes a human genome.

[0084] The terms “adaptor(s)”, “adapter(s)” and “tag(s)” are used synonymously throughout this specification. An adaptor or tag can be coupled to a polynucleotide sequence to be “tagged” by any approach including ligation, hybridization, or other approaches.

[0085] The term “library adaptor” or “library adapter” as used herein, generally refers to a molecule (e.g., polynucleotide) whose identity (e.g., sequence) can be used to differentiate polynucleotides in a biological sample (also “sample” herein).

[0086] The term “sequencing adaptor,” as used herein, generally refers to a molecule (e.g., polynucleotide) that is adapted to permit a sequencing instrument to sequence a target polynucleotide, such as by interacting with the target polynucleotide to enable sequencing. The sequencing adaptor permits the target polynucleotide to be sequenced by the sequencing

instrument. In an example, the sequencing adaptor comprises a nucleotide sequence that hybridizes or binds to a capture polynucleotide attached to a solid support of a sequencing system, such as a flow cell. In another example, the sequencing adaptor comprises a nucleotide sequence that hybridizes or binds to a polynucleotide to generate a hairpin loop, which permits the target polynucleotide to be sequenced by a sequencing system. The sequencing adaptor can include a sequencer motif, which can be a nucleotide sequence that is complementary to a flow cell sequence of other molecule (e.g., polynucleotide) and usable by the sequencing system to sequence the target polynucleotide. The sequencer motif can also include a primer sequence for use in sequencing, such as sequencing by synthesis. The sequencer motif can include the sequence(s) needed to couple a library adaptor to a sequencing system and sequence the target polynucleotide.

[0087] As used herein the terms “at least”, “at most” or “about”, when preceding a series, refers to each member of the series, unless otherwise identified.

[0088] The term “about” and its grammatical equivalents in relation to a reference numerical value can include a range of values up to plus or minus 10% from that value. For example, the amount “about 10” can include amounts from 9 to 11. In other embodiments, the term “about” in relation to a reference numerical value can include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[0089] The term “at least” and its grammatical equivalents in relation to a reference numerical value can include the reference numerical value and greater than that value. For example, the amount “at least 10” can include the value 10 and any numerical value above 10, such as 11, 100, and 1,000.

[0090] The term “at most” and its grammatical equivalents in relation to a reference numerical value can include the reference numerical value and less than that value. For example, the amount “at most 10” can include the value 10 and any numerical value under 10, such as 9, 8, 5, 1, 0.5, and 0.1.

[0091] **1. Methods for processing and/or analyzing a nucleic acid sample**

[0092] An aspect of the present disclosure provides methods for determining a genomic alternation in a nucleic acid sample of a subject. **FIG. 1** shows a method of determining copy

number variation (CNV). The method can be implemented to determine other genomic alternations, such as SNVs.

[0093] A. Polynucleotide Isolation

[0094] Methods disclosed herein can comprise isolating one or more polynucleotides. A polynucleotide can comprise any type of nucleic acid, for example, a sequence of genomic nucleic acid, or an artificial sequence (*e.g.*, a sequence not found in genomic nucleic acid). For example, an artificial sequence can contain non-natural nucleotides. Also, a polynucleotide can comprise both genomic nucleic acid and an artificial sequence, in any portion. For example, a polynucleotide can comprise 1 to 99% of genomic nucleic acid and 99% to 1% of artificial sequence, where the total adds up to 100%. Thus, fractions of percentages are also contemplated. For example, a ratio of 99.1% to 0.9% is contemplated.

[0095] A polynucleotide can comprise any type of nucleic acids, such as DNA and/or RNA. For example, if a polynucleotide is DNA, it can be genomic DNA, complementary DNA (cDNA), or any other deoxyribonucleic acid. A polynucleotide can also be cell-free DNA (cfDNA). For example, the polynucleotide can be circulating DNA. The circulating DNA can comprise circulating tumor DNA (ctDNA). A polynucleotide can be double-stranded or single-stranded. Alternatively, a polynucleotide can comprise a combination of a double-stranded portion and a single-stranded portion.

[0096] Polynucleotides do not have to be cell-free. In some cases, the polynucleotides can be isolated from a sample. For example, in step (102) (**FIG. 1**), double-stranded polynucleotides are isolated from a sample. A sample can be any biological sample isolated from a subject. For example, a sample can comprise, without limitation, bodily fluid, whole blood, platelets, serum, plasma, stool, red blood cells, white blood cells or leucocytes, endothelial cells, tissue biopsies, synovial fluid, lymphatic fluid, ascites fluid, interstitial or extracellular fluid, the fluid in spaces between cells, including gingival crevicular fluid, bone marrow, cerebrospinal fluid, saliva, mucous, sputum, semen, sweat, urine, or any other bodily fluids. A bodily fluid can include saliva, blood, or serum. For example, a polynucleotide can be cell-free DNA isolated from a bodily fluid, *e.g.*, blood or serum. A sample can also be a tumor sample, which can be obtained from a subject by various approaches, including, but not limited to, venipuncture, excretion,

ejaculation, massage, biopsy, needle aspirate, lavage, scraping, surgical incision, or intervention or other approaches.

[0097] A sample can comprise various amount of nucleic acid that contains genome equivalents. For example, a sample of about 30 ng DNA can contain about 10,000 (10^4) haploid human genome equivalents and, in the case of cfDNA, about 200 billion (2×10^{11}) individual polynucleotide molecules. Similarly, a sample of about 100 ng of DNA can contain about 30,000 haploid human genome equivalents and, in the case of cfDNA, about 600 billion individual molecules.

[0098] A sample can comprise nucleic acids from different sources. For example, a sample can comprise germline DNA or somatic DNA. A sample can comprise nucleic acids carrying mutations. For example, a sample can comprise DNA carrying germline mutations and/somatic mutations, . A sample can also comprise DNA carrying cancer-associated mutations (*e.g.*, cancer-associated somatic mutations).

[0099] B. Tagging

[00100] Polynucleotides disclosed herein can be tagged. For example, in step (104) (**FIG. 1**) the double-stranded polynucleotides are tagged with duplex tags, tags that differently label the complementary strands (*i.e.*, the “Watson” and “Crick” strands) of a double-stranded molecule. In one embodiment the duplex tags are polynucleotides having complementary and non-complementary portions.

[00101] Tags can be any types of molecules attached to a polynucleotide, including, but not limited to, nucleic acids, chemical compounds, florescent probes, or radioactive probes. Tags can also be oligonucleotides (*e.g.*, DNA or RNA). Tags can comprise known sequences, unknown sequences, or both. A tag can comprise random sequences, pre-determined sequences, or both. A tag can be double-stranded or single-stranded. A double-stranded tag can be a duplex tag. A double-stranded tag can comprise two complementary strands. Alternatively, a double-stranded tag can comprise a hybridized portion and a non-hybridized portion. The double-stranded tag can be Y-shaped, *e.g.*, the hybridized portion is at one end of the tag and the non-hybridized portion is at the opposite end of the tag. One such example are the “Y adapters” used in Illumina sequencing. Other examples include hairpin shaped adapters or bubble shaped

adapters. Bubble shaped adapters have non-complementary sequences flanked on both sides by complementary sequences.

[00102] Tagging disclosed herein can be performed using any method. A polynucleotide can be tagged with an adaptor by hybridization. For example, the adaptor can have a nucleotide sequence that is complementary to at least a portion of a sequence of the polynucleotide. As an alternative, a polynucleotide can be tagged with an adaptor by ligation.

[00103] For example, tagging can comprise using one or more enzymes. The enzyme can be a ligase. The ligase can be a DNA ligase. For example, the DNA ligase can be a T4 DNA ligase, *E. coli* DNA ligase, and/or mammalian ligase. The mammalian ligase can be DNA ligase I, DNA ligase III, or DNA ligase IV. The ligase can also be a thermostable ligase. Tags can be ligated to a blunt-end of a polynucleotide (blunt-end ligation). Alternatively, tags can be ligated to a sticky end of a polynucleotide (sticky-end ligation). Efficiency of ligation can be increased by optimizing various conditions. Efficiency of ligation can be increased by optimizing the reaction time of ligation. For example, the reaction time of ligation can be less than 12 hours, *e.g.*, less than 1, less than 2, less than 3, less than 4, less than 5, less than 6, less than 7, less than 8, less than 9, less than 10, less than 11, less than 12, less than 13, less than 14, less than 15, less than 16, less than 17, less than 18, less than 19, or less than 20 hours. In a particular example, reaction time of ligation is less than 20 hours. Efficiency of ligation can be increased by optimizing the ligase concentration in the reaction. For example, the ligase concentration can be at least 10, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 400, at least 500, or at least 600 unit/microliter. Efficiency can also be optimized by adding or varying the concentration of an enzyme suitable for ligation, enzyme cofactors or other additives, and/or optimizing a temperature of a solution having the enzyme. Efficiency can also be optimized by varying the addition order of various components of the reaction. The end of tag sequence can comprise dinucleotide to increase ligation efficiency. When the tag comprises a non-complementary portion (*e.g.*, Y-shaped adaptor), the sequence on the complementary portion of the tag adaptor can comprise one or more selected sequences that promote ligation efficiency. Preferably such sequences are located at the terminal end of the tag. Such sequences can comprise 1, 2, 3, 4, 5, or 6 terminal bases. Reaction solution with high viscosity (*e.g.*, a low Reynolds number) can also be used to increase ligation efficiency. For example, solution can

have a Reynolds number less than 3000, less than 2000, less than 1000, less than 900, less than 800, less than 700, less than 600, less than 500, less than 400, less than 300, less than 200, less than 100, less than 50, less than 25, or less than 10. It is also contemplated that roughly unified distribution of fragments (*e.g.*, tight standard deviation) can be used to increase ligation efficiency. For example, the variation in fragment sizes can vary by less than 20%, less than 15%, less than 10%, less than 5%, or less than 1%. Tagging can also comprise primer extension, for example, by polymerase chain reaction (PCR). Tagging can also comprise any of ligation-based PCR, multiplex PCR, single strand ligation, or single strand circularization.

[00104] In some instances, the tags herein comprise molecular barcodes. Such molecular barcodes can be used to differentiate polynucleotides in a sample. Preferably molecular barcodes are different from one another. For example, molecular barcodes can have a difference between them that can be characterized by a predetermined edit distance or a Hamming distance. In some instances, the molecular barcodes herein have a minimum edit distance of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. To further improve efficiency of conversion (*e.g.*, tagging) of untagged molecular to tagged molecules, one preferably utilizes short tags. For example, in some embodiments, a library adapter tag can be up to 65, 60, 55, 50, 45, 40, or 35 nucleotide bases in length. A collection of such short library barcodes preferably includes a number of different molecular barcodes, *e.g.*, at least 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 different barcodes with a minimum edit distance of 1, 2, 3 or more.

[00105] Thus, a collection of molecules can include one or more tags. In some instances, some molecules in a collection can include an identifying tag (“identifier”) such as a molecular barcode that is not shared by any other molecule in the collection. For example, in some instances of a collection of molecules, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the molecules in the collection

can include an identifier or molecular barcode that is not shared by any other molecule in the collection. As used herein, a collection of molecules is considered to be “uniquely tagged” if each of at least 95% of the molecules in the collection bears an identifier that is not shared by any other molecule in the collection (“unique tag” or “unique identifier”). A collection of molecules is considered to be “non-uniquely tagged” if each of at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least or about 50% of the molecules in the collection bears an identifying tag or molecular barcode that is shared by at least one other molecule in the collection (“non-unique tag” or “non-unique identifier”). Accordingly, in a non-uniquely tagged population no more than 1% of the molecules are uniquely tagged. For example, in a non-uniquely tagged population, no more than 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the molecules can be uniquely tagged.

[00106] A number of different tags can be used based on the estimated number of molecules in a sample. In some tagging methods, the number of different tags can be at least the same as the estimated number of molecules in the sample. In other tagging methods, the number of different tags can be at least two, three, four, five, six, seven, eight, nine, ten, one hundred or one thousand times as many as the estimated number of molecules in the sample. In unique tagging, at least two times (or more) as many different tags can be used as the estimated number of molecules in the sample.

[00107] The molecules in the sample may be non-uniquely tagged. In such instances a fewer number of tags or molecular barcodes is used than the number of molecules in the sample to be tagged. For example, no more than 100, 50, 40, 30, 20 or 10 unique tags or molecular barcodes are used to tag a complex sample such as a cell free DNA sample with many more different fragments.

[00108] The polynucleotide to be tagged can be fragmented, such as either naturally or using other approaches, such as, for example, shearing. The polynucleotides can be fragmented by certain methods, including but not limited to, mechanical shearing, passing the sample through a syringe, sonication, heat treatment (*e.g.*, for 30 minutes at 90°C), and/or nuclease treatment (*e.g.*, using DNase, RNase, endonuclease, exonuclease, and/or restriction enzyme).

[00109] The polynucleotides fragments (prior to tagging) can comprise sequences of any length. For example, polynucleotide fragments (prior to tagging) can comprise at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 or more nucleotides in length. The polynucleotide fragment are preferably about the average length of cell-free DNA. For example, the polynucleotide fragments can comprise about 160 bases in length. The polynucleotide fragment can also be fragmented from a larger fragment into smaller fragments about 160 bases in length.

[00110] Polynucleotides tagged can comprise sequences associated with cancer. The cancer-associated sequences can comprise single nucleotide variation (SNV), copy number variation (CNV), insertions, deletions, and/or rearrangements.

[00111] The polynucleotides can comprise sequences associated with cancer, such as acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, Kaposi Sarcoma, anal cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, malignant fibrous histiocytoma, brain stem glioma, brain cancer, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumor, breast cancer, bronchial tumor, Burkitt lymphoma, Non-Hodgkin lymphoma, carcinoid tumor, cervical cancer, chordoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), colon cancer, colorectal cancer, cutaneous T-cell lymphoma, ductal carcinoma in situ, endometrial cancer, esophageal cancer, Ewing Sarcoma, eye cancer, intraocular melanoma, retinoblastoma, fibrous histiocytoma, gallbladder cancer, gastric cancer, glioma, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, kidney cancer, laryngeal cancer, lip cancer, oral cavity cancer, lung cancer, non-small cell carcinoma, small cell carcinoma, melanoma, mouth cancer, myelodysplastic syndromes, multiple myeloma, medulloblastoma, nasal cavity cancer, paranasal sinus cancer, neuroblastoma, nasopharyngeal cancer, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary tumor, plasma

cell neoplasm, prostate cancer, rectal cancer, renal cell cancer, rhabdomyosarcoma, salivary gland cancer, Sezary syndrome, skin cancer, nonmelanoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, testicular cancer, throat cancer, thymoma, thyroid cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, and/or Wilms Tumor.

[00112] A haploid human genome equivalent has about 3 picograms of DNA. A sample of about 1 microgram of DNA contains about 300,000 haploid human genome equivalents.

Improvements in sequencing can be achieved as long as at least some of the duplicate or cognate polynucleotides bear unique identifiers with respect to each other, that is, bear different tags. However, in certain embodiments, the number of tags used is selected so that there is at least a 95% chance that all duplicate molecules starting at any one position bear unique identifiers. For example, in a sample comprising about 10,000 haploid human genome equivalents of fragmented genomic DNA, *e.g.*, cfDNA, z is expected to be between 2 and 8. Such a population can be tagged with between about 10 and 100 different identifiers, for example, about 2 identifiers, about 4 identifiers, about 9 identifiers, about 16 identifiers, about 25 identifiers, about 36 different identifiers, about 49 different identifiers, about 64 different identifiers, about 81 different identifiers, or about 100 different identifiers.

[00113] Nucleic acid barcodes having identifiable sequences including molecular barcodes, can be used for tagging. For example, a plurality of DNA barcodes can comprise various numbers of sequences of nucleotides. A plurality of DNA barcodes having 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more identifiable sequences of nucleotides can be used. When attached to only one end of a polynucleotide, the plurality of DNA barcodes can produce 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more different identifiers. Alternatively, when attached to both ends of a polynucleotide, the plurality DNA barcodes can produce 4, 9, 16, 25, 36, 49, 64, 81, 100, 121, 144, 169, 196, 225, 256, 289, 324, 361, 400 or more different identifiers (which is the z^2 of when the DNA barcode is attached to only 1 end of a polynucleotide). In one example, a plurality of DNA barcodes having 6, 7, 8, 9 or 10 identifiable sequences of nucleotides can be used. When attached to both ends of a polynucleotide, they produce 36, 49, 64, 81 or 100 possible different identifiers, respectively. In

a particular example, the plurality of DNA barcodes can comprise 8 identifiable sequences of nucleotides. When attached to only one end of a polynucleotide, the plurality of DNA barcodes can produce 8 different identifiers. Alternatively, when attached to both ends of a polynucleotide, the plurality of DNA barcodes can produce 64 different identifiers. Samples tagged in such a way can be those with a range of about 10 ng to any of about 100 ng, about 1 µg, about 10 µg of fragmented polynucleotides, *e.g.*, genomic DNA, *e.g.*, cfDNA.

[00114] A polynucleotide can be uniquely identified in various ways. A polynucleotide can be uniquely identified by a unique DNA barcode. For example, any two polynucleotides in a sample are attached two different DNA barcodes. Alternatively, a polynucleotide can be uniquely identified by the combination of a DNA barcode and one or more endogenous sequences of the polynucleotide. For example, any two polynucleotides in a sample can be attached the same DNA barcode, but the two polynucleotides can still be identified by different endogenous sequences. The endogenous sequence can be on an end of a polynucleotide. For example, the endogenous sequence can be adjacent (*e.g.*, base in between) to the attached DNA barcode. In some instances the endogenous sequence can be at least 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 bases in length. Preferably, the endogenous sequence is a terminal sequence of the fragment/polynucleotides to be analyzed. The endogenous sequence may be the length of the sequence. For example, a plurality of DNA barcodes comprising 8 different DNA barcodes can be attached to both ends of each polynucleotide in a sample. Each polynucleotide in the sample can be identified by the combination of the DNA barcodes and about 10 base pair endogenous sequence on an end of the polynucleotide. Without being bound by theory, the endogenous sequence of a polynucleotide can also be the entire polynucleotide sequence.

[00115] Also disclosed herein are compositions of tagged polynucleotides. The tagged polynucleotide can be single-stranded. Alternatively, the tagged polynucleotide can be double-stranded (*e.g.*, duplex-tagged polynucleotides). Accordingly, this invention also provides compositions of duplex-tagged polynucleotides. The polynucleotides can comprise any types of nucleic acids (DNA and/or RNA). The polynucleotides comprise any types of DNA disclosed herein. For example, the polynucleotides can comprise DNA, *e.g.*, fragmented DNA or cfDNA. A set of polynucleotides in the composition that map to a mappable base position in a genome can be non-uniquely tagged, that is, the number of different identifiers can be at least 2 and fewer

than the number of polynucleotides that map to the mappable base position. The number of different identifiers can also be at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and fewer than the number of polynucleotides that map to the mappable base position.

[00116] In some instances, as a composition goes from about 1 ng to about 10 µg or higher, a larger set of different molecular barcodes can be used. For example, between 5 and 100 different library adaptors can be used to tag polynucleotides in a cfDNA sample.

[00117] The systems and methods disclosed herein may be used in applications that involve the assignment of molecular barcodes. The molecular barcodes can be assigned to any types of polynucleotides disclosed in this invention. For example, the molecular barcodes can be assigned to cell-free polynucleotides (*e.g.*, cfDNAs). Often, an identifier disclosed herein can be a barcode oligonucleotide that is used to tag the polynucleotide. The barcode identifier may be a nucleic acid oligonucleotide (*e.g.*, a DNA oligonucleotide). The barcode identifier can be single-stranded. Alternatively, the barcode identifier can be double-stranded. The barcode identifier can be attached to polynucleotides using any method disclosed herein. For example, the barcode identifier can be attached to the polynucleotide by ligation using an enzyme. The barcode identifier can also be incorporated into the polynucleotide through PCR. In other cases, the reaction may comprise addition of a metal isotope, either directly to the analyte or by a probe labeled with the isotope. Generally, assignment of unique or non-unique identifiers or molecular barcodes in reactions of this disclosure may follow methods and systems described by, for example, U.S. patent applications 2001/0053519, 2003/0152490, 2011/0160078 and U.S. Patent No. 6,582,908, each of which is entirely incorporated herein by reference.

[00118] Identifiers or molecular barcodes used herein may be completely endogenous whereby circular ligation of individual fragments may be performed followed by random shearing or targeted amplification. In this case, the combination of a new start and stop point of the molecule and the original intramolecular ligation point can form a specific identifier.

[00119] Identifiers or molecular barcodes used herein can comprise any types of oligonucleotides. In some cases, identifiers may be predetermined, random, or semi-random sequence oligonucleotides. Identifiers can be barcodes. For example, a plurality of barcodes may be used such that barcodes are not necessarily unique to one another in the plurality.

Alternatively, a plurality of barcodes may be used such that each barcode is unique to any other barcode in the plurality. The barcodes can comprise specific sequences (*e.g.*, predetermined sequences) that can be individually tracked. Further, barcodes may be attached (*e.g.*, by ligation) to individual molecules such that the combination of the barcode and the sequence it may be ligated to creates a specific sequence that may be individually tracked. As described herein, detection of barcodes in combination with sequence data of beginning (start) and/or end (stop) portions of sequence reads can allow assignment of a unique identity to a particular molecule. The length or number of base pairs of an individual sequence read may also be used to assign a unique identity to such a molecule. As described herein, fragments from a single strand of nucleic acid having been assigned a unique identity, may thereby permit subsequent identification of fragments from the parent strand. In this way the polynucleotides in the sample can be uniquely or substantially uniquely tagged. A duplex tag can include a degenerate or semi-degenerate nucleotide sequence, *e.g.*, a random degenerate sequence. The nucleotide sequence can comprise any number of nucleotides. For example, the nucleotide sequence can comprise 1 (if using a non-natural nucleotide), 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more nucleotides. In a particular example, the sequence can comprise 7 nucleotides. In another example, the sequence can comprise 8 nucleotides. The sequence can also comprise 9 nucleotides. The sequence can comprise 10 nucleotides.

[00120] A barcode can comprise contiguous or non-contiguous sequences. A barcode that comprises at least 1, 2, 3, 4, 5 or more nucleotides is a contiguous sequence or non-contiguous sequence. If the 4 nucleotides are uninterrupted by any other nucleotide. For example, if a barcode comprises the sequence TTGC, a barcode is contiguous if the barcode is TTGC. On the other hand, a barcode is non-contiguous if the barcode is TTXGC, where X is a nucleic acid base.

[00121] An identifier or molecular barcode can have an n-mer sequence which may be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more nucleotides in length. A tag herein can comprise any range of nucleotides in length. For example, the sequence can be between 2 to 100, 10 to 90, 20 to 80, 30 to 70, 40 to 60, or about 50 nucleotides in length.

[00122] The tag can comprise a double-stranded fixed reference sequence downstream of the identifier or molecular barcode. Alternatively, the tag can comprise a double-stranded fixed reference sequence upstream or downstream of the identifier or molecular barcode. Each strand of a double-stranded fixed reference sequence can be, for example, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 nucleotides in length.

[00123] C. Adaptors

[00124] A library of polynucleotide molecules can be synthesized for use in sequencing. For example, a library of polynucleotides comprising a plurality of polynucleotide molecules that are each less than or equal to 100, 90, 80, 70, 60, 50, 45, 40, or 35 nucleic acid (or nucleotide) bases in length can be made. A plurality of polynucleotide molecules can be each less than or equal to 35 nucleic acid bases in length. A plurality of polynucleotide molecules can be each less than or equal to 30 nucleic acid bases in length. A plurality of polynucleotide molecules can also be less than or equal to 250, 200, 150, 100, or 50 nucleic acid bases. Additionally, the plurality of polynucleotide molecules can also be less than or equal to 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleic acid bases.

[00125] A library of polynucleotides comprising a plurality of polynucleotide molecules can also have distinct (with respect to each other) molecular barcode sequences (or molecular barcodes) with respect to at least 4 nucleic acid bases. A molecular barcode (also “barcode” or “identifier” herein) sequence is a nucleotide sequence that distinguishes one polynucleotide from another. In other embodiments, the polynucleotide molecules can also have different barcode sequences with respect to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more nucleic acid bases.

[00126] A library of polynucleotides comprising a plurality of polynucleotide molecules can also have a plurality of different barcode sequences. For example, a plurality of polynucleotide molecules can have at least 4 different molecular barcode sequences. In some cases, the plurality

of polynucleotide molecules has from 2-100, 4-50, 4-30, 4-20, or 4-10 different molecular barcode sequences. The plurality of polynucleotides molecules can also have other ranges of different barcode sequences such as, 1-4, 2-5, 3-6, 4-7, 5-8, 6-9, 7-10, 8-11, 9-12, 10-13, 11-14, 12-15, 13-16, 14-17, 15-18, 16-19, 17-20, 18-21, 19-22, 20-23, 21-24, or 22-25 different barcode sequences. In other cases, a plurality of polynucleotide molecules can have at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 more different barcode sequences. In a particular example, the plurality library adapters comprise at least 8 different sequences.

[00127] The location of the different barcode sequences can vary within the plurality of polynucleotides. For example, the different barcode sequences can be within 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, or 2 nucleic acid bases from a terminal end of a respective one of the plurality of polynucleotide molecules. In an example, a plurality of polynucleotide molecules has distinct barcode sequences that are within 10 nucleic acid bases from the terminal end. In another example, a plurality of polynucleotide molecules has distinct barcode sequences that are within 5 or 1 nucleic acid bases from the terminal end. In other instances, the distinct barcode sequences can be at the terminal end of a respective one of the plurality of polynucleotide molecules. Other variations include that the distinct molecular barcode sequences can be within 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, or more nucleic acid bases from a terminal end of a respective one of the plurality of polynucleotide molecules.

[00128] The terminal end of the plurality of polynucleotide molecules can be adapted for ligation to a target nucleic acid molecule. For example, the terminal end can be a blunt end. In some other cases, the terminal end is adapted for hybridization to a complementary sequence of a target nucleic acid molecule.

[00129] A library of polynucleotides comprising a plurality of polynucleotide molecules can also have an edit distance of at least 1. In some cases, the edit distance is with respect to individual bases of the plurality of polynucleotide molecules. In other cases, the plurality of polynucleotide molecules can have an edit distance of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more. The edit distance can be a Hamming distance.

[00130] In some cases, the plurality of polynucleotides does not contain sequencing adaptors. A sequence adaptor can be a polynucleotide that comprises a sequence that hybridizes to one or more sequencing adaptors or primers. A sequencing adaptor can further comprise a sequence hybridizing to a solid support, *e.g.*, a flow cell sequence. The term “flow cell sequence” and its grammatical equivalents as used herein, refers to a sequence that permits hybridization to a substrate, for example, by way of a primer attached to the substrate. The substrate can be bead or a planar surface. In some embodiments, a flow cell sequence can allow a polynucleotide to attach to a flow cell or surface (*e.g.*, surface of a bead, for example, an Illumina flow cell).

[00131] When a plurality of polynucleotide molecules does not contain sequencing adaptors or primers, each polynucleotide molecule of the plurality does not contain a nucleic acid sequence or other moiety that is adapted to permit sequencing of a target nucleic acid molecule with a given sequencing approach, such as Illumina, SOLiD, Pacific Biosciences, GeneReader, Oxford Nanopore, Complete Genomics, Gnu-Bio, Ion Torrent, Oxford Nanopore or Genia. In some examples, when a plurality of polynucleotide molecules does not contain sequencing adaptors or primers, the plurality of polynucleotide molecules does not contain flow cell sequences. For example, the plurality of polynucleotide molecules cannot bind to flow cells, such as used in Illumina flow cell sequencers. However, these flow cell sequences, if desired, can be added to the plurality of polynucleotide molecules by methods such as PCR amplification or ligation. At this point, Illumina flow cell sequencers can be used. Alternatively, when the

plurality of polynucleotide molecules does not contain sequencing adaptors or primers, the plurality of polynucleotide molecules does not contain hairpin shaped adaptors or adaptors for generating hairpin loops in a target nucleic acid molecule, such as Pacific Bioscience SMRTbell™ adaptors. However, these hairpin shaped adaptors, if desired, can be added to the plurality of polynucleotide molecules by methods such as PCR amplification or ligation. The plurality of polynucleotide molecules can be circular or linear.

[00132] A plurality of polynucleotide molecules can be double stranded. In some cases, the plurality of polynucleotide molecules can be single stranded, or can comprise hybridized and non-hybridized regions. A plurality of polynucleotide molecules can be non-naturally occurring polynucleotide molecules.

[00133] Adaptors can be polynucleotide molecules. The polynucleotide molecules can be Y-shaped, bubble-shaped or hairpin-shaped. A hairpin adaptor may contain a restriction site(s) or a Uracil containing base. Adaptors can comprise a complementary portion and a non-complementary portion. The non-complementary portion can have an edit distance (*e.g.*, Hamming distance). For example, the edit distance can be at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30. The complementary portion of the adaptor can comprise sequences that are selected to enable and/or promote ligation to a polynucleotide, *e.g.*, a sequence to enable and/or promote ligation to a polynucleotide at a high yield.

[00134] A plurality of polynucleotide molecules as disclosed herein can be purified. In some cases, a plurality of polynucleotide molecules as disclosed herein can be isolated polynucleotide molecules. In other cases, a plurality of polynucleotide molecules as disclosed herein can be purified and isolated polynucleotide molecules.

[00135] In certain aspects, each of the plurality of polynucleotide molecules is Y-shaped or hairpin-shaped. Each of the plurality of polynucleotide molecules can comprise a different barcode. The different barcode can be a randomer in the complementary portion (*e.g.*, double stranded portion) of the Y-shaped or hairpin-shaped adaptor. Alternatively, the different barcode can be in one strand of the non-complementary portion (*e.g.*, one of the Y-shaped arms). As

discussed above, the different barcode can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more (or any length as described throughout) nucleic acid bases, *e.g.*, 7 bases. The barcode can be contiguous or non-contiguous sequences, as described above. The plurality of polynucleotide molecules is from 10 nucleic acid bases to 35 nucleic acid bases (or any length as described above) in length. Further, the plurality of polynucleotide molecules can comprise an edit distance (as described above), that is a Hamming distance. A plurality of polynucleotide molecules can have distinct barcode sequences that are within 10 nucleic acid bases from the terminal end.

[00136] In another aspect, a plurality of polynucleotide molecules can be sequencing adaptors. A sequencing adaptor can comprise a sequence hybridizing to one or more sequencing primers. A sequencing adaptor can further comprise a sequence hybridizing to a solid support, *e.g.*, a flow cell sequence. For example, a sequencing adaptor can be a flow cell adaptor. The sequencing adaptors can be attached to one or both ends of a polynucleotide fragment. In another example, a sequencing adaptor can be hairpin shaped. For example, the hairpin shaped adaptor can comprise a complementary double-stranded portion and a loop portion, where the double-stranded portion can be attached (*e.g.*, ligated) to a double-stranded polynucleotide. Hairpin shaped sequencing adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times. A sequencing adaptor can be up to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more bases from end to end. For example, a sequencing adaptor can be up to 70 bases from end to end. The sequencing adaptor can comprise 20-30, 20-40, 30-50, 30-60, 40-60, 40-70, 50-60, 50-70, bases from end to end. In a particular example, the sequencing adaptor can comprise 20-30 bases from end to end. In another example, the sequencing adaptor can comprise 50-60 bases from end to end. A sequencing adaptor can comprise one or more barcodes. For example, a sequencing adaptor can comprise a sample barcode. The sample barcode can comprise a pre-determined sequence. The sample barcodes can be used to identify the source of the polynucleotides. The sample barcode can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,

or more (or any length as described throughout) nucleic acid bases, *e.g.*, at least 8 bases. The barcode can be contiguous or non-contiguous sequences, as described above.

[00137] The plurality of polynucleotide molecules as described herein can be used as adaptors. Adaptors can comprise one or more identifiers. An adaptor can comprise an identifier with a random sequence. Alternatively, an adaptor can comprise an identifier with pre-determined sequences. Some adaptors can comprise an identifier with a random sequence and another identifier with a pre-determined sequence. The adaptors comprising identifiers can be double-stranded or single-stranded adaptors. The adaptors comprising identifiers can be Y-shaped adaptors. A Y-shaped adaptor can comprise one or more identifiers with a random sequence. The one or more identifiers can be on the hybrid portion and/or non-hybridized portion of the Y-shaped adaptor. A Y-shaped adaptor can comprise one or more identifiers with a pre-determined sequence. The one or more identifiers with pre-determined sequence can be on the hybridized portion and/or non-hybridized portion of the Y-shaped adaptor. A Y-shaped adaptor can comprise one or more identifiers with a random sequence and one or more identifiers with a pre-determined sequence. For example, the one or more identifiers with a random sequence can be on the hybridized portion of the Y-shaped adaptor and/or the non-hybridized portion of the Y-shaped adaptor. The one or more identifiers with a pre-determined sequence can be on the hybridized portion of the Y-shaped adaptor and/or the non-hybridized portion of the Y-shaped adaptor. In a particular example, a Y-shaped adaptor can comprise an identifier with a random sequence on its hybridized portion and an identifier with a pre-determined sequence on its non-hybridized portion. The identifiers can be in any length disclosed herein. For example, a Y-shaped adaptor can comprise an identifier with a random sequence of 7 nucleotides on its hybridized portion and an identifier with a pre-determined sequence of 8 nucleotides on its non-hybridized portion.

[00138] An adaptor can include a double-stranded portion with a molecular barcode and at least one or two single-stranded portion. For example, the adaptor can be Y-shaped and include a double-stranded portion and two single-stranded portions. The single-stranded portions can include sequences that are not complementary to one another.

[00139] The adaptor can include a terminal end that has a sequence that is selected to permit the adaptor to be efficiently (*e.g.*, at an efficiency of at least about 20%, 30%, 40%, 50%) ligated

or otherwise coupled to a polynucleotide. In some examples, terminal nucleotides in a double-stranded portion of an adaptor are selected from a combination of purines and pyrimidines to provide for efficient ligation.

[00140] In some examples, a set of library adaptors comprises a plurality of polynucleotide molecules (library adaptors) with molecular barcodes. The library adaptors are less than or equal to 80, 70, 60, 50, 45, or 40 nucleotide bases in length. The molecular barcodes can be at least 4 nucleotide bases in length, but may be from 4 to 20 nucleotide bases in length. The molecular barcodes can be different from one another and have an edit distance of at least 1, 2, 3, 4, or 5 between one another. The molecular barcodes are located at least 1, 2, 3, 4, 5, 10, or 20 nucleotide bases away from a terminal end of their respective library adaptors. In some cases, the at least one terminal base is identical in all of the library adaptors.

[00141] The library adaptors can be identical but for the molecular barcodes. For example, the library adaptors can have identical sequences but differ only with respect to nucleotide sequences of the molecular barcodes.

[00142] Each of the library adaptors can have a double stranded portion and at least one single-stranded portion. By “single stranded portion” is meant an area of non-complementarity or an overhang. In some cases, each of the library adaptors has a double-stranded portion and two single-stranded portions. The double-stranded portion can have a molecular barcode. In some cases, the molecular barcode is a randomer. Each of the library adaptors can further include a strand-identification barcode on a single-stranded portion. The strand-identification barcode can include at least 4 nucleotide bases, in some cases from 4 to 20 nucleotide bases.

[00143] In some examples, each of the library adaptors has a double-stranded portion with a molecular barcode and two single-stranded portions. The single-stranded portions may not hybridize to one another. The single-stranded portions may not be completely complementary to one another.

[00144] The library adaptors can have a sequence of terminal nucleotides in a double-stranded portion that are the same. The sequence of terminal nucleotides can be at least 2, 3, 4, 5 or 6 nucleotide bases in length. For example, one strand of a double-stranded portion of the library adaptor can have the sequence ACTT, TCGC, or TACC at the terminal end, while the other strand can have a complementary sequence. In some cases, such a sequence is selected to

optimize the efficiency at which the library adaptors ligate to target polynucleotides. Such sequences can be selected to optimize a binding interaction between the ends of the library adaptors and the target polynucleotides.

[00145] In some cases, none of the library adaptors contains a sample identification motif (or sample molecular barcode). Such sample identification motif can be provided via sequencing adaptors. A sample identification motif can include a sequencer of at least 4, 5, 6, 7, 8, 9, 10, 20, 30, or 40 nucleotide bases that permits the identification of polynucleotide molecules from a given sample from polynucleotide molecules from other samples. For example, this can permit polynucleotide molecules from two subjects to be sequenced in the same pool and sequence reads for the subjects subsequently identified.

[00146] A sequencer motif includes nucleotide sequence(s) needed to couple a library adaptor to a sequencing system and sequence a target polynucleotide coupled to the library adaptor. The sequencer motif can include a sequence that is complementary to a flow cell sequence and a sequence (sequencing initiation sequence) that is selectively hybridizable to a primer (or priming sequence) for use in sequencing. For example, such sequencing initiation sequence can be complementary to a primer that is employed for use in sequence by synthesis (e.g., Illumina). Such primer can be included in a sequencing adaptor. A sequencing initiation sequence can be a primer hybridization site.

[00147] In some cases, none of the library adaptors contains a complete sequencer motif. The library adaptors can contain partial or no sequencer motifs. In some cases, the library adaptors include a sequencing initiation sequence. The library adaptors can include a sequencing initiation sequence but no flow cell sequence. The sequence initiation sequence can be complementary to a primer for sequencing. The primer can be a sequence specific primer or a universal primer. Such sequencing initiation sequences may be situated on single-stranded portions of the library adaptors. As an alternative, such sequencing initiation sequences may be priming sites (e.g., kinks or nicks) to permit a polymerase to couple to the library adaptors during sequencing.

[00148] In some cases, partial or complete sequencer motifs are provided by sequencing adaptors. A sequencing adaptor can include a sample molecular barcode and a sequencer motif. The sequencing adaptors can be provided in a set that is separate from the library adaptors. The

sequencing adaptors in a given set can be identical – i.e., they contain the same sample barcode and sequencer motif.

[00149] Sequencing adaptors can include sample identification motifs and sequencer motifs. Sequencer motifs can include primers that are complementary to a sequencing initiation sequence. In some cases, sequencer motifs also include flow cell sequences or other sequences that permit a polynucleotide to be configured or arranged in a manner that permits the polynucleotide to be sequenced by a sequencer.

[00150] Library adaptors and sequencing adaptors can each be partial adaptors, that is, containing part but not all of the sequences necessary to enable sequencing by a sequencing platform. Together they provide complete adaptors. For example, library adaptors can include partial or no sequencer motifs, but such sequencer motifs are provided by sequencing adaptors.

[00151] **FIGs. 9A-9C** schematically illustrate a method for tagging a target polynucleotide molecule with library adaptors. **FIG. 9A** shows a library adaptor as a partial adaptor containing a primer hybridization site on one of the strands and a molecular barcode towards another end. The primer hybridization site can be a sequencing initiation sequence for subsequent sequencing. The library adaptor is less than or equal to 80 nucleotide bases in length. In **FIG. 9B**, the library adaptors are ligated at both ends of the target polynucleotide molecule to provide a tagged target polynucleotide molecule. The tagged target polynucleotide molecule may be subjected to nucleic acid amplification to generate copies of the target. Next, in **FIG. 9C**, sequencing adaptors containing sequencer motifs are provided and hybridized to the tagged target polynucleotide molecule. The sequencing adaptors contain sample identification motifs. The sequencing adaptors can contain sequences to permit sequencing of the tagged target with a given sequencer.

[00152] **D. Sequencing**

[00153] Tagged polynucleotides can be sequenced to generate sequence reads (*e.g.*, as shown in step (106), **FIG. 1**). For example, a tagged duplex polynucleotide can be sequenced. Sequence reads can be generated from only one strand of a tagged duplex polynucleotide. Alternatively, both strands of a tagged duplex polynucleotide can generate sequence reads. The two strands of the tagged duplex polynucleotide can comprise the same tags. Alternatively, the two strands of the tagged duplex polynucleotide can comprise different tags. When the two

strands of the tagged duplex polynucleotide are differently tagged, sequence reads generated from one strand (*e.g.*, a Watson strand) can be distinguished from sequence reads generated from the other strands (*e.g.*, a Crick strand). Sequencing can involve generating multiple sequence reads for each molecule. This occurs, for example, as a result the amplification of individual polynucleotide strands during the sequencing process, *e.g.*, by PCR.

[00154] Methods disclosed herein can comprise amplifying of polynucleotides.

Polynucleotides amplification can result in the incorporation of nucleotides into a nucleic acid molecule or primer thereby forming a new nucleic acid molecule complementary to a template nucleic acid. The newly formed polynucleotide molecule and its template can be used as templates to synthesize additional polynucleotides. The polynucleotides being amplified can be any nucleic acids, for example, deoxyribonucleic acids, including genomic DNAs, cDNAs (complementary DNA), cfDNAs, and circulating tumor DNAs (ctDNAs). The polynucleotides being amplified can also be RNAs. As used herein, one amplification reaction may comprise many rounds of DNA replication. DNA amplification reactions can include, for example, polymerase chain reaction (PCR). One PCR reaction may comprise 2-100 “cycles” of denaturation, annealing, and synthesis of a DNA molecule. For example, 2-7, 5-10, 6-11, 7-12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, or 15-20 cycles can be performed during the amplification step. The condition of the PCR can be optimized based on the GC content of the sequences, including the primers.

[00155] Nucleic acid amplification techniques can be used with the assays described herein. Some amplification techniques are the PCR methodologies which can include, but are not limited to, solution PCR and *in situ* PCR. For example, amplification may comprise PCR-based amplification. Alternatively, amplification may comprise non PCR-based amplification. Amplification of the template nucleic acid may comprise use of one or more polymerases. For example, the polymerase may be a DNA polymerase or an RNA polymerase. In some cases, high fidelity amplification is performed such as with the use of high fidelity polymerase (*e.g.*, Phusion® High-Fidelity DNA Polymerase) or PCR protocols. In some cases, the polymerase may be a high fidelity polymerase. For example, the polymerase may be KAPA HiFi DNA polymerase. The polymerase may also be Phusion DNA polymerase. The polymerase may be

used under reaction conditions that reduce or minimize amplification biases, e.g., due to fragment length, GC content, etc.

[00156] Amplification of a single strand of a polynucleotide by PCR will generate copies both of that strand and its complement. During sequencing, both the strand and its complement will generate sequence reads. However, sequence reads generated from the complement of, for example, the Watson strand, can be identified as such because they bear the complement of the portion of the duplex tag that tagged the original Watson strand. In contrast, a sequence read generated from a Crick strand or its amplification product will bear the portion of the duplex tag that tagged the original Crick strand. In this way, a sequence read generated from an amplified product of a complement of the Watson strand can be distinguished from a complement sequence read generated from an amplification product of the Crick strand of the original molecule.

[00157] All amplified polynucleotides can be submitted to a sequencing device for sequencing. Alternatively, a sampling, or subset, of all of the amplified polynucleotides is submitted to a sequencing device for sequencing. With respect to any original double-stranded polynucleotide there can be three results with respect to sequencing. First, sequence reads can be generated from both complementary strands of the original molecule (that is, from both the Watson strand and from the Crick strand). Second, sequence reads can be generated from only one of the two complementary strands (that is, either from the Watson strand or from the Crick strand, but not both). Third, no sequence read may be generated from either of the two complementary strands. Consequently, counting unique sequence reads mapping to a genetic locus will underestimate the number of double-stranded polynucleotides in the original sample mapping to the locus. Described herein are methods of estimating the unseen and uncounted polynucleotides.

[00158] The sequencing method can be massively parallel sequencing, that is, simultaneously (or in rapid succession) sequencing any of at least 100, 1000, 10,000, 100,000, 1 million, 10 million, 100 million, or 1 billion polynucleotide molecules. Sequencing methods may include, but are not limited to: high-throughput sequencing, pyrosequencing, sequencing-by-synthesis, single-molecule sequencing, nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, RNA-Seq (Illumina), Digital Gene Expression (Helicos), Next generation sequencing, Single Molecule Sequencing by Synthesis (SMSS)(Helicos),

massively-parallel sequencing, Clonal Single Molecule Array (Solexa), shotgun sequencing, Maxam-Gilbert or Sanger sequencing, primer walking, sequencing using PacBio, SOLiD, Ion Torrent, or Nanopore platforms and any other sequencing methods known in the art.

[00159] For example, duplex-tagged polynucleotides can be amplified, by for example PCR (*see e.g.*, **FIG. 4A** duplex-tagged polynucleotides are referred to as mm' and nn'). In **Fig. 4A**, the strand of the duplex polynucleotide including sequence m bears sequence tags w and y , while the strand of the duplex polynucleotide including sequence m' bears sequence tags x and z . Similarly, the strand of the duplex polynucleotide including sequence n bears sequence tags a and c , while the strand of the duplex polynucleotide including sequence n' bears sequence tags b and d . During amplification, each strand produces itself and its complementary sequence. However, for example, an amplification progeny of original strand m that includes the complementary sequence, m' , is distinguishable from an amplification progeny of original strand m' because the progeny from original strand m will have the sequence $5'-y'm'w'-3'$ and the progeny of the original m' strand one strand will have the sequence $5'-zm'x'-3'$. **FIG. 4B** shows amplification in more detail. During amplification, errors can be introduced into the amplification progeny, represented by dots. The application progeny are sampled for sequencing, so that not all strands produce sequence reads, resulting in the sequence reads indicated. Because sequence reads can come from either of a strand or its complement, both sequences and complement sequences will be included in the set of sequence reads. It should be noted that it is possible that a polynucleotide would bear the same tag on each end. Thus, for a tag “a”, and polynucleotide “m”, a first strand could be tagged $a-m-a'$, and the complement could be tagged $a-m'-a$.

[00160] E. Determining consensus sequence reads

[00161] Methods disclosed herein can comprise determining consensus sequence reads in sequence reads (*e.g.*, as shown in step (108), **FIG. 1**), such as by reducing or tracking redundancy. Sequencing of amplified polynucleotides can produce reads of the several amplification products from the same original polynucleotide, referred to as “redundant reads”. By identifying redundant reads, unique molecules in the original sample can be determined. If the molecules in a sample are uniquely tagged, then reads generated from amplification of a

single unique original molecule can be identified based on their distinct barcode. Ignoring barcodes, reads from unique original molecules can be determined based on sequences at the beginning and end of a read, optionally in combination with the length of the read. In certain cases, however, a sample may be expected to have a plurality of original molecules having the same start stop sequences and the same length. Without barcoding, these molecules are difficult to distinguish from one another. However, if a collection of polynucleotides is non-uniquely tagged (that is, an original molecule shares the same identifier with at least one other original molecule), combining information from a barcode with start/stop sequence and/or polynucleotide length significantly increases the probability that any sequence read can be traced back to an original polynucleotide. This is because, in part, even without unique tagging, it is unlikely that any two original polynucleotides having the same start/stop sequence and length also will be tagged with the same identifier.

[00162] F. Collapsing

[00163] Collapsing allows for reduction in noise (*i.e.*, background) that is generated at each step of the process. Methods disclosed herein can comprise collapsing, *e.g.*, generating a consensus sequence by comparing multiple sequence reads. For example, sequence reads generated from a single original polynucleotide can be used to generate a consensus sequence of that original polynucleotide. Iterative rounds of amplification can introduce errors into progeny polynucleotides. Also, sequencing typically may not be performed with perfect fidelity so sequencing errors are introduced at this stage as well. However, comparison of sequence reads of molecules derived from a single original molecule, including those that have sequence variants, can be analyzed so as to determine the original, or “consensus” sequence. This can be done phylogenetically. Consensus sequences can be generated from families of sequence reads by any of a variety of methods. Such methods include, for example, linear or non-linear methods of building consensus sequences (such as voting (*e.g.*, biased voting), averaging, statistical, maximum a posteriori or maximum likelihood detection, dynamic programming, Bayesian, hidden Markov or support vector machine methods, etc.) derived from digital communication theory, information theory, or bioinformatics. For example, if all or most of the sequence reads tracking back to an original molecule bear the same sequence variant, that variant probably existed in the original molecule. On the other hand, if a sequence variant exists in a subset of

redundant sequence reads, that variant may have been introduced during amplification/sequencing and represents an artifact not existing in the original. Furthermore, if only sequence reads derived from the Watson or Crick strand of an original polynucleotide contain the variant, the variant may have been introduced through single-sided DNA damage, first-cycle PCR error or through contaminating polynucleotides that were amplified from a different sample.

[00164] After fragments are amplified and the sequences of amplified fragments are read and aligned, the fragments are subjected to base calling, *e.g.*, determining for each locus the most likely nucleotide. However, variations in the number of amplified fragments and unseen amplified fragments (*e.g.*, those without being read their sequences; reasons could be too many such as amplification errors, sequencing reading errors, too long, too short, being chopped, etc.) may introduce errors in base calling. If there are too many unseen amplified fragments with respect to the seen amplified fragments (amplified fragments actually being read), the reliability of base calling may be diminished.

[00165] Therefore, disclosed herein is a method to correct for the number of unseen fragments in base calling. For example, when base calling for locus A (an arbitrary locus), it is first assumed that there are N amplified fragments. The sequence readouts can come from two types of fragments: double-strand fragments and single-strand fragments. Therefore, we assign N_1 , N_2 , and N_3 as the numbers of double-strands, single-strands, and unseen fragments, respectively. Thus, $N=N_1+N_2+N_3$ (N_1 and N_2 are known from the sequence readouts, and N and N_3 are unknown). If the formula is solved for N (or N_3), then N_3 (or N) will be inferred.

[00166] Probability is used to estimate N . For example, we assign “ p ” to be the probability of having detected (or having read) a nucleotide of locus A in a sequence readout of a single-strand.

[00167] For sequence readouts from double-strands, the nucleotide call from a double-strand amplified fragment has a probability of $p * p=p^2$, seeing all N_1 double-strands has the following equation: $N_1=N * (p^2)$.

[00168] For sequence readouts from a single-strand. Assuming that one of the 2 strands is seen, and the other is unseen, the probability of seeing one strand is “ p ”, but the probability of missing the other strand is $(1-p)$. Furthermore, by not distinguishing the single strand sourcing from 5-primer and sourcing from 3-primer, there is a factor of 2. Therefore, the nucleotide call

from a single-strand amplified fragment has a probability $2 \times p \times (1-p)$. Thus, seeing all N_2 single-strands has the following equation: $N_2 = N \times 2 \times p \times (1-p)$.

[00169] “p” is also unknown. To solve p, the ratio of N_1 to N_2 is used to solve for “p”:

$$R = \frac{N_1}{N_2} = \frac{Np^2}{2Np(1-p)} = \frac{p^2}{2p(1-p)} = \frac{p}{2(1-p)}$$

Once “p” is found, N can be found. After N is found, can be found $N_3 = N - N_1 - N_2$.

[00170] Besides the ratio of paired versus unpaired strands (which is a measure post-collapsing), there is useful information in the pre-collapsing read depth at each locus. This information can be used to further improve the call for total molecule count and/or increase confidence of calling variants.

[00171] For example, **FIG. 4C** demonstrates sequence reads corrected for complementary sequences. Sequences generated from an original Watson strand or an original Crick strand can be differentiated on the basis of their duplex tags. Sequences generated from the same original strand can be grouped. Examination of the sequences can allow one to infer the sequence of the original strand (the “consensus sequence”). In this case, for example, the sequence variant in the nn' molecule is included in the consensus sequence because it included in every sequence read while other variants are seen to be stray errors. After collapsing sequences, original polynucleotide pairs can be identified based on their complementary sequences and duplex tags.

[00172] **FIG. 5** demonstrates increased confidence in detecting sequence variants by pairing reads from Watson and Crick strands. Sequence nn' can include a sequence variant indicated by a dot. In some cases, sequence pp' does not include a sequence variant. Amplification, sequencing, redundancy reduction and pairing can result in both Watson and Crick strands of the same original molecule including the sequence variant. In contrast, as a result of errors introduced during amplification and sampling during sequencing, the consensus sequence of the Watson strand p can contain a sequence variant, while the consensus sequence of the Crick strand p' does not. It is less likely that amplification and sequencing will introduce the same variant into both strands (nn' sequence) of a duplex than onto one strand (pp' sequence). Therefore, the variant in the pp' sequence is more likely to be an artifact, and the variant in the nn' sequence is more likely to exist in the original molecule.

[00173] Methods disclosed herein can be used to correct errors resulted from experiments, *e.g.*, PCR, amplification, and/or sequencing. For example, such a method can comprises attaching one or more double stranded adaptors to both ends of a double stranded polynucleotide, thereby providing a tagged double stranded polynucleotide; amplifying the double stranded tagged polynucleotide; sequencing both strands of the tagged polynucleotide; comparing the sequence of one strand with its complement to determine any errors introduced during sequencing; and correcting errors in the sequence based on (d). The adaptors used in this method can be any adaptors disclosed herein, *e.g.*, Y-shaped adaptors. The adaptor can comprise any barcodes (*e.g.*, distinct barcodes) disclosed herein.

[00174] G. Mapping

[00175] Sequence reads or consensus sequences can be mapped to one or more selected genetic loci (*e.g.*, as shown step (110), **FIG. 1**). A genetic locus can be, for example, a specific nucleotide position in the genome, a sequence of nucleotides (for example, an open reading frame), a fragment of a chromosome, a whole chromosome, or an entire genome. A genetic locus can be a polymorphic locus. Polymorphic locus can be a locus at which sequence variation exists in the population and/or exists in a subject and/or a sample. A polymorphic locus can be generated by two or more distinct sequences coexisting at the same location of the genome. The distinct sequences can differ from one another by one or more nucleotide substitutions, a deletion/insertion, and/or a duplication of any number of nucleotides, generally a relatively small number of nucleotides, such as less than 50, 45, 40, 35, 30, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide(s), among others. A polymorphic locus can be created by a single nucleotide position that varies within the population, *e.g.* a single nucleotide variation (SNV) or a single nucleotide polymorphism (SNP).

[00176] A reference genome for mapping can include the genome of any species of interest. Human genome sequences useful as references can include the hg19 assembly or any previous or available hg assembly. Such sequences can be interrogated using the genome browser available at genome.ucsc.edu/index.html. Other species genomes include, for example PanTro2 (chimp) and mm9 (mouse).

[00177] In methods disclosed herein, collapsing can be performed before or after mapping. In some aspects, collapsing can be performed before mapping. For example, sequence reads can be

grouped into families based on their tags and one or more endogenous sequences, without regard to where the reads map in the genome. Then, the members of a family can be collapsed into a consensus sequence. The consensus sequence can be generated using any collapsing method disclosed herein. Then the consensus sequence can be mapped to locations in the genome. Reads mapped to a locus can be quantified (*e.g.*, counted). Percentage of reads carrying a mutation at a locus can also be determined. Alternatively, collapsing can be performed after mapping. For example, all reads can first be mapped to the genome. Then the reads can be grouped into families based on their tags and one or more endogenous sequences. Since the reads have been mapped to the genome, consensus bases can be determined for each family at each locus. In other aspects, consensus sequence can be generated for one strand of a DNA molecule (*e.g.*, for a Watson strand or a Crick strand). Mapping can be performed before or after the consensus sequence for one strand of the DNA molecule is determined. Numbers of Doublets and Singlets can be determined. These numbers can be used to calculate unseen molecules. For example, the unseen molecules can be calculated using the following equation: $N = D + S + U$; $D = Np(2)$, $S = N2pq$, where $p = 1 - q$, where p is the probability of seeing; q is the probability of missing a strand.

[00178] H. Grouping

[00179] Methods disclosed herein can also comprise grouping sequence reads. Sequence reads can be grouped based on various types of sequences, *e.g.*, sequences of an oligonucleotide tag (*e.g.*, a barcode), sequence of a polynucleotide fragments, or any combinations. For example, as shown in step (112) (**FIG. 1**), sequence reads can be grouped as follows: Sequence reads generated from a “Watson” strand and those generated from a “Crick” strand of a double-stranded polynucleotide in the sample are identifiable based on the duplex tags that they bear. In this way, a sequence read or consensus sequence from a Watson strand of a duplex polynucleotide can be paired with a sequence read or consensus sequence from its complementary Crick strand. Paired sequence reads are referred to as a “Pair”.

[00180] Sequence reads for which no sequence read corresponding to a complementary strand can be found among the sequence reads are termed “Singlets”.

[00181] Double-stranded polynucleotides for which a sequence read for neither of the two complementary strands has been generated are referred to as “Unseen” molecules.

[00182] I. Quantifying

[00183] Methods disclosed herein also comprise quantifying sequence reads. For example, as shown in step (114) (**FIG. 1**), Pairs and Singlets mapping to a selected genetic locus, or to each of a plurality of selected genetic loci, are quantified, *e.g.*, counted.

[00184] The quantifying can comprise estimating number of polynucleotides in the sample (*e.g.*, Pairs polynucleotides, Singlets polynucleotides, or Unseen polynucleotides. For example, as shown in step (116) (**FIG. 1**), the number of double-stranded polynucleotides in the sample for which no sequence reads were generated (“Unseen” polynucleotides) is estimated. The probability that a double strand polynucleotide generates no sequence reads can be determined based on the relative number of Pairs and Singlets at any locus. Using this probability, the number of Unseen polynucleotide can be estimated.

[00185] In step (118) an estimate for the total number of double-stranded polynucleotides in a sample mapping to a selected locus is the sum of the number of Pairs, the number of Singlets and the number of Unseen molecules mapping to the locus.

[00186] The number of Unseen original molecules in a sample can be estimated based on the relative number of Pairs and Singlets (**FIG. 2**). Referring to **FIG. 2**, as an example, counts for a particular genomic locus, Locus A, are recorded, where 1000 molecules are paired and 1000 molecules are unpaired. Assuming a uniform probability, p , for an individual Watson or Crick strand to make it through the process subsequent to conversion, one can calculate the proportion of molecules that fail to make it through the process (Unseen) as follows: Let R = ratio of paired to unpaired molecules = 1, so $R=1=p^2/(2p(1-p))$. This implies that $p=2/3$ and that the quantity of lost molecules is equal to $(1-p)^2 = 1/9$. Thus in this example, approximately 11% of converted molecules are lost and never detected. Consider another genomic locus, Locus B, in the same sample where 1440 molecules are paired and 720 are unpaired. Using the same method, we can infer the number of molecules that are lost, is only 4%. Comparing the two areas, it may be assumed that Locus A had 2000 unique molecules as compared to 2160 molecules in Locus B – a difference of almost 8%. However, by correctly adding in the lost molecules in each region, we infer there are $2000/(8/9)=2250$ molecules in Locus A and $2160/.96=2250$ molecules in Locus B. Hence, the counts in both regions are actually equal. This correction and thus much higher sensitivity can be achievable by converting the original double-stranded nucleic acid

molecules and bioinformatically keeping track of all those that are paired and unpaired at the end of the process. Similarly, the same procedure can be used to infer true copy number variations in regions that appear to have similar counts of observed unique molecules. By taking the number of unseen molecules into consideration in the two or more regions, the copy number variation becomes apparent.

[00187] In addition to using binomial distribution, other methods of estimating numbers of unseen molecules include exponential, beta, gamma or empirical distributions based on the redundancy of sequence reads observed. In the latter case, the distribution of read counts for paired and unpaired molecules can be derived from such redundancy to infer the underlying distribution of original polynucleotide molecules at a particular locus. This can often lead to a better estimation of the number of unseen molecules.

[00188] J. CNV Detection

[00189] Methods disclosed herein also comprise detecting CNV. For example, as shown in step (120) (**FIG. 1**), once the total number of polynucleotides mapping to a locus is determined, this number can be used in standard methods of determining CNV at the locus. A quantitative measure can be normalized against a standard. The standard can be an amount of any polynucleotides. In one method, a quantitative measure at a test locus can be standardized against a quantitative measure of polynucleotides mapping to a control locus in the genome, such as gene of known copy number. Quantitative measures can be compared against the amount of nucleic acid in any sample disclosed herein. For example, in another method, the quantitative measure can be compared against the amount of nucleic acid in the original sample. For example, if the original sample contained 10,000 haploid gene equivalents, the quantitative measure can be compared against an expected measure for diploidy. In another method, the quantitative measure can be normalized against a measure from a control sample, and normalized measures at different loci can be compared.

[00190] In some cases, in which copy number variation analysis is desired, sequence data may be: 1) aligned with a reference genome; 2) filtered and mapped; 3) partitioned into windows or bins of sequence; 4) coverage reads counted for each window; 5) coverage reads can then be normalized using a stochastic or statistical modeling algorithm; 6) and an output file can be generated reflecting discrete copy number states at various positions in the genome. In other

cases, in which rare mutation analysis is desired, sequence data may be 1) aligned with a reference genome; 2) filtered and mapped; 3) frequency of variant bases calculated based on coverage reads for that specific base; 4) variant base frequency normalized using a stochastic, statistical or probabilistic modeling algorithm; 5) and an output file can be generated reflecting mutation states at various positions in the genome.

[00191] After the sequence read coverage ratios have been determined, a stochastic modeling algorithm can be optionally applied to convert the normalized ratios for each window region into discrete copy number states. In some cases, this algorithm may comprise a Hidden Markov Model. In other cases, the stochastic model may comprise dynamic programming, support vector machine, Bayesian modeling, probabilistic modeling, trellis decoding, Viterbi decoding, expectation maximization, Kalman filtering methodologies, or neural networks.

[00192] Methods disclosed herein can comprise detecting SNVs, CNVs, insertions, deletions, and/or rearrangements at a specific region in a genome. The specific genomic region can comprise a sequence in a gene, such as ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, or NTRK1.

[00193] In some cases, the method uses a panel which comprises exons of one or more genes. The panel can comprise introns of one or more genes as well. The panel can also comprise exons and introns of one or more genes. The one or more genes can be those disclosed above. The panel can comprise about 80,000 bases which cover a panel of genes. The panel can comprise about 1000, 2000, 3000, 4000, 5000, 10000, 15000, 20000, 25000, 30000, 35000, 40000, 45000, 50000, 55000, 60000, 65000, 70000, 75000, 80000, 85000, 90000, 95000, 100000, 105000, 110000, 115000, 120000, 125000, or more bases.

[00194] In some aspects, copy number of a gene can be reflected in the frequency of a genetic form of the gene in a sample. For example, in a healthy individual, no copy number variation is

reflected in a variant in a gene in one chromosome (*e.g.*, heterozygosity) being detected in about 50% of detected molecules in a sample. Also, in a healthy individual, duplication of a gene bearing a variant can be reflected in the variant being detected in about 66% of detected molecules in a sample. Accordingly, if the tumor burden in a DNA sample is 10%, the frequency of a somatic mutation in a gene in one chromosome of cancer cells, without CNV, can be about 5%. The converse can be true in the case of aneuploidy.

[00195] The methods disclosed herein can be used to determine whether a sequence variant is more likely present in the germ line level or resulted from a somatic cell mutation, *e.g.*, in a cancer cell. For example, a sequence variant in a gene detected at levels arguably consistent with heterozygosity in the germ line is more likely the product of a somatic mutation if CNV is also detected in that gene. In some cases, to the extent we expect that a gene duplication in the germ line bears a variant consistent with genetic dose (*e.g.*, 66% for trisomy at a locus), detection gene amplification with a sequence variant dose that deviates significantly from this expected amount indicates that the CNV is more likely present as a result of somatic cell mutation.

[00196] The methods disclosed herein can also be used to infer tumor heterogeneity in a situation in which sequence variants in two genes are detected at different frequencies. For example, tumor heterogeneity can be inferred when two genes are detected at different frequencies but their copy numbers are relatively equal. Alternatively, tumor homogeneity can be inferred when the difference in frequency between two sequence variants is consistent with difference in copy number for the two genes. Thus, for example, if an EGFR variant is detected at 11% and a KRAS variant is detected at 5%, and no CNV is detected at these genes, the difference in frequency likely reflects tumor heterogeneity (*e.g.*, all tumor cells carry an EGFR mutant and half the tumor cells also carry a KRAS mutant). Alternatively, if the EGFR gene carrying the mutant is detected at 2-times normal copy number, one interpretation is a homogenous population of tumor cells, each cell carrying a mutant in the EGFR and KRAS genes, but in which the KRAS gene is duplicated.

[00197] In response to chemotherapy, a dominant tumor form can eventually give way through Darwinian selection to cancer cells carrying mutants that render the cancer unresponsive to the therapy regimen. Appearance of these resistance mutants can be delayed through methods of this invention. In one embodiment of this method, a subject is subjected to one or more

pulsed therapy cycles, each pulsed therapy cycle comprising a first period during which a drug is administered at a first amount and a second cycle during which the drug is administered at a second, reduced amount. The first period can be characterized by a tumor burden detected above a first clinical level. The second period can be characterized by a tumor burden detected below a second clinical level. First and second clinical levels can be different in different pulsed therapy cycles. For example, the first clinical level can be lower in succeeding cycles. A plurality of cycles can include at least 2, 3, 4, 5, 6, 7, 8 or more cycles. For example, the BRAF mutant V600E may be detected in polynucleotides of a disease cell at an amount indicating a tumor burden of 5% in cfDNA. Chemotherapy can commence with dabrafenib. Subsequent testing can show that the amount of the BRAF mutant in the cfDNA falls below 0.5% or to undetectable levels. At this point, dabrafenib therapy can stop or be significantly curtailed. Further subsequent testing may find that DNA bearing the BRAF mutation has risen to 2.5% of polynucleotides in cfDNA. At this point, dabrafenib therapy can be re-started, *e.g.*, at the same level as the initial treatment. Subsequent testing may find that DNA bearing the BRAF mutation has decreased to 0.5% of polynucleotides in cfDNA. Again, dabrafenib therapy can be stopped or reduced. The cycle can be repeated a number of times.

[00198] A therapeutic intervention can also be changed upon detection of the rise of a mutant form resistant to an original drug. For example, cancers with the EGFR mutation L858R respond to therapy with erlotinib. However, cancers with the EGFR mutation T790M are resistant to erlotinib. However, they are responsive to ruxolitinib. A method of this invention involves monitoring changes in tumor profile and changing a therapeutic intervention when a genetic variant associated with drug resistance rises to a predetermined clinical level.

[00199] Methods disclosed in this invention can comprise a method of detecting disease cell heterogeneity from a sample comprising polynucleotides from somatic cells and disease cells, the method comprising: a) quantifying polynucleotides in the sample bearing a sequence variant at each of a plurality of genetic loci; b) determining CNV at each of the plurality of genetic loci; different relative amounts of disease molecules at a locus, wherein the CNV indicates a genetic dose of a locus in the disease cell polynucleotides; c) determining a relative measure of quantity of polynucleotides bearing a sequence variant at a locus per genetic dose at the locus for each of a plurality of the loci; and d) comparing the relative measures at each of the plurality of loci,

wherein different relative measures indicates tumor heterogeneity. In the methods disclosed herein, the genetic dose can be determined on a total molecule basis. For example, if there are 1X total molecules at a first locus, and 1.2X molecules mapped to a second locus, then the genetic dose is 1.2. Variants at this locus can be divided by 1.2. In some aspects, the method disclosed herein can be used to detect any disease cell heterogeneity, *e.g.*, tumor cell heterogeneity. The methods can be used to detect disease cell heterogeneity from a sample comprising any types of polynucleotides, *e.g.*, cfDNA, genomic DNA, cDNA, or ctDNA. In the methods, the quantifying can comprise, for example, determining the number or relative amount of the polynucleotides. Determining CNV can comprise mapping and normalizing different relative amounts of total molecules to a locus.

[00200] In another aspect, in response to chemotherapy, a dominant tumor form can eventually give way through Darwinian selection to cancer cells carrying mutants that render the cancer unresponsive to the therapy regimen. Appearance of these resistance mutants can be delayed through methods disclosed throughout. The methods disclosed herein can comprise a method comprising: a) subjecting a subject to one or more pulsed therapy cycles, each pulsed therapy cycle comprising (i) a first period during which a drug is administered at a first amount and (ii) a second period during which the drug is administered at a second, reduced amount; wherein (A) the first period is characterized by a tumor burden detected above a first clinical level; and (B) the second period is characterized by a tumor burden detected below a second clinical level.

[00201] K. Sequence Variant Detection

[00202] Systems and methods disclosed herein can be used to detect sequence variants, *e.g.*, SNVs. For example, a sequence variant can be detected from consensus sequences from multiple sequence reads, for example, from at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56, at least 57, at

least 58, at least 59, at least 60, at least 61, at least 62, at least 63, at least 64, at least 65, at least 66, at least 67, at least 68, at least 69, at least 70, at least 71, at least 72, at least 73, at least 74, at least 75, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 2000, at least 3000, at least 4000, at least 5000, at least 6000, at least 7000, at least 8000, at least 9000, at least 10000 or more sequence reads. A consensus sequence can be from sequence reads of a single strand polynucleotide. A consensus sequence can also be from sequence reads of one strand of a double-stranded polynucleotide (*e.g.*, pairing reads). In an exemplary method, pairing reads allows one to identify with increased confidence the existence of a sequence variant in a molecule. For example, if both strands of a Pair include the same variant, one can be reasonably sure that the variant existed in the original molecule, as the chance that the same variant is introduced into both strands during amplification/sequencing is rare. In contrast, if only one strand of a Pair includes the sequence variant, this is more likely to be an artifact. Similarly, the confidence that a Singlet bearing a sequence variant existed in the original molecule is less than the confidence if the variant exists in a Duplex, as there is higher probability that the variant can be introduced once than twice during amplification/sequencing.

[00203] Other methods of copy number variation detection and the sequence variant detection are described in PCT/US2013/058061, which is entirely incorporated herein by reference.

[00204] Sequence reads can be collapsed to generate a consensus sequence, which can be mapped to a reference sequence to identify genetic variants, such as CNV or SNV. As an alternative, the sequence reads are mapped prior to or even without mapping. In such a case, the sequence reads can be individually mapped to the reference to identify a CNV or SNV.

[00205] **FIG. 3** shows a reference sequence encoding a genetic Locus A. The polynucleotides in FIG. 3 may be Y-shaped or have other shapes, such as hairpin.

[00206] In some cases, an SNV or multiple-nucleotide variant (MNV) can be determined across multiple sequence reads at a given locus (*e.g.*, nucleotide base) by aligning sequence reads that correspond to that locus. Next, a plurality of sequential nucleotide bases from at least a

subset of the sequence reads are mapped to the reference to a SNV or MNV in a polynucleotide molecule or portion thereof that corresponds to the reads. The plurality of sequential nucleotide bases can span an actual, inferred or suspected location of the SNV or MNV. The plurality of sequential nucleotide bases can span at least 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide bases.

[00207] L. Detecting/Quantifying Nucleic Acids

[00208] The methods described throughout can be used to tag nucleic acids fragments, such as deoxyribonucleic acid (DNA), at extremely high efficiency. This efficient tagging allows a person to efficiently and accurately detect rare DNA in heterogenous populations of original DNA fragments (such as in cfDNA). A rare polynucleotide (e.g., rare DNA) can be a polynucleotide that comprises a genetic variant occurring in a population of polynucleotides at a frequency of less than 10%, 5%, 4%, 3%, 2%, 1%, or 0.1%. A rare DNA can be a polynucleotide with a detectable property at a concentration less than 50%, 25%, 10%, 5%, 1%, or 0.1%

[00209] Tagging can occur in a single reaction. In some cases, two or more reactions can be performed and pooled together. Tagging each original DNA fragments in a single reaction can result in tagging such that greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, or 99%) of the original DNA fragments are tagged at both ends with tags that comprise molecular barcodes, thereby providing tagged DNA fragments. Tagging can also result in greater than 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the original DNA fragments tagged at both ends with tags that comprise molecular barcodes. Tagging can also result in 100% of the original DNA fragments tagged at both ends with tags that comprise molecular barcodes. Tagging can also result in single end tagging.

[00210] Tagging can also occur by using an excess amount of tags as compared to the original DNA fragments. For example, the excess can be at least 5-fold excess. In other cases, the excess can be at least 1.25, 1.5, 1.75, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more fold excess. Tagging can comprise attachment to blunt ends or sticky ends. Tagging can also be

performed by hybridization PCR. Tagging can also be performed in low reaction volumes, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 pico- and/or microliters.

[00211] The method can also include performing high fidelity amplification on the tagged DNA fragments. Any high fidelity DNA polymerases can be used. For example, the polymerase may be KAPA HiFi DNA polymerase or Phusion DNA polymerase.

[00212] Further, the method can comprise selectively enriching a subset of the tagged DNA fragments. For example, selective enrichment can be performed by hybridization or amplification techniques. The selective enrichment can be performed using a solid support (*e.g.*, beads). The solid support (*e.g.*, beads) can comprise probes (*e.g.*, oligonucleotides specifically hybridizing to certain sequences. For example, the probes can hybridize with certain genomic regions, *e.g.*, genes. In some cases, the genomic regions, *e.g.*, genes, can be regions associated with diseases, *e.g.*, cancer. After enrichment, the selected fragmented can be attached any sequencing adaptor disclosed in this invention. For example, a sequence adaptor can comprise a flow cell sequence, a sample barcode, or both. In another example, a sequence adaptor can be a hairpin shaped adaptor and/or comprises a sample barcode. Further, the resulting fragments can be amplified and sequenced. In some cases, the adaptor does not comprise a sequencing primer region.

[00213] The method can include sequencing one or both strands of the DNA fragments. In one case, both strands of the DNA fragment are independently sequenced. The tagged, amplified, and/or selectively enriched DNA fragments are sequenced to obtain sequence reads that comprise sequence information of the molecular barcodes and at least a portion of the original DNA fragments.

[00214] The method can include reducing or tracking redundancy (as described above) in the sequence reads to determine consensus reads that are representative of single-strands of the original DNA fragments. For example, to reduce or track redundancy, the method can include comparing sequence reads having the same or similar molecular barcodes and the same or

similar end of fragment sequences. The method can comprise performing a phylogenetic analysis on the sequence reads having the same or similar molecular barcodes. The molecular barcodes can have a barcode with varying edit distances (including any edit distances as described throughout), for example, an edit distance of up to 3. The end of the fragment sequences can include fragment sequences having an edit distance with varying distances (including any edit distances as described throughout), for example, an edit distance of up to 3.

[00215] The method can comprise binning the sequence reads according to the molecular barcodes and sequence information. For example, binning the sequence reads according to the molecular barcodes and sequence information can be performed from at least one end of each of the original DNA fragments to create bins of single stranded reads. The method can further comprise in each bin, determining a sequence of a given original DNA fragment among the original DNA fragments by analyzing sequence reads.

[00216] In some cases, sequence reads in each bin can be collapsed to a consensus sequence and subsequently mapped to a genome. As an alternative, sequence reads can be mapped to a genome prior to binning and subsequently collapsed to a consensus sequence.

[00217] The method can also comprise sorting sequence reads into paired reads and unpaired reads. After sorting, the number of paired reads and unpaired reads that map to each of one or more genetic loci can be quantified.

[00218] The method can include quantifying the consensus reads to detect and/or quantify the rare DNA, which are described throughout. The method can comprise detecting and/or quantifying the rare DNA by comparing a number of times each base occurs at each position of a genome represented by the tagged, amplified, and/or enriched DNA fragments.

[00219] The method can comprise tagging the original DNA fragments in a single reaction using a library of tags. The library can include at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 50, at least 100, at least 500, at least 1000, at least 5000, at least 10000, or any number of tags as disclosed throughout. For example, the library of tags can include at least 8 tags. The library of tags can include 8 tags (which can generate 64 different possible combinations). The method can be conducted such

that a high percentage of fragments, *e.g.*, greater than 50% (or any percentages as described throughout) are tagged at both ends, wherein each of the tags comprises a molecular barcode.

[00220] M. Processing and/or Analyzing Nucleic Acids

[00221] The methods described throughout can be used for processing and/or analyzing a nucleic acid sample of a subject. The method can comprising exposing polynucleotide fragments of the nucleic acid sample to a plurality of polynucleotide molecules to yield tagged polynucleotide fragments. The plurality of polynucleotide molecules that can be used are described throughout the application.

[00222] For example, the plurality of polynucleotide molecules can be each less than or equal to 40 nucleic acid bases in length and have distinct barcode sequences with respect to at least 4 nucleic acid bases and an edit distance of at least 1, wherein each of the distinct barcode sequences is within 20 nucleic acid bases from a terminal end of a respective one of the plurality of polynucleotide molecules, and wherein the plurality of polynucleotide molecules are not sequencing adaptors.

[00223] The tagged polynucleotide fragments can be subjected to nucleic acid amplification reactions under conditions that yield amplified polynucleotide fragments as amplification products of the tagged polynucleotide fragments. After amplification, the nucleotide sequence of the amplified tagged polynucleotide fragments is determined. In some cases, the nucleotide sequences of the amplified tagged polynucleotide fragments are determined without the use of polymerase chain reaction (PCR).

[00224] The method can comprise analyzing the nucleotide sequences with a programmed computer processor to identify one or more genetic variants in the nucleotide sample of the subject. Any genetic alterations can be identified, including but not limited to, base change(s), insertion(s), repeat(s), deletion(s), copy number variation(s), epigenetic modification(s), nucleosome binding site(s), copy number change(s) due to origin(s) of replication, and transversion(s). Other genetic alterations can include, but are not limited to, one or more tumor associated genetic alterations.

[00225] The subject of the methods can be suspected of having a disease. For example, the subject can be suspected of having cancer. The method can comprise collecting a nucleic acid sample from a subject. The nucleic acid sample can be collected from blood, plasma, serum,

urine, saliva, mucosal excretions, sputum, stool, cerebral spinal fluid, skin, hair, sweat, and/or tears. The nucleic acid sample can be a cell-free nucleic acid sample. In some cases, the nucleic acid sample is collected from no more than 100 nanograms (ng) of double-stranded polynucleotide molecules of the subject.

[00226] The polynucleotide fragments can comprise double-stranded polynucleotide molecules. In some cases, the plurality of polynucleotide molecules are coupled to the polynucleotide fragments via blunt end ligation, sticky end ligation, molecular inversion probes, polymerase chain reaction (PCR), ligation-based PCR, multiplex PCR, single strand ligation, or single strand circularization.

[00227] The method as described herein results in high efficiency tagging of nucleic acids. For example, exposing the polynucleotide fragments of the nucleic acid sample to the plurality of polynucleotide molecules yields the tagged polynucleotide fragments with a conversion efficiency of at least 30%, *e.g.*, of at least 50% (*e.g.*, 60%, 70%, 80%, 90%, 95%, or 99%).

Conversion efficiency of at least 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% can be achieved.

[00228] The method can result in a tagged polynucleotide fragment that share common polynucleotide molecules. For example, any of at least 5%, 6%, 7%, 8%, 9%, 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of the tagged polynucleotide fragments share a common polynucleotide molecule. The method can comprise generating the polynucleotide fragments from the nucleic acid sample.

[00229] In some cases, the subjecting of the method comprises amplifying the tagged polynucleotide fragments in the presence primers corresponding to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4,

CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. Additionally, any combination of these genes can be amplified. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, or all 54 of these genes can be amplified.

[00230] The methods described herein can comprise generating a plurality of sequence reads from a plurality of polynucleotide molecules. The plurality of polynucleotide molecules can cover genomic loci of a target genome. For example, the genomic loci can correspond to a plurality of genes as listed above. Further, the genomic loci can be any combination of these genes. Any given genomic locus can comprise at least two nucleic acid bases. Any given genomic locus can also comprise a plurality of nucleic acid bases, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more nucleic acid bases.

[00231] The method can comprise grouping with a computer processor the plurality of sequence reads into families. Each of the family can comprises sequence reads from one of the template polynucleotides. Each family can comprise sequence reads from only one of the template polynucleotides. For each of the family, the sequence reads can be merged to generate a consensus sequence. The grouping can comprise classifying the plurality of sequence reads into families by identifying (i) distinct molecular barcodes coupled to the plurality of polynucleotide molecules and (ii) similarities between the plurality of sequence reads, wherein each family includes a plurality of nucleic acid sequences that are associated with a distinct combination of molecular barcodes and similar or identical sequence reads.

[00232] Once merged, a consensus sequence can be called at a given genomic locus among the genomic loci. At any given genomic loci, any of the following can be determined: i) genetic variants among the calls; ii) frequency of a genetic alteration among the calls; iii) total number of calls; and iv) total number of alterations among the calls. The calling can comprise calling at least one nucleic acid base at the given genomic locus. The calling can also comprise calling a plurality of nucleic acid bases at the given genomic locus. In some cases, the calling can comprise phylogenetic analysis, voting (*e.g.*, biased voting), weighing, assigning a probability to

each read at the locus in a family, or calling the base with the highest probability. The consensus sequence can be generated by evaluating a quantitative measure or a statistical significance level for each of the sequence reads. If a quantitative measure is performed, the method can comprise use of a binomial distribution, exponential distribution, beta distribution, or empirical distribution. However, frequency of the base at the particular location can also be used for calling, for example, if 51% or more of the reads is a “A” at the location, then the base may be called an “A” at that particular location. The method can further comprise mapping a consensus sequence to a target genome.

[00233] The method can further comprising performing consensus calling at an additional genomic locus among the genomic loci. The method can comprise determining a variation in copy number at one of the given genomic locus and additional genomic locus based on counts at the given genomic locus and additional genomic locus.

[00234] The methods described herein can comprise providing template polynucleotide molecules and a library of adaptor polynucleotide molecules in a reaction vessel. The adaptor polynucleotide molecules can have from 2 to 1,000 different barcode sequences and in some cases are not sequencing adaptors. Other variations of adaptor polynucleotide molecules are described throughout, which can also be used in the methods.

[00235] The polynucleotide molecules of the adaptors can have the same sample tag. The adaptor polynucleotide molecules can be coupled to both ends of the template polynucleotide molecules. The method can comprise coupling the adaptor polynucleotide molecules to the template polynucleotide molecules at an efficiency of at least 30%, *e.g.*, of at least 50% (*e.g.*, 60%, 70%, 80%, 90%, 95%, or 99%), thereby tagging each template polynucleotide with a tagging combination that is among 4 to 1,000,000 different tagging combinations, to produce tagged polynucleotide molecules. In some cases, the reaction can occur in a single reaction vessel. Coupling efficiency can also be at least 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. Tagging can be non-unique tagging.

[00236] The tagged polynucleotide molecules can then be subject to an amplification reaction under conditions that will yield amplified polynucleotide molecules as amplification products of the tagged polynucleotide molecules. The template polynucleotide molecules can be double-stranded. Further, the template polynucleotide molecules can be blunt ended. In some cases, the amplification reaction comprises non-specifically amplifying the tagged polynucleotide molecules. The amplification reaction can also comprise using a priming site to amplify each of the tagged polynucleotide molecules. The priming site can be a primer, *e.g.*, a universal primer. The priming site can also be a nick.

[00237] The method can also comprise sequencing the amplified polynucleotide molecules. The sequencing can comprise (i) subjecting the amplified polynucleotide molecules to an additional amplification reaction under conditions that yield additional amplified polynucleotide molecules as amplification products of the amplified polynucleotide molecules, and/or (ii) sequencing the additional amplified polynucleotide molecules. The additional amplification can be performed in the presence of primers comprising flow cells sequences, which will produce polynucleotide molecules that are capable of binding to a flow cell. The additional amplification can also be performed in the presence of primers comprising sequences for hairpin shaped adaptors. The hairpin shaped adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times. The method can further comprise identifying genetic variants upon sequencing the amplified polynucleotide molecules.

[00238] The method can further comprising separating polynucleotide molecules comprising one or more given sequences from the amplified polynucleotide molecules, to produce enriched polynucleotide molecules. The method can also comprise amplifying the enriched polynucleotide molecules with primers comprising the flow cell sequences. This amplification with primers comprising flow cell sequences will produce polynucleotide molecules that are capable of binding to a flow cell. The amplification can also be performed in the presence of primers comprising sequences for hairpin shaped adaptors. The hairpin shaped adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times.

[00239] Flow cell sequences or hairpin shaped adaptors can be added by non-amplification methods such as through ligation of such sequences. Other techniques such as hybridization methods can be used, *e.g.*, nucleotide overhangs.

[00240] The method can be performed without aliquoting the tagged polynucleotide molecules. For example, once the tagged polynucleotide molecule is made, the amplification and sequencing can occur in the same tube without any further preparation.

[00241] The methods described herein can be useful in detecting single nucleotide variations (SNV), copy number variations (CNV), insertions, deletions, and/or rearrangements. In some cases, the SNVs, CNVs, insertions, deletions, and/or rearrangements, can be associated with disease, for example, cancer.

[00242] **N. Monitoring a Patient's Status**

[00243] Methods disclosed herein can also be used to monitor a patient's disease status. The disease of a subject can be monitored over time to determine a progression of the disease (*e.g.*, regression). Markers indicative of the disease can be monitored in a biological sample of the subject, such as a cell-free DNA sample.

[00244] For example, monitoring a subject's cancer status can comprise (a) determining an amount of one or more SNVs or copy numbers of a plurality of genes (*e.g.*, in an exon), (b) repeating such determination at different points in time, and (c) determining if there is a difference in the number of SNVs, level of SNVs, number or level of genomic rearrangements, or copy numbers between (a) and (b). The genes can be selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. The genes can be selected from any 5, 10, 15, 20, 30, 40, 50, or all of the genes in this group.

[00245] O. Sensitivity and Specificity

[00246] Methods disclosed herein can be used to detect cancer polynucleotides in a sample, and cancer in a subject, with high measures of agreement, *e.g.*, high sensitivity and/or specificity. For example, such methods can detect cancer polynucleotides (*e.g.*, rare DNA) in a sample at a concentration that is less than 5%, 1%, 0.5%, 0.1%, 0.05%, or 0.01%, at a specificity of at least 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or 99.99999%. Such polynucleotides may be indicative of cancer or other disease. Further, such methods can detect cancer polynucleotides in a sample with a positive predictive value of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, or 99.9999%.

[00247] Subjects identified as positive in a test that are in reality positive are referred as true positives (TP). Subjects identified as positive in a test that are in reality negative are referred as false positives (FP). Subjects identified as negative in a test that are in reality negative are referred as true negatives (TN). Subjects identified as negative in a test that are in reality positive are referred as false negatives (FN). Sensitivity is the percentage of actual positives identified in a test as positive. This includes, for example, instances in which one should have found a cancer genetic variant and did. (Sensitivity = $TP/(TP+FN)$.) Specificity is the percentage of actual negatives identified in a test as negative. This includes, for example, instances in which one should have found no cancer genetic variant and did not. Specificity can be calculated using the following equation: Specificity = $TN/(TN+FP)$. Positive predictive value (PPV) can be measured by the percentage of subjects who test positive that are true positives. PPV can be calculated using the following equation: $PPV = TP/(TP+FP)$. Positive predictive value can be increased by increasing sensitivity (*e.g.*, chance of an actual positive being detected) and/or specificity (*e.g.*, chance of not mistaking an actual negative for a positive).

[00248] Low conversion rates of polynucleotides into adaptor-tagged polynucleotides can compromise sensitivity as it decreases the chance of converting, and therefore detecting, rare polynucleotide targets. Noise in a test can compromise specificity as it increases the number of false positives detected in a test. Both low conversion rate and noise compromise positive predictive value as they decrease the percentage of true positives and increase the percentage of false positives.

[00249] The methods disclosed herein can achieve high levels of agreement, *e.g.*, sensitivity and specificity, leading to high positive predictive values. Methods of increasing sensitivity include high efficiency conversion of polynucleotides into adaptor-tagged polynucleotides in a sample. Methods of increasing specificity include reducing sequencing errors, for example, by molecular tracking.

[00250] Methods of the present disclosure can be used to detect genetic variation in non-uniquely tagged initial starting genetic material (*e.g.*, rare DNA) at a concentration that is less than 5%, 1%, 0.5%, 0.1%, 0.05%, or 0.01%, at a specificity of at least 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or 99.99999%. In some aspects, the methods can further comprise converting polynucleotides in the initial starting material at an efficiency of at least at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%. Sequence reads of tagged polynucleotides can be subsequently tracked to generate consensus sequences for polynucleotides with an error rate of no more than 2%, 1%, 0.1%, or 0.01%.

[00251] **2. Pooling Methods**

[00252] Disclosed herein are methods of detecting copy number variation and/or sequence variants at one or more genetic loci in a test sample. One embodiment is shown in **FIG. 8**. Typically, detecting copy number variation involves determining a quantitative measure (*e.g.*, an absolute or relative number) of polynucleotides mapping to a genetic locus of interest in a genome of a test sample, and comparing that number to a quantitative measure of polynucleotides mapping to that locus in a control sample. In certain methods, the quantitative measure is determined by comparing the number of molecules in the test sample that map to a locus of interest with a number of molecules in the test sample mapping to a reference sequence, *e.g.*, a sequence expected to be present at wild type ploidy number. In some examples, the reference sequence is HG19, build 37, or build 38. The comparison could involve, for example, determining a ratio. Then, this measure is compared with a similar measure determined in a control sample. So, for example, if a test sample has a ratio of 1.5:1 for locus of interest versus reference locus, and a control sample has a ratio of 1:1 for the same loci, one may conclude that the test sample exhibits polyploidy at the locus of interest.

[00253] When the test sample and the control sample are analyzed separately, the work flow can introduce distortions between final numbers in the control and test samples.

[00254] In one method disclosed herein (*e.g.*, flow chart 800), polynucleotides are provided from a test and a control sample (802). Polynucleotides in a test sample and those in a control sample are tagged with tags that identify the polynucleotides as originating from the test or control sample (a source tag). (804.) The tag can be, for example, a polynucleotide sequence or barcode that unambiguously identifies the source.

[00255] The polynucleotides in each of the control and test samples also can be tagged with identifier tags that will be carried by all amplification progeny of a polynucleotide. Information from start and end sequences of a polynucleotide and identifier tags can identify sequence reads from polynucleotides amplified from an original parent molecule. Each molecule can be uniquely tagged compared with other molecules in the sample. Alternatively, each molecule need not be uniquely tagged compared with other molecules in the sample. That is, the number of different identifier sequences can be fewer than that the number of molecules in sample. By combining identifier information with start/stop sequence information, the probability of confusing two molecules having the same start/stop sequence is significantly diminished.

[00256] Number of different identifiers used to tag a nucleic acid (*e.g.*, cfDNA) can dependent on the number of different haploid genome equivalents. Different identifiers can be used to tag at least 2, least 10, least 100, least 200, least 300, least 400, least 500, least 600, least 700, least 800, least 900, least 1,000, least 2,000, least 3,000, least 4,000, least 5,000, least 6,000, least 7,000, least 8,000, least 9,000, least 10,000 or more different haploid genome equivalents. Accordingly, the number of different identifiers used to tag a nucleic acid sample, *e.g.*, cell-free DNA from 500 to 10,000 different haploid genome equivalents and be between any of 1, 2, 3, 4 and 5 and no more than 100, 90, 80, 70, 60, 50, 40 or 30. For example, the number of different identifier used to tag a nucleic acid sample from 500 to 10,000 different haploid genome equivalents can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or less.

[00257] Polynucleotides can be tagged by ligation of adaptors comprising the tags or identifiers before amplification. Ligation can be performed using an enzyme, *e.g.*, a ligase. For example, tagging can be performed using a DNA ligase. The DNA ligase can be a T4 DNA ligase, *E. coli* DNA ligase, and/or mammalian ligase. The mammalian ligase can be DNA ligase I, DNA ligase III, or DNA ligase IV. The ligase may also be a thermostable ligase. Tags can be ligated to a blunt-end of a polynucleotide (blunt-end ligation). Alternatively, tags can be ligated to a sticky end of a polynucleotide (sticky-end ligation). The polynucleotides can be tagged by blunt end ligation using adaptors (*e.g.*, adaptors having forked ends). High efficiency of ligation can be achieved using high excess of adaptors (*e.g.*, more than 1.5X, more than 2X, more than 3X, more than 4X, more than 5X, more than 6X, more than 7X, more than 8X, more than 9X, more than 10X, more than 11X, more than 12X, more than 13X, more than 14X, more than 15X, more than 20X, more than 25X, more than 30X, more than 35X, more than 40X, more than 45X, more than 50X, more than 55X, more than 60X, more than 65X, more than 70X, more than 75X, more than 80X, more than 85X, more than 90X, more than 95X, or more than 100).

[00258] Once tagged with tags that identify source of the polynucleotides, polynucleotides from different sources (*e.g.*, different samples) can be pooled. After pooling, polynucleotides from different sources (*e.g.*, different samples) can be distinguished by any measurement using the tags, including any process of quantitative measurement. For example, as shown in (806) (**FIG. 8**), polynucleotides from the control sample and the test sample can be pooled. The pooled molecules can be subject to the sequencing (808) and bioinformatic work flow. Both will be subject to the same variations in the process and, therefore, any differential bias is reduced. Because molecules originating from control and test samples are differently tagged, they can be distinguished in any process of quantitative measurement.

[00259] The relative amount of control and test sample pooled can be varied. The amount of control sample can be same as the amount of test sample. The amount of control sample can also be larger than the amount of test sample. Alternatively, the amount of control sample can be smaller than the amount of test sample. The smaller the relative amount of one sample to the total, the fewer identifying tags needed in the original tagging process. A number can be selected to reduce to acceptable levels the probability that two parent molecules having the same start/end sequences will bear the same identifying tag. This probability can be less than 10%,

less than 1%, less than 0.1% or less than 0.01%. The probability can be less than 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

[00260] Methods disclosed herein can also comprise grouping sequence reads. For example, bioinformatic workflow can include grouping sequence reads produced from progeny of a single parent molecule, as shown in (810) (**FIG. 8**). This can involve any of the redundancy reduction methods described herein. Molecules sourced from test and control samples can be differentiated based on source tags they carry (812). Molecules mapping to a target locus are quantified for both test-sourced and control-sourced molecules (812). This can include the normalization methods discussed herein, *e.g.*, in which numbers at a target locus are normalized against numbers at a reference locus.

[00261] Normalized (or raw) quantities at a target locus from test and control samples are compared to determine presence of copy number variation (814).

[00262] **3. Computer Control Systems**

[00263] The present disclosure provides computer control systems that are programmed to implement methods of the disclosure. **FIG. 6** shows a computer system 1501 that is programmed or otherwise configured to implement the methods of the present disclosure. The computer system 1501 can regulate various aspects sample preparation, sequencing and/or analysis. In some examples, the computer system 1501 is configured to perform sample preparation and sample analysis, including nucleic acid sequencing. The computer system 1501 can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device.

[00264] The computer system 1501 includes a central processing unit (CPU, also “processor” and “computer processor” herein) 1505, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system 1501 also includes memory or memory location 1510 (*e.g.*, random-access memory, read-only memory, flash memory), electronic storage unit 1515 (*e.g.*, hard disk), communication interface 1520 (*e.g.*, network adapter) for communicating with one or more other systems, and peripheral devices 1525, such as cache, other memory, data storage and/or electronic display adapters. The memory 1510, storage unit 1515, interface 1520 and peripheral devices 1525 are in

communication with the CPU 1505 through a communication bus (solid lines), such as a motherboard. The storage unit 1515 can be a data storage unit (or data repository) for storing data. The computer system 1501 can be operatively coupled to a computer network (“network”) 1530 with the aid of the communication interface 1520. The network 1530 can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network 1530 in some cases is a telecommunication and/or data network. The network 1530 can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network 1530, in some cases with the aid of the computer system 1501, can implement a peer-to-peer network, which may enable devices coupled to the computer system 1501 to behave as a client or a server.

[00265] The CPU 1505 can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory 1510. The instructions can be directed to the CPU 1505, which can subsequently program or otherwise configure the CPU 1505 to implement methods of the present disclosure. Examples of operations performed by the CPU 1505 can include fetch, decode, execute, and writeback.

[00266] The CPU 1505 can be part of a circuit, such as an integrated circuit. One or more other components of the system 1501 can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00267] The storage unit 1515 can store files, such as drivers, libraries and saved programs. The storage unit 1515 can store user data, *e.g.*, user preferences and user programs. The computer system 1501 in some cases can include one or more additional data storage units that are external to the computer system 1501, such as located on a remote server that is in communication with the computer system 1501 through an intranet or the Internet.

[00268] The computer system 1501 can communicate with one or more remote computer systems through the network 1530. For instance, the computer system 1501 can communicate with a remote computer system of a user (*e.g.*, an operator). Examples of remote computer systems include personal computers (*e.g.*, portable PC), slate or tablet PC’s (*e.g.*, Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (*e.g.*, Apple® iPhone, Android-enabled

device, Blackberry®), or personal digital assistants. The user can access the computer system 1501 via the network 1530.

[00269] Methods as described herein can be implemented by way of machine (*e.g.*, computer processor) executable code stored on an electronic storage location of the computer system 1501, such as, for example, on the memory 1510 or electronic storage unit 1515. The machine executable or machine readable code can be provided in the form of software. During use, the code can be executed by the processor 1505. In some cases, the code can be retrieved from the storage unit 1515 and stored on the memory 1510 for ready access by the processor 1505. In some situations, the electronic storage unit 1515 can be precluded, and machine-executable instructions are stored on memory 1510.

[00270] The code can be pre-compiled and configured for use with a machine have a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[00271] Aspects of the systems and methods provided herein, such as the computer system 1501, can be embodied in programming. Various aspects of the technology may be thought of as “products” or “articles of manufacture” typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such memory (*e.g.*, read-only memory, random-access memory, flash memory) or a hard disk. “Storage” type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry

such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible “storage” media, terms such as computer or machine “readable medium” refer to any medium that participates in providing instructions to a processor for execution.

[00272] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[00273] The computer system 1501 can include or be in communication with an electronic display 1535 that comprises a user interface (UI) 1540. The UI can allow a user to set various conditions for the methods described herein, for example, PCR or sequencing conditions. Examples of UI’s include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00274] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the

central processing unit 1505. The algorithm can, for example, process the reads to generate a consequence sequence.

[00275] FIG. 7 schematically illustrates another system for analyzing a sample comprising nucleic acids from a subject. The system includes a sequencer, bioinformatic software and internet connection for report analysis by, for example, a hand held device or a desktop computer

[00276] Disclosed herein is a system for analyzing a target nucleic acid molecule of a subject, comprising: a communication interface that receives nucleic acid sequence reads for a plurality of polynucleotide molecules that cover genomic loci of a target genome; computer memory that stores the nucleic acid sequence reads for the plurality of polynucleotide molecules received by the communication interface; and a computer processor operatively coupled to the communication interface and the memory and programmed to (i) group the plurality of sequence reads into families, wherein each family comprises sequence reads from one of the template polynucleotides, (ii) for each of the families, merge sequence reads to generate a consensus sequence, (iii) call the consensus sequence at a given genomic locus among the genomic loci, and (iv) detect at the given genomic locus any of genetic variants among the calls, frequency of a genetic alteration among the calls, total number of calls; and total number of alterations among the calls, wherein the genomic loci correspond to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. The different variations of each component of the system are described throughout the disclosure within the methods and compositions. These individual components and variations thereof, are also applicable in this system.

[00277] **4. Kits**

[00278] Kits comprising the compositions as described herein. The kits can be useful in performing the methods as described herein. Disclosed herein is a kit comprising a plurality of

oligonucleotide probes that selectively hybridize to least 5, 6, 7, 8, 9, 10, 20, 30, 40 or all genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. The number genes to which the oligonucleotide probes can selectively hybridize can vary. For example, the number of genes can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, or 54. The kit can include a container that includes the plurality of oligonucleotide probes and instructions for performing any of the methods described herein.

[00279] The oligonucleotide probes can selectively hybridize to exon regions of the genes, *e.g.*, of the at least 5 genes. In some cases, the oligonucleotide probes can selectively hybridize to at least 30 exons of the genes, *e.g.*, of the at least 5 genes. In some cases, the multiple probes can selectively hybridize to each of the at least 30 exons. The probes that hybridize to each exon can have sequences that overlap with at least 1 other probe. In some embodiments, the oligoprobes can selectively hybridize to non-coding regions of genes disclosed herein, for example, intronic regions of the genes. The oligoprobes can also selectively hybridize to regions of genes comprising both exonic and intronic regions of the genes disclosed herein.

[00280] Any number of exons can be targeted by the oligonucleotide probes. For example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, , 295, 300, 400, 500, 600, 700, 800, 900, 1,000, or more, exons can be targeted.

[00281] The kit can comprise at least 4, 5, 6, 7, or 8 different library adaptors having distinct molecular barcodes and identical sample barcodes. The library adaptors may not be sequencing

adaptors. For example, the library adaptors do not include flow cell sequences or sequences that permit the formation of hairpin loops for sequencing. The different variations and combinations of molecular barcodes and sample barcodes are described throughout, and are applicable to the kit. Further, in some cases, the adaptors are not sequencing adaptors. Additionally, the adaptors provided with the kit can also comprise sequencing adaptors. A sequencing adaptor can comprise a sequence hybridizing to one or more sequencing primers. A sequencing adaptor can further comprise a sequence hybridizing to a solid support, *e.g.*, a flow cell sequence. For example, a sequencing adaptor can be a flow cell adaptor. The sequencing adaptors can be attached to one or both ends of a polynucleotide fragment. In some cases, the kit can comprise at least 8 different library adaptors having distinct molecular barcodes and identical sample barcodes. The library adaptors may not be sequencing adaptors. The kit can further include a sequencing adaptor having a first sequence that selectively hybridizes to the library adaptors and a second sequence that selectively hybridizes to a flow cell sequence. In another example, a sequencing adaptor can be hairpin shaped. For example, the hairpin shaped adaptor can comprise a complementary double stranded portion and a loop portion, where the double stranded portion can be attached (*e.g.*, ligated) to a double-stranded polynucleotide. Hairpin shaped sequencing adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times. A sequencing adaptor can be up to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more bases from end to end. The sequencing adaptor can comprise 20-30, 20-40, 30-50, 30-60, 40-60, 40-70, 50-60, 50-70, bases from end to end. In a particular example, the sequencing adaptor can comprise 20-30 bases from end to end. In another example, the sequencing adaptor can comprise 50-60 bases from end to end. A sequencing adaptor can comprise one or more barcodes. For example, a sequencing adaptor can comprise a sample barcode. The sample barcode can comprise a pre-determined sequence. The sample barcodes can be used to identify the source of the polynucleotides. The sample barcode can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

21, 22, 23, 24, 25, or more (or any length as described throughout) nucleic acid bases, *e.g.*, at least 8 bases. The barcode can be contiguous or non-contiguous sequences, as described above.

[00282] The library adaptors can be blunt ended and Y-shaped and can be less than or equal to 40 nucleic acid bases in length. Other variations of the can be found throughout and are applicable to the kit.

EXAMPLES

[00283] **Example 1. Methods for copy number variation detection.**

[00284] Blood collection

[00285] 10-30 mL Blood samples are collected at room temperature. The samples are centrifuged to remove cells. Plasma is collected after centrifugation.

[00286] cfDNA extraction

[00287] The sample is subjected to proteinase K digestion. DNA is precipitated with isopropanol. DNA is captured on a DNA purification column (*e.g.*, a QIAamp DNA Blood Mini Kit) and eluted in 100 µl solution. DNAs below 500 bp are selected with Ampure SPRI magnetic bead capture (PEG/salt). The resulting production is suspended in 30 µl H₂O. Size distribution is checked (major peak = 166 nucleotides; minor peak = 330 nucleotides) and quantified. 5 ng of extracted DNA contain approximately 1700 haploid genome equivalents (“HGE”). The general correlation between the amount of DNA and HGE is as follow: 3 pg DNA = 1 HGE; 3 ng DNA = 1K HGE; 3 µg DNA = 1M HGE; 10 pg DNA = 3 HE; 10 ng DNA = 3K HGE; 10 µg DNA = 3M HGE.

[00288] “Single Molecule” library prep

[00289] High-efficiency DNA tagging (>80%) is performed by blunt-end repair and ligation with 8 different octomers (*i.e.*, 64 combinations) with overloaded hairpin adaptors. 2.5 ng DNA (*i.e.* approximately 800 HGE) is used as the starting material. Each hairpin adaptor comprises a random sequence on its non-complementary portion. Both ends of each DNA fragment are attached with hairpin adaptors. Each tagged fragment can be identified by the random sequence on the hairpin adaptors and a 10 p endogenous sequence on the fragment.

[00290] Tagged DNA is amplified by 10 cycles of PCR to produce about 1-7 µg DNAs that contain approximately 500 copies of each of the 800 HGE in the starting material.

[00291] Buffer optimization, polymerase optimization and cycle reduction may be performed to optimize the PCR reactions. Amplification bias, *e.g.*, non-specific bias, GC bias, and/or size bias are also reduced by optimization. Noise(s) (*e.g.*, polymerase-introduced errors) are reduced by using high-fidelity polymerases.

[00292] The Library may be prepared using Verniata or Sequenom methods.

[00293] Sequences may be enriched as follow: DNAs with regions of interest (ROI) are captured using biotin-labeled bead with probe to ROIs. The ROIs are amplified with 12 cycles of PCR to generate a 2000 times amplification. The resulting DNA is then denatured and diluted to 8 pM and loaded into an Illumina sequencer.

[00294] Massively parallel sequencing

[00295] 0.1 to 1% of the sample (approximately 100pg) are used for sequencing.

[00296] Digital bioinformatics

[00297] Sequence reads are grouped into families, with about 10 sequence reads in each family. Families are collapsed into consensus sequences by voting (*e.g.*, biased voting) each position in a family. A base is called for consensus sequence if 8 or 9 members agree. A base is not called for consensus sequence if no more than 60% of the members agree.

[00298] The resulting consensus sequences are mapped to a reference genome. Each base in a consensus sequence is covered by about 3000 different families. A quality score for each sequence is calculated and sequences are filtered based on the their quality scores.

[00299] Sequence variation is detected by counting distribution of bases at each locus. If 98% of the reads have the same base (homozygous) and 2% have a different base, the locus is likely to have a sequence variant, presumably from cancer DNA.

[00300] CNV is detected by counting the total number of sequences (bases) mapping to a locus and comparing with a control locus. To increase CNV detection, CNV analysis is performed specific regions, including regions on ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A,

BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, or NTRK1 genes.

[00301] Example 2. Method for Correcting Base Calling by Determining the Total Number Unseen Molecules in a Sample

[00302] After fragments are amplified and the sequences of amplified fragments are read and aligned, the fragments are subjected to base calling. Variations in the number of amplified fragments and unseen amplified fragments can introduce errors in base calling. These variations are corrected by calculating the number of unseen amplified fragments.

[00303] When base calling for locus A (an arbitrary locus), it is first assumed that there are N amplified fragments. The sequence readouts can come from two types of fragments: double-strand fragments and single-strand fragments. The following is a theoretical example of calculating the total number of unseen molecules in a sample.

[00304] N is the total number of molecules in the sample.
Assuming 1000 is the number of duplexes detected.
Assuming 500 is the number of single-stranded molecule detected.
P is the probability of seeing a strand.
Q is the probability of not detecting a strand.

[00305] Since $Q = 1 - P$.
 $1000 = NP(2)$.
 $500 = N2PQ$.
 $1000 / P(2) = N$.
 $500 \div 2 PQ = N$.
 $1000 / P(2) = 500 \div 2PQ$.
 $1000 * 2 PQ = 500 P(2)$.
 $2000 PQ = 500 P(2)$.
 $2000 Q = 500 P$.
 $2000 (1-P) = 500P$
 $2000 - 2000 P = 500P$.
 $2000 = 500P + 2000 P$.
 $2000 = 2500 P$.
 $2000 \div 2500 = P$.
 $0.8 = P$.
 $1000 / P(2) = N$.
 $1000 \div 0.64 = N$.
 $1562 = N$.
Number of unseen fragments = 62.

[00306] Example 3. Identification of genetic variants in cancer-associated somatic variants in a patient.

[00307] An assay is used to analyze a panel of genes to identify genetic variants in cancer-associated somatic variants with high sensitivity.

[00308] Cell-free DNA is extracted from plasma of a patient and amplified by PCR. Genetic variants are analyzed by massively parallel sequencing of the amplified target genes. For one set of genes, all exons are sequenced as such sequencing coverage had shown to have clinically utility (Table 1). For another set of genes, sequencing coverage included those exons with a previously reported somatic mutation (Table 2). The minimum detectable mutant allele (limit of detection) is dependent on the patient's sample cell-free DNA concentration, which varied from less than 10 to over 1,000 genomic equivalents per mL of peripheral blood. Amplification may not be detected in samples with lower amounts of cell-free DNA and/or low-level gene copy amplification. Certain sample or variant characteristics resulted in reduced analytic sensitivity, such as low sample quality or improper collection.

[00309] The percentage of genetic variants found in cell-free DNA circulating in blood is related to the unique tumor biology of this patient. Factors that affected the amount/percentages of detected genetic variants in circulating cell-free DNA in blood include tumor growth, turnover, size, heterogeneity, vascularization, disease progression or treatment. Table 3 annotates the percentage, or allele frequency, of altered circulating cell-free DNA (% cfDNA) detected in this patient. Some of the detected genetic variants are listed in descending order by % cfDNA.

[00310] Genetic variants are detected in the circulating cell-free DNA isolated from this patient's blood specimen. These genetic variants are cancer-associated somatic variants, some of which have been associated with either increased or reduced clinical response to specific treatment. "Minor Alterations" are defined as those alterations detected at less than 10% the allele frequency of "Major Alterations". The detected allele frequencies of these alterations (Table 3) and associated treatments for this patient are annotated.

[00311] All genes listed in Tables 1 and 2 are analyzed as part of the Guardant360™ test. Amplification is not detected for *ERBB2*, *EGFR*, or *MET* in the circulating cell-free DNA isolated from this patient's blood specimen.

[00312] Patient test results comprising the genetic variants are listed in Table 4.

Table 1. Genes in which all exons are sequenced

GENES IN WHICH ALL EXONS ARE SEQUENCED			
ALK	< 0.1%	APC	< 0.1%
AR	< 0.1%	BRAF	< 0.1%
CDKN2A	< 0.1%	EGFR	< 0.1%
ERBB2	< 0.1%	FBXW7	< 0.1%
KRAS	< 0.1%	MET	< 0.1%
MYC	< 0.1%	NOTCH1	< 0.1%
NRAS	< 0.1%	PIK3CA	< 0.1%
PTEN	< 0.1%	PROC	< 0.1%
RB1	< 0.1%	TP53	< 0.1%

LOD: Limit of Detection. The minimum detectable mutant allele frequency for this specimen in which 80% of somatic variants is detected.

Table 2. Genes in which exons with a previously reported somatic mutation are sequenced

GENES IN WHICH EXONS WITH A PREVIOUSLY REPORTED SOMATIC MUTATION ARE SEQUENCED			
ABL1	< 0.1%	AKT1	< 0.1%
ATM	< 0.1%	CDH1	< 0.1%
CSF1R	< 0.1%	CTNNB1	< 0.1%
ERBB4	< 0.1%	EZH2	< 0.1%
FGFR1	< 0.1%	FGFR2	< 0.1%
FGFR3	< 0.1%	FLT3	< 0.1%
GNA11	< 0.1%	GNAQ	< 0.1%
GNAS	< 0.1%	HNF1A	< 0.1%
HRAS	< 0.1%	IDH1	< 0.1%
IDH2	< 0.1%	JAK2	< 0.1%
JAK3	< 0.1%	KDR	< 0.1%
KIT	< 0.1%	MLH1	< 0.1%
MPL	< 0.1%	NPM1	< 0.1%
PDGFRA	< 0.1%	PTPN11	< 0.1%
RET	< 0.1%	SMAD4	< 0.1%
SMARCB1	< 0.1%	SMO	< 0.1%
SRC	< 0.1%	STK11	< 0.1%
TERT	< 0.1%	VHL	< 0.1%

LOD: Limit of Detection. The minimum detectable mutant allele frequency for this specimen in which 80% of somatic variants is detected.

Table 3. Allele frequency of altered circulating cell-free DNA detected in this patient

Gene	cfDNA with alterations (%)	cfDNA without alterations (%)
BRAF V600E	8.9	91.1
NRAS Q61K	6.2	93.8
JAK V617F	1.5	98.6

Table 4. Genomic alterations detected in selected genes

Detected: 51 Genomic Alterations							
Gene	Chromosome	Position	Mutation (nt)	Mutation (AA)	Percentage	Cosmic ID	DBSNP ID
KRAS	12	25368462	C>T		100.0%		rs4362222
ALK	2	29416572	T>C	I1461V	100.0%		rs1670283
ALK	2	29444095	C>T		100.0%		rs1569156
ALK	2	29543663	T>C	Q500Q	100.0%		rs2293564
ALK	2	29940529	A>T	P234P	100.0%		rs2246745
APC	5	112176756	T>A	V1822D	100.0%		rs459552
CDKN2A	9	21968199	C>G		100.0%	COSM14251	rs11515
FGFR3	4	1807894	G>A	T651T	100.0%		rs7688609
NOTCH1	9	139410424	A>G		100.0%		rs3125006
PDGFRA	4	55141055	A>G	P567P	100.0%		rs1873778
HRAS	11	534242	A>G	H27H	100.0%	COSM249860	rs12628
EGFR	7	55214348	C>T	N158N	99.9%	COSM42978	rs2072454
TP53	17	7579472	G>C	P72R	99.8%		rs1042522
APC	5	112162854	T>C	Y486Y	55.0%		rs2229992
APC	5	112177171	G>A	P1960P	53.8%		rs465899
EGFR	7	55266417	T>C	T903T	53.6%		rs1140475
APC	5	112176325	G>A	G1678G	53.2%		rs42427
APC	5	112176559	T>G	S1756S	53.0%		rs866006
EGFR	7	55229255	G>A	R521K	53.0%		
MET	7	116397572	A>G	Q648Q	52.7%		
APC	5	112175770	G>A	T1493T	52.7%		rs41115
EGFR	7	55249063	G>A	Q787Q	52.6%		rs1050171
NOTCH1	9	139411714	T>C		52.4%		rs11145767
EGFR	7	55238874	T>A	T629T	52.0%		rs2227984
ERBB2	17	37879588	A>G	I655V	51.6%		rs1136201
NOTCH1	9	139397707	G>A	D1698D	51.3%	COSM33747	rs10521
ALK	2	30143499	G>C	L9L	51.0%		rs4358080
APC	5	112164561	G>A	A545A	51.0%		rs351771
FLT3	13	28610183	A>G		50.8%		rs2491231
NOTCH1	9	139418260	A>G	N104N	50.5%		rs4489420
ALK	2	29444076	G>T		50.4%		rs1534545
PIK3CA	3	178917005	A>G		50.3%		rs3729674
NOTCH1	9	139412197	G>A		50.2%		rs9411208
ALK	2	29455267	A>G	G845G	50.0%	COSM148825	rs2256740
KIT	4	55593464	A>C	M541L	49.9%	COSM28026	

NOTCH1	9	139391636	G>A	D2185D	48.9%		rs2229974
PDGFRA	4	55152040	C>T	V824V	48.9%	COSM22413	rs2228230
ALK	2	29416481	T>C	K1491R	48.9%	COSM1130802	rs1881420
ALK	2	29445458	G>T	G1125G	48.6%		rs3795850
NOTCH1	9	139410177	T>C		48.5%		rs3124603
RET	10	43613843	G>T	L769L	48.2%		rs1800861
EGFR	7	55214443	G>A		48.0%		rs7801956
ALK	2	29416366	G>C	D1529E	47.2%		rs1881421
EGFR	7	55238087	C>T		45.5%		rs10258429
RET	10	43615633	C>G	S904S	44.8%		rs1800863
BRAF	7	140453136	A>T	V600E	8.9%	COSM476	
NRAS	1	115256530	G>T	Q61K	6.2%	COSM580	rs121913254
JAK2	9	5073770	G>T	V617F	1.5%	COSM12600	rs77375493

[00313] Example 4. Determining patient-specific limits of detection for genes analyzed by Guardant360™ assays.

[00314] Using the method of Example 3, Genetic alterations in cell-free DNA of a patient are detected. The sequence reads of these genes include exon and/or intron sequences.

[00315] Limits of detection of the test are shown in Table 5. The limits of detection values are dependent on cell-free DNA concentration and sequencing coverage for each gene.

Table 5. Limits of Detection of selected genes in a patient using Guardant

Complete Exon and Partial Intron Coverage					
APC	0.1%	AR *	0.2%	ARID1A	
BRAF *	0.1%	BRCA1		BRCA2	
CCND1 *		CCND2 *		CCNE1 *	
CDK4 *		CDK6 *		CDKN2A	0.1%
CDKN2B		EGFR *	< 0.1%	ERBB2 *	0.1%
FGFR1 *	< 0.1%	FGFR2 *	0.1%	HRAS	0.1%
KIT *	0.1%	KRAS *	0.1%	MET *	0.1%
MYC *	0.1%	NF1		NRAS	0.1%
PDGFRA *	0.1%	PIK3CA*	0.1%	PTEN	0.1%
RAF1 *		TP53	0.1%		
Exons Covered with Reported Somatic Mutations					
AKT1	0.1%	ALK	< 0.1%	ARAF	
ATM	0.1%	CDH1	0.1%	CTNNB1	0.1%
ESR1		EZH2	0.1%	FBXW7	0.1%
FGFR3	0.1%	GATA3		GNA11	0.1%
GNAQ	0.1%	GNAS	0.1%	HNF1A	0.1%

IDH1	0.1%	IDH2	0.1%	JAK2	0.1%
JAK3	0.1%	MAP2K1		MAP2K2	
MLH1	0.1%	MPL	0.2%	NFE2L2	
NOTCH1	0.1%	NPM1	0.1%	PTPN11	0.1%
RET	0.1%	RHEB		RHOA	
RIT1		ROS1		SMAD4	0.1%
SMO	0.1%	SRC	< 0.1%	STK11	0.2%
TERT	0.1%	VHL	0.2%		
Fusions					
ALK	< 0.1%	RET	0.1%	ROS1	
NTRK1					

LOD: Limit of Detection. The minimum detectable mutant allele frequency for this specimen in which 80% of somatic variants is detected. * indicates CNV genes.

[00316] Example 5. Correcting Sequence Errors Comparing Watson and Crick Sequences

[00317] Double-stranded cell-free DNA is isolated from the plasma of a patient. The cell-free DNA fragments are tagged using 16 different bubble-containing adaptors, each of which comprises a distinctive barcode. The bubble-containing adaptors are attached to both ends of each cell-free DNA fragment by ligation. After ligation, each of the cell-free DNA fragment can be distinctly identified by the sequence of the distinct barcodes and two 20 bp endogenous sequences at each end of the cell-free DNA fragment.

[00318] The tagged cell-free DNA fragments are amplified by PCR. The amplified fragments are enriched using beads comprising oligonucleotide probes that specifically bind to a group of cancer-associated genes. Therefore, cell-free DNA fragments from the group of cancer-associated genes are selectively enriched.

[00319] Sequencing adaptors, each of which comprises a sequencing primer binding site, a sample barcode, and a cell-flow sequence, are attached to the enriched DNA molecules. The resulting molecules are amplified by PCR.

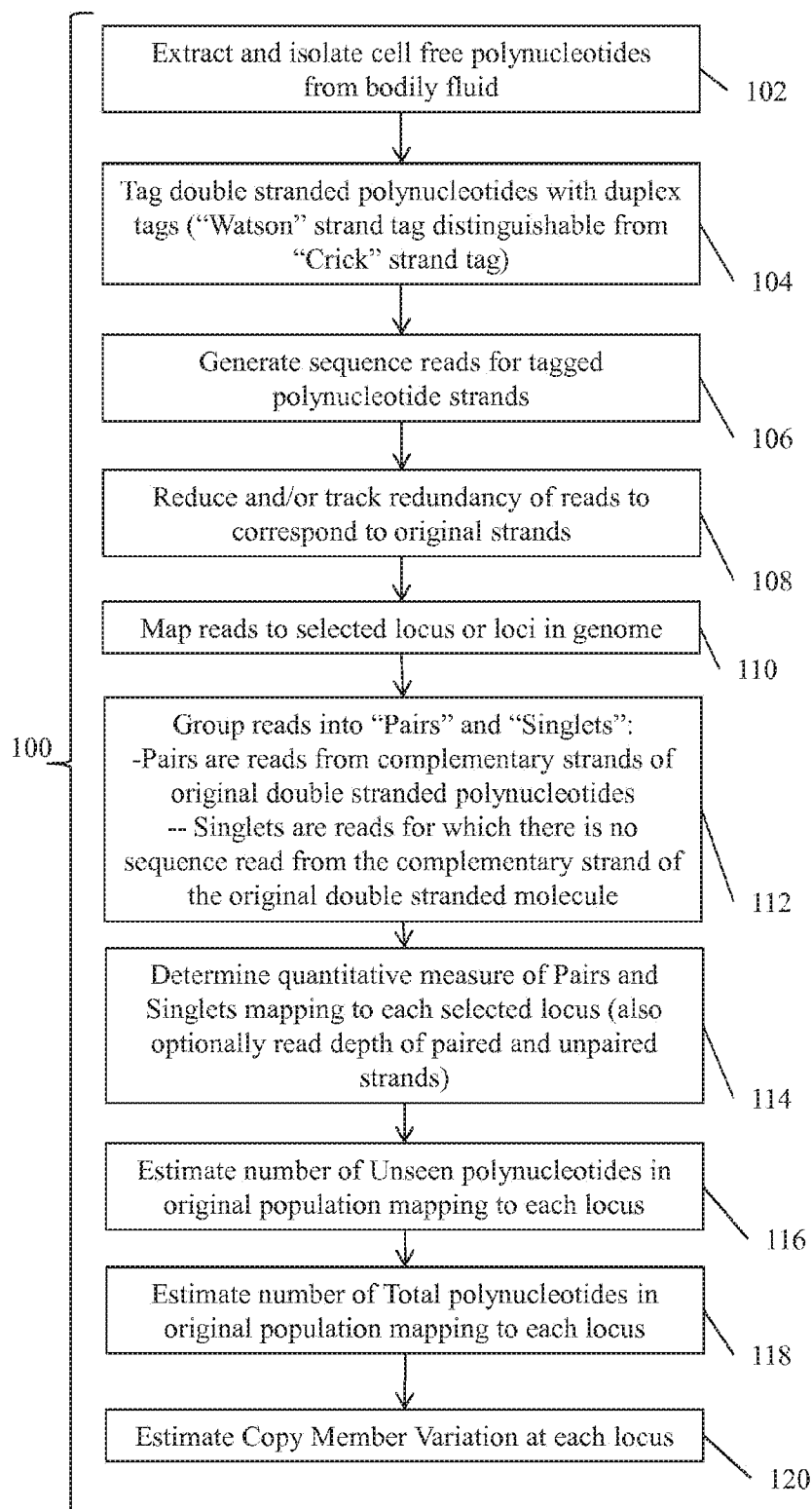
[00320] Both strands of the amplified fragments are sequenced. Because each bubble-containing adaptor comprises a non-complementary portion (*e.g.*, the bubble), the sequence of the one strand of the bubble-containing adaptor is different from the sequence of the other strand (complement). Therefore, the sequence reads of amplicons derived from the Watson strand of an

original cell-free DNA can be distinguished from amplicons from the Crick strand of the original cell-free DNA by the attached bubble-containing adaptor sequences.

[00321] The sequence reads from a strand of an original cell-free DNA fragment are compared to the sequence reads from the other strand of the original cell-free DNA fragment. If a variant occurs in only the sequence reads from one strand, but not other strand, of the original cell-free DNA fragment, this variant will be identified as an error (*e.g.*, resulted from PCR and/or amplification), rather than a true genetic variant.

[00322] The sequence reads are grouped into families. Errors in the sequence reads are corrected. The consensus sequence of each family is generated by collapsing.

[00323] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

**Fig. 1**

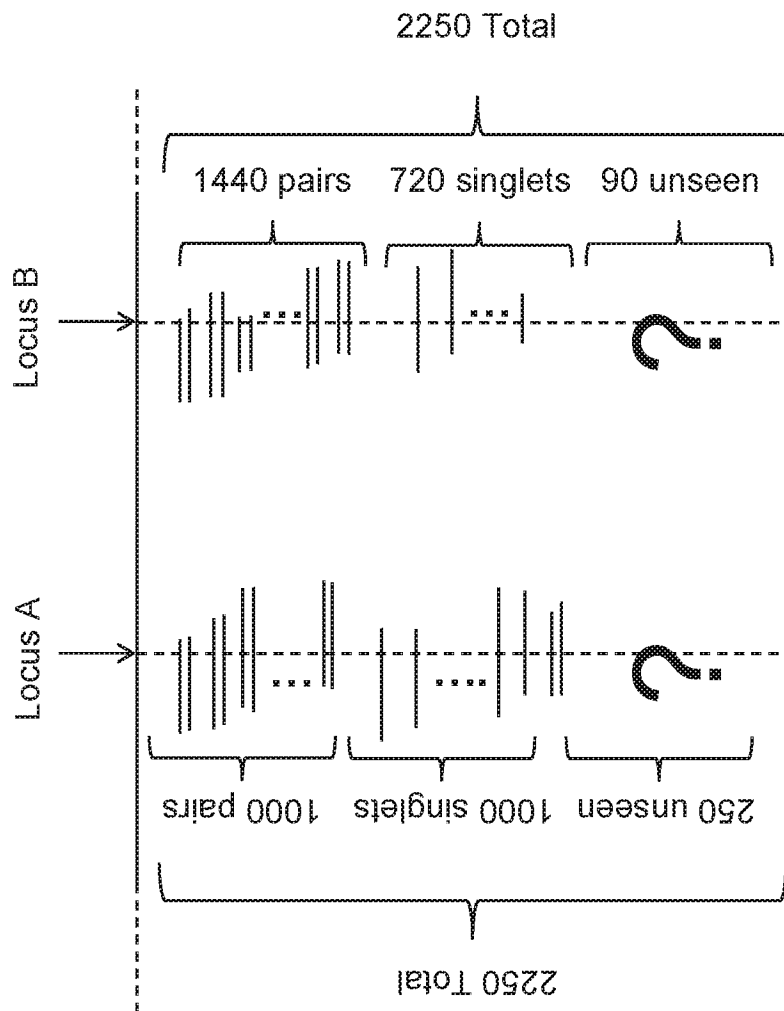
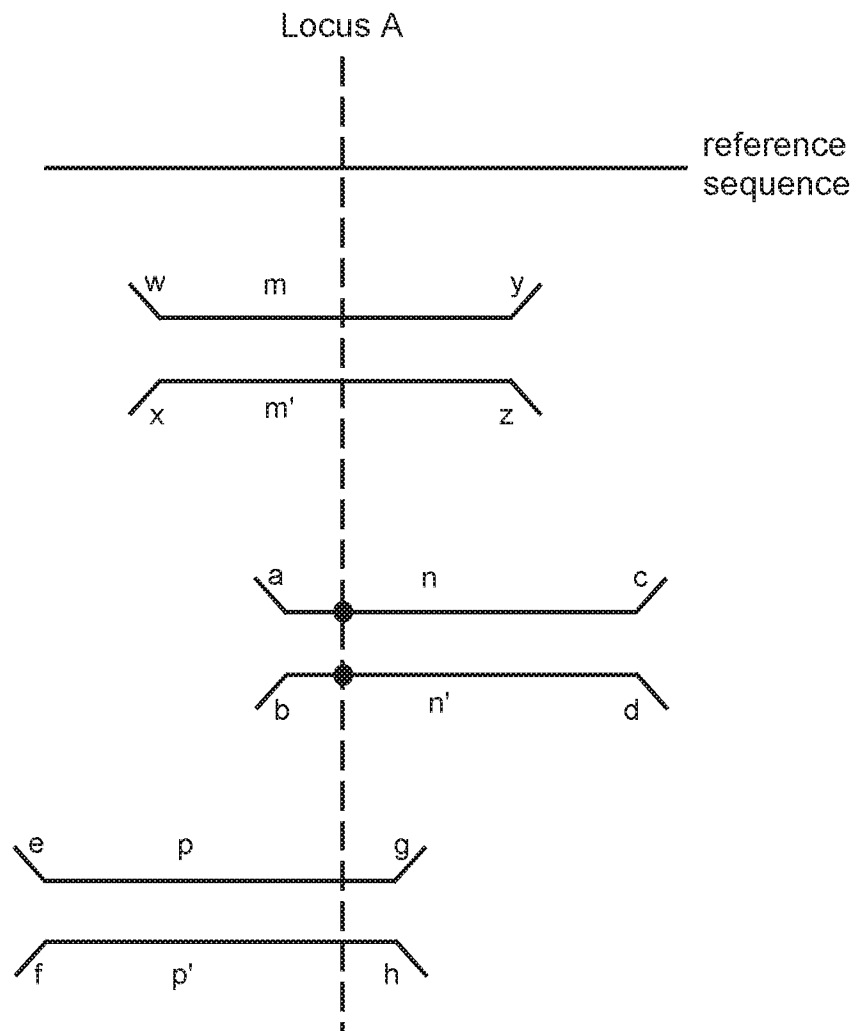


Fig. 2

**Fig. 3**

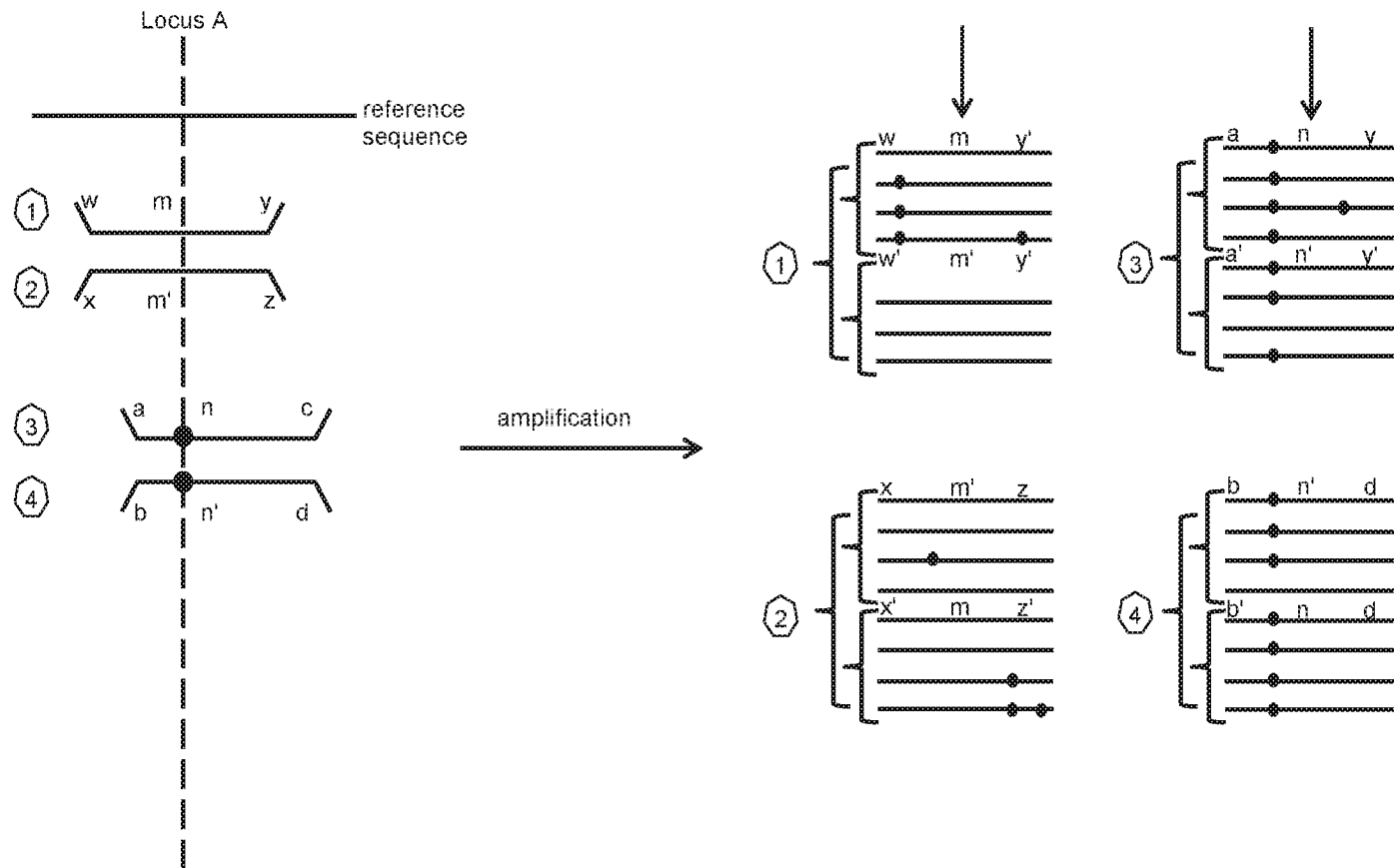


Fig. 4A

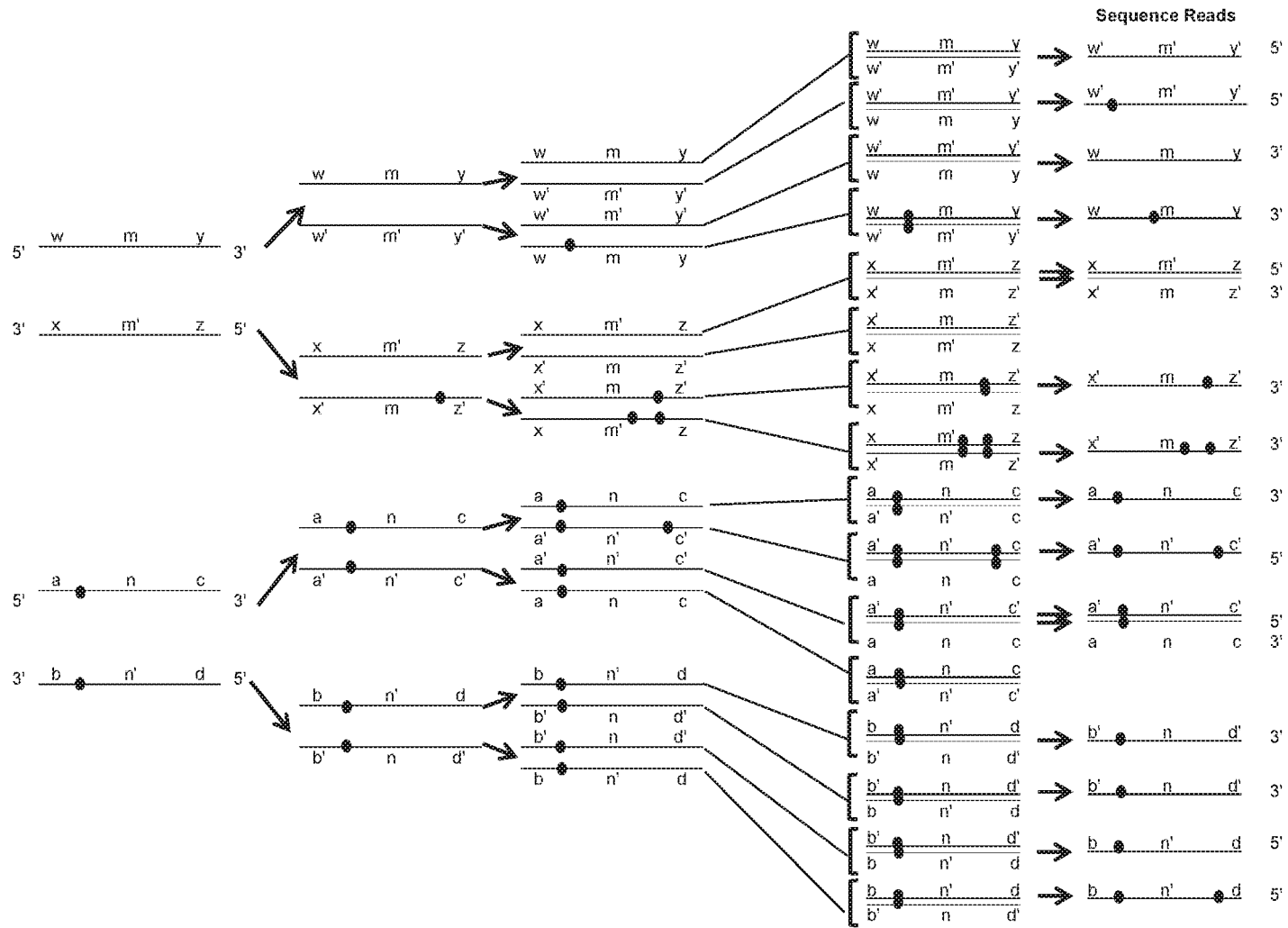


Fig. 4B

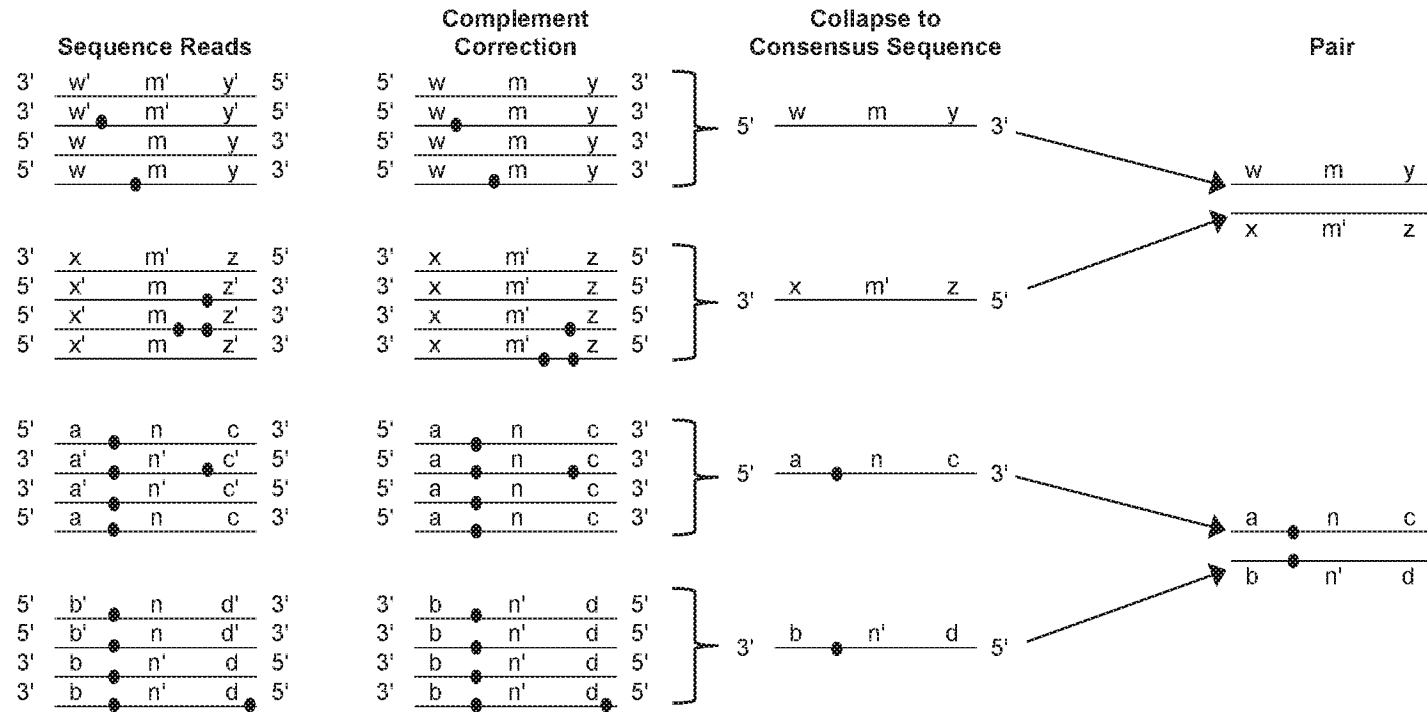


Fig. 4C

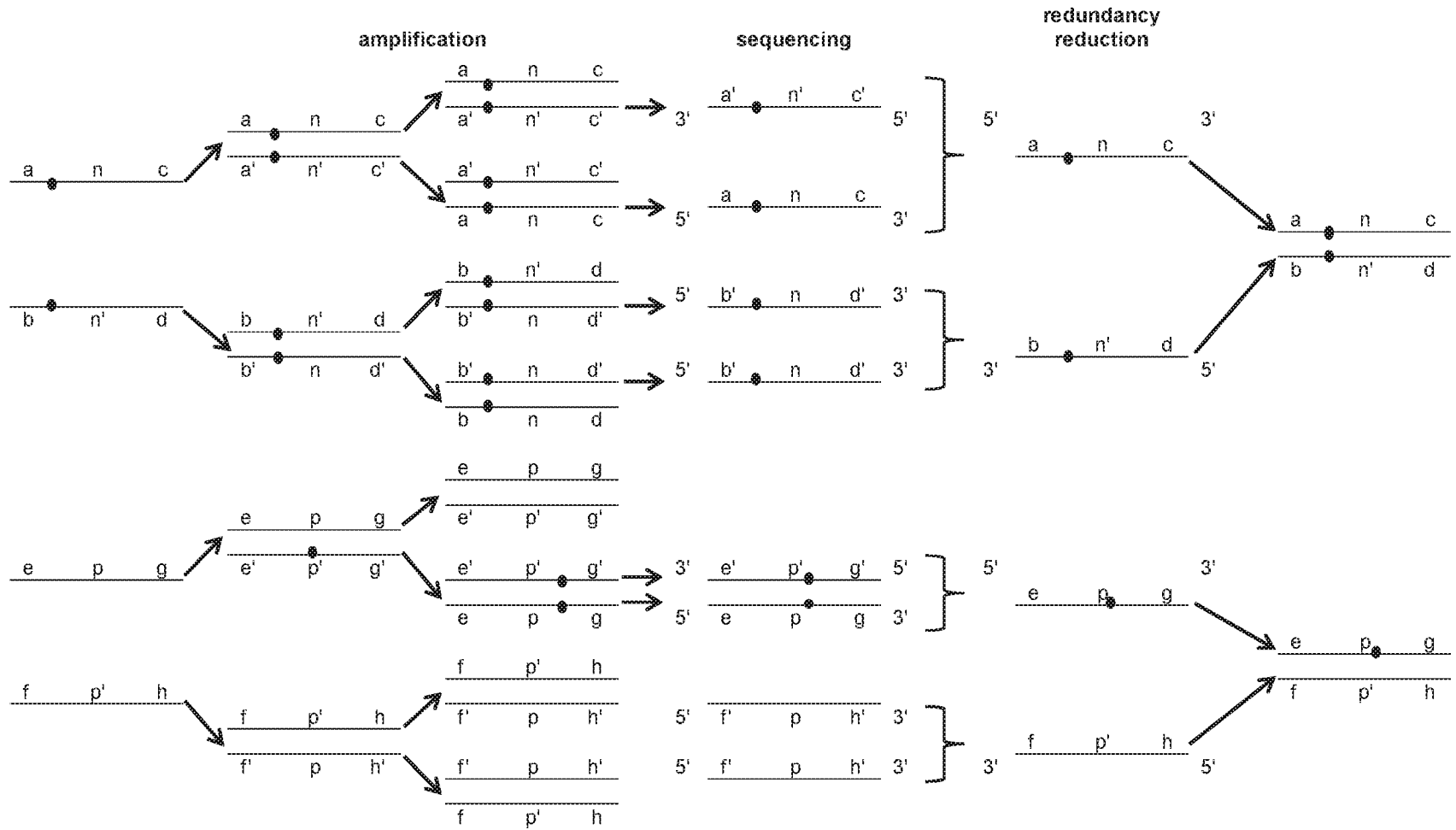


Fig. 5

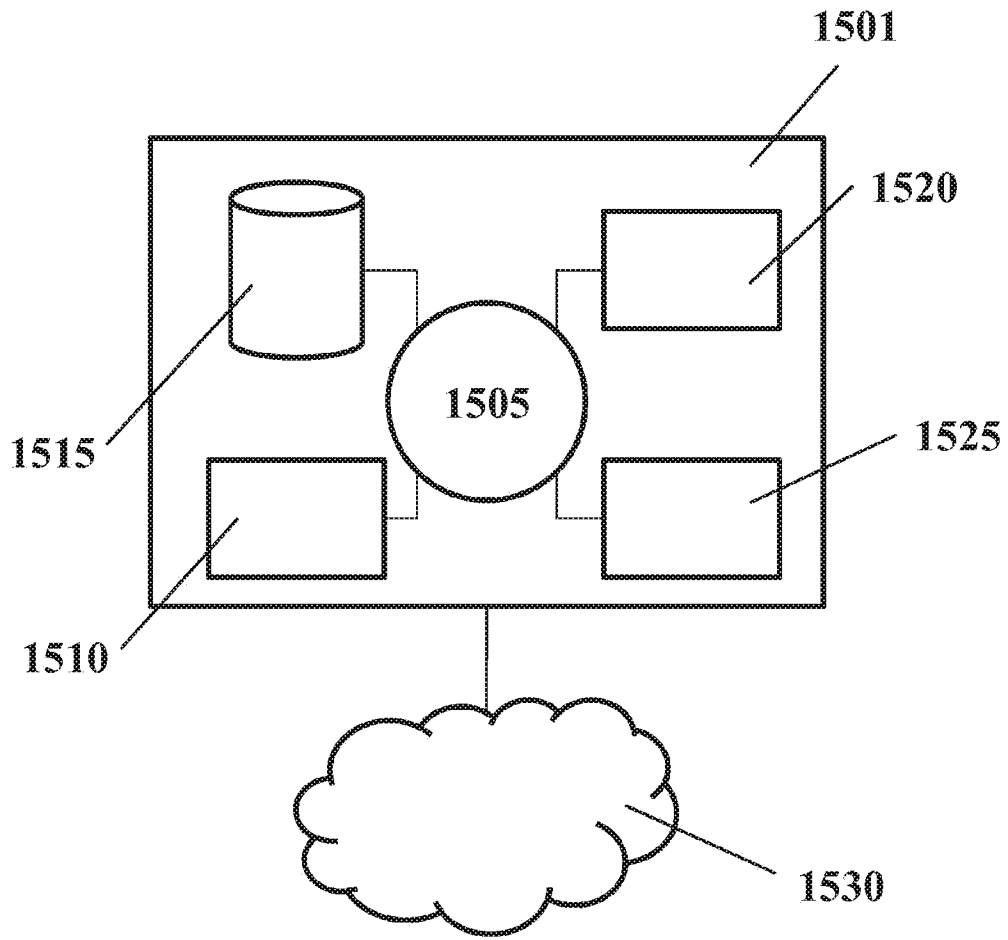


Fig. 6

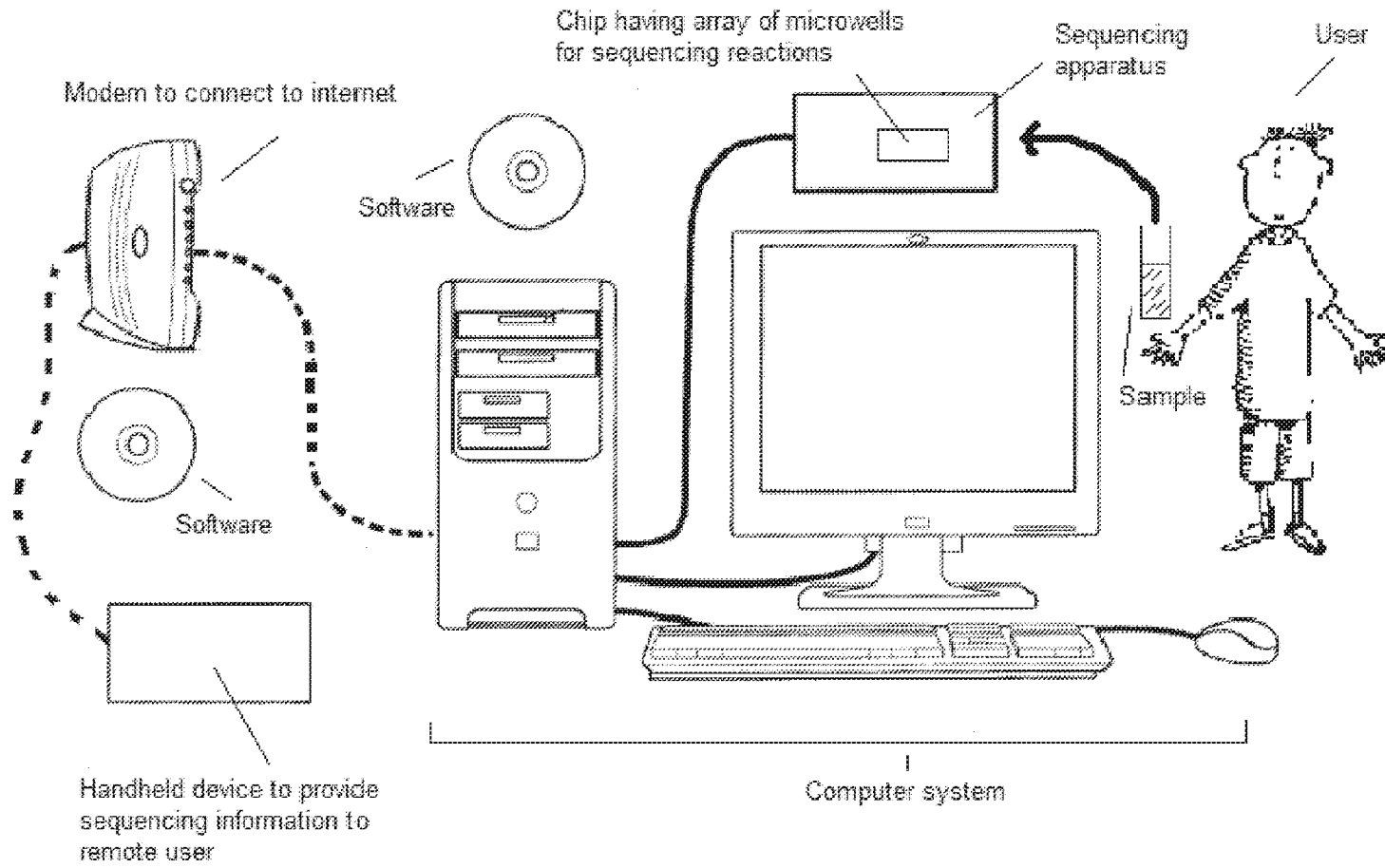


Fig. 7

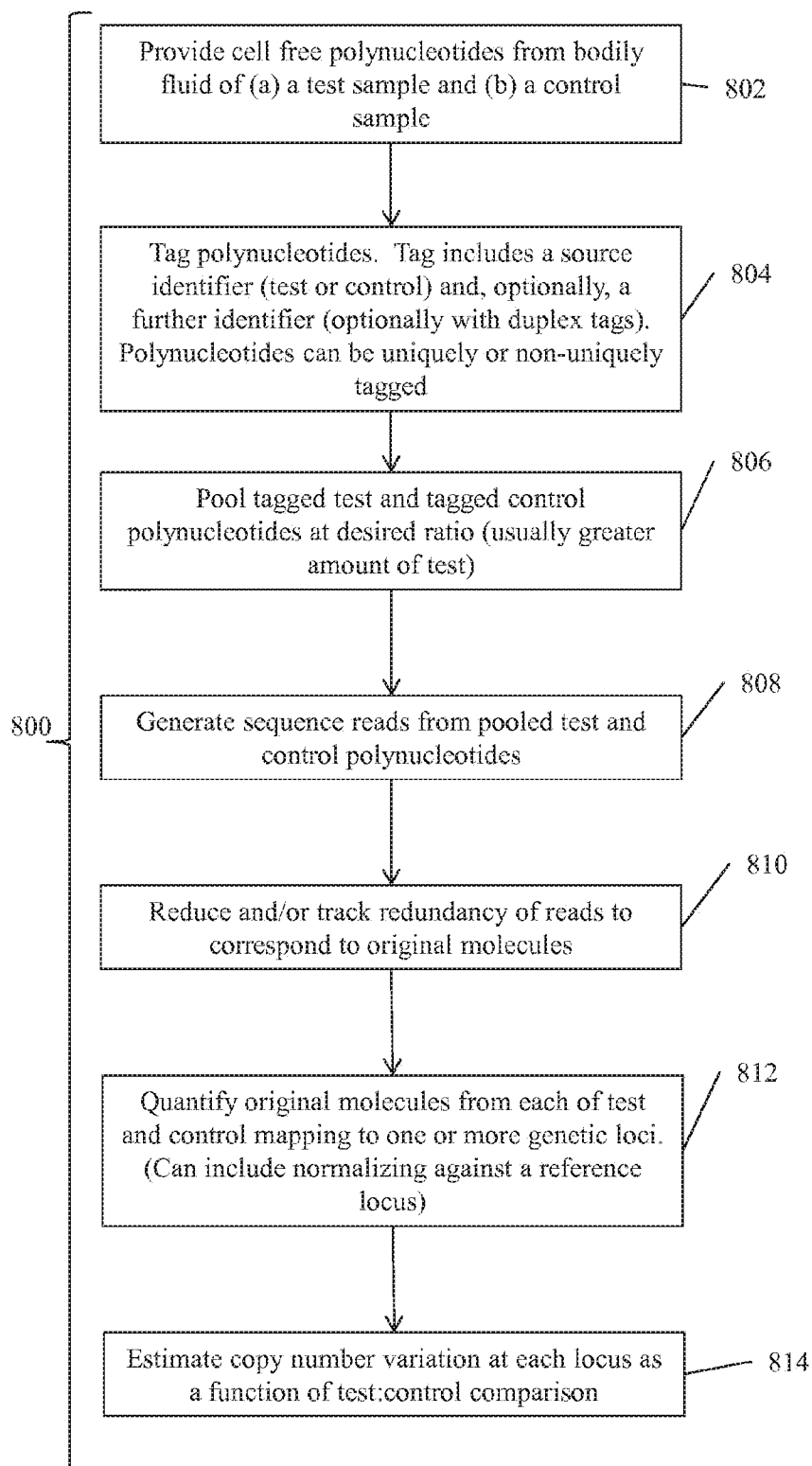
**Fig. 8**

Fig. 9A

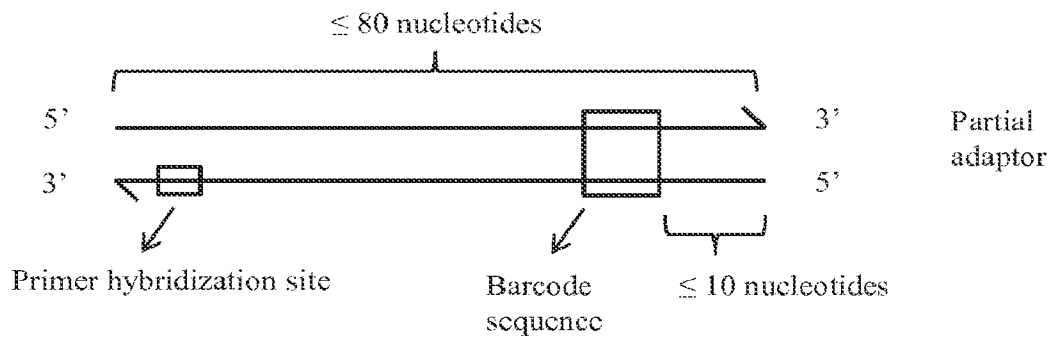


Fig. 9B

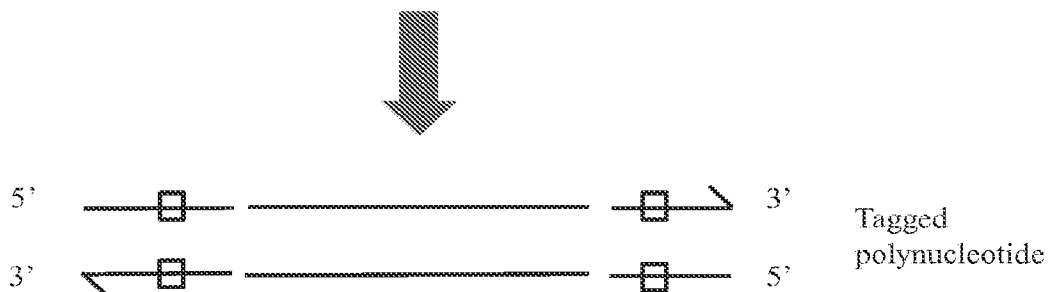
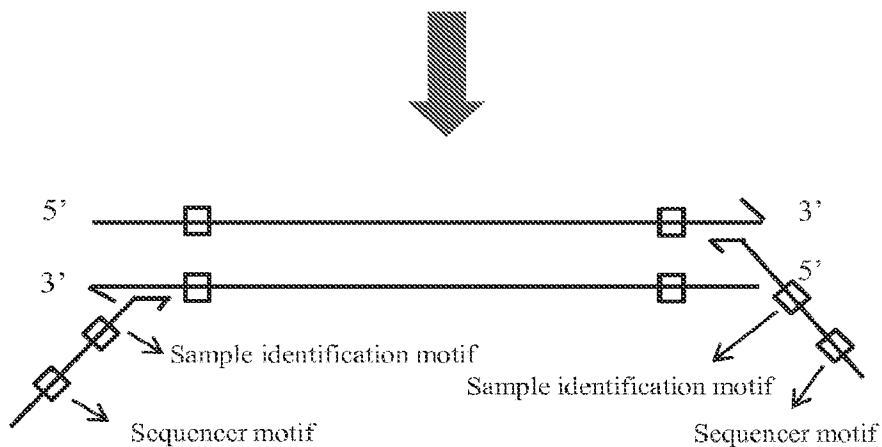


Fig. 9C



Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	GH0004US-C4/42534-708.305

Filed as Large Entity

Filing Fees for Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
UTILITY APPLICATION FILING	1011	1	300	300
UTILITY SEARCH FEE	1111	1	660	660
UTILITY EXAMINATION FEE	1311	1	760	760
REQUEST FOR PRIORITIZED EXAMINATION	1817	1	4000	4000

Pages:

Claims:

CLAIMS IN EXCESS OF 20	1202	10	100	1000
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Miscellaneous-Filing:

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				6860

Electronic Acknowledgement Receipt

EFS ID:	38031557
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	GH0004US-C4/42534-708.305
Receipt Date:	13-DEC-2019
Filing Date:	
Time Stamp:	19:44:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$6860
RAM confirmation Number	E2019BCJ45566205
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	TrackOne Request	2019-12-13_GH0004US-CON4_T1Req.pdf	129107	no	2
			497f2607b9ea5d6e97964ad4bcd221297f9e5134		

Warnings:

Information:

2	Transmittal of New Application	2019-12-13_GH0004US-CON4_AppTrans.pdf	303176	no	2
			06ceb6da9a00d6f826e6c458ea64844fa5aa5a74		

Warnings:

Information:

3	Application Data Sheet	2019-12-13_GH0004US-CON4_ADS.pdf	1874571	no	9
			4bf1f4faafa57cebbb1bc22e9925a273a64ba099		

Warnings:

Information:

4	Oath or Declaration filed	2019-12-13_GH0004US-CON4_ParentDec.pdf	198149	no	2
			9b1fdd215bf3349ea7dfbe0758e33756228765fo		

Warnings:

Information:

5		2019-12-13_GH0004US-CON4_SpecAsFiled.pdf	627997	yes	91
			879472c9971cd242cbd6f796eafaf19a49373f8f		

Multipart Description/PDF files in .zip description

Document Description	Start	End
Abstract	91	91
Claims	86	90
Specification	1	85

Warnings:					
Information:					
6	Drawings-only black and white line drawings	2019-12-13_GH0004US-CON4_Figs.pdf	2550181 97cf1eb2e8fae70ef9de5a96fb471e6f046d9975	no	11
Warnings:					
Information:					
7	Power of Attorney	2019-12-13_GH0004US-CON4_ParentPoA.pdf	190194 ff69efd3f616ea4d4f0569e8551fc9e7bce85453	no	3
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	41626 a5c4f8e204b4c0be7ac0fad4065fbc886106bd49	no	2
Warnings:					
Information:					
Total Files Size (in bytes):				5915001	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

**CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION
UNDER 37 CFR 1.102(e) (Page 1 of 1)**

First Named Inventor:	AmirAli TALASAZ	Nonprovisional Application Number (if known):	
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently \$0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.
2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.
3. The applicable box is checked below:

I. Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

- i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.
- ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, or the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

- i. A request for continued examination has been filed with, or prior to, this form.
- ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
- iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
- iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
- v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature / Timothy A. Hott/	Date 12/13/2019
Name (Print/Typed) Timothy A. Hott	Practitioner Registration Number 67740

Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.*

*Total of _____ forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

Practitioners associated with Customer Number: 115823

OR

Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number

Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

The address associated with Customer Number: 115823

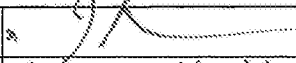
OR

<input type="checkbox"/>	Firm or Individual Name	
	Address	
	City	
	Country	
	Telephone	Email

Assignee
Guardant Health, Inc.
 2686 Middlefield Rd, Suite D
 Redwood City, CA 94063

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/SB/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record
 The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	9/5/13
Name	Helmy Ehtoukhy	Telephone	
Title	CEO		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)Applicant/Patent Owner: Guardant Health, Inc.Application No./Patent No.: 14/861,989 Filed/Issue Date: September 22, 2015Titled: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTSGuardant Health, Inc., a corporation of the State of Delaware

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. The assignee of the entire right, title, and interest.
2. An assignee of less than the entire right, title, and interest (check applicable box):
- The extent (by percentage) of its ownership interest is ___%. Additional Statement(s) by the owners holding the balance of the interest **must be submitted** to account for 100% of the ownership interest.
- There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest **must be submitted** to account for the entire right, title, and interest.

3. The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest **must be submitted** to account for the entire right, title, and interest.

4. The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

- A. An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 037444, Frame 0265, or for which a copy thereof is attached.
- B. A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel __, Frame __, or for which a COPY thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel __, Frame __, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel ____, Frame ____, or for which a COPY thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel ____, Frame ____, or for which a COPY thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel ____, Frame ____, or for which a COPY thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel ____, Frame ____, or for which a COPY thereof is attached. Additional documents in the chain of title are listed on a supplemental sheet(s). As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Ali Alemozafar/ _____

Signature

February 22, 2016 _____

Date

Ali R. Alemozafar _____

Printed or Typed Name

68,180 _____

Title or Registration Number



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

Table with 7 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY. DOCKET NO, TOT CLAIMS, IND CLAIMS. Row 1: 16/714,579, 12/13/2019, 1636, 2720, 42534-708.305, 30, 3

CONFIRMATION NO. 3111

FILING RECEIPT

115823
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304



Date Mailed: 01/09/2020

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

AmirAli TALASAZ, Atherton, CA;
Helmy ELTOUKHY, Atherton, CA;

Applicant(s)

GUARDANT HEALTH, INC., Redwood City, CA;

Power of Attorney: The patent practitioners associated with Customer Number 115823

Domestic Priority data as claimed by applicant

This application is a CON of 16/672,267
which is a CON of 16/601,168 10/14/2019
which is a CON of 15/892,178 02/08/2018
which is a CON of 14/861,989 09/22/2015 PAT 9920366
which is a CON of PCT/US2014/072383 12/24/2014
which claims benefit of 61/948,509 03/05/2014
and claims benefit of 61/921,456 12/28/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the Patent Prosecution Highway program at the USPTO. Please see http://www.uspto.gov for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 01/07/2020

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/714,579**

Projected Publication Date: To Be Determined - pending completion of Corrected Papers

Non-Publication Request: No

Early Publication Request: No

Title

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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Table with 4 columns: APPLICATION NUMBER (16/714,579), FILING OR 371(C) DATE (12/13/2019), FIRST NAMED APPLICANT (AmirAli TALASAZ), ATTY. DOCKET NO./TITLE (42534-708.305)

CONFIRMATION NO. 3111

FORMALITIES LETTER

115823
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304



Date Mailed: 01/09/2020

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Filing Date Granted

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- A substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required. The substitute specification must be submitted with markings and be accompanied by a clean version (without markings) as set forth in 37 CFR 1.125(c) and a statement that the substitute specification contains no new matter (see 37 CFR 1.125(b)). The specification, claims, and/or abstract page(s) submitted is not acceptable and cannot be scanned or properly stored because:
- The line spacing on page(s) 79 of the specification is not 1 1/2 or double spaced (see 37 CFR 1.52(b)). A proper substitute specification in compliance with 37 CFR 1.121(b)(3) and 1.125, having the proper line spacing throughout, is required to satisfy this requirement. A statement that the substitute specification contains no new matter is required. However, a marked up version of the specification is not required for this correction.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

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/elbanaybanay/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli TALASAZ et al.	Confirmation No.: 3111
Serial Number: 16/714,579	Customer No.: 115823
Filing Date: December 13, 2019	Group Art Unit: 1636
Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: To be assigned

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Alexandria, VA 22313-1450

**RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS AND
PRELIMINARY AMENDMENT**

Sir:

This paper is in response to the Notice to File Corrected Application Papers mailed on January 9, 2020. The shortened statutory period for reply expires March 9, 2020; therefore, this response is timely filed. Applicant respectfully requests consideration of the above-referenced application in view of the following amendments and remarks:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 7 of this paper.

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS**CROSS-REFERENCE**

[0001] This application is a continuation of U.S. Application No. 16/672,267, filed November 1, 2019, which is a continuation of U.S. Application No. 16/601,168, filed October 14, 2019, which is a continuation of U.S. Application No. 15/892,178, filed February 8, 2018, which is a continuation of U.S. Application No. 14/861,989, filed September 22, 2015 (now U.S. Patent 9,920,366), which is a continuation application of International Application No. PCT/US2014/072383, filed December 24, 2014, which application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/921,456, filed December 28, 2013, and U.S. Provisional Application No. 61/948,509, filed March 5, 2014, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] The detection and quantification of polynucleotides is important for molecular biology and medical applications, such as diagnostics. Genetic testing is particularly useful for a number of diagnostic methods. For example, disorders that are caused by rare genetic alterations (*e.g.*, sequence variants) or changes in epigenetic markers, such as cancer and partial or complete aneuploidy, may be detected or more accurately characterized with DNA sequence information.

[0003] Early detection and monitoring of genetic diseases, such as cancer, is often useful and needed in the successful treatment or management of the disease. One approach may include the monitoring of a sample derived from cell-free nucleic acids, a population of polynucleotides that can be found in different types of bodily fluids. In some cases, disease may be characterized or detected based on detection of genetic aberrations, such as copy number variation and/or sequence variation of one or more nucleic acid sequences, or the development of other certain rare genetic alterations. Cell-free DNA (cfDNA) may contain genetic aberrations associated with a particular disease. With improvements in sequencing and techniques to manipulate nucleic acids, there is a need in the art for improved methods and systems for using cell-free DNA to detect and monitor disease.

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[0004] In particular, many methods have been developed for accurate copy number variation estimation, especially for heterogeneous genomic samples, such as tumor-derived gDNA or for cfDNA for many applications (*e.g.*, prenatal, transplant, immune, metagenomics or cancer diagnostics). Most of these methods include sample preparation whereby the original nucleic acids are converted into a sequenceable library, followed by massively parallel sequencing, and finally bioinformatics to estimate copy number variation at one or more loci.

SUMMARY

[0005] Although many of these methods are able to reduce or combat the errors introduced by the sample preparation and sequencing processes for all molecules that are converted and sequenced, these methods are not able to infer the counts of molecules that were converted but not sequenced. Since this count of converted by unsequenced molecules can be highly variable from genomic region to region, these counts can dramatically and adversely affect the sensitivity that can be achieved.

[0006] To address this issue, input double-stranded deoxyribonucleic acid (DNA) can be converted by a process that tags both halves of the individual double-stranded molecule, in some cases differently. This can be performed using a variety of techniques, including ligation of hairpin, bubble, or forked adapters or other adaptors having double-stranded and single stranded segments (the unhybridized portion of a bubble, forked or hairpin adapter are deemed single-stranded herein). If tagged correctly, each original Watson and Crick (*i.e.*, strand) side of the input double-stranded DNA molecule can be differently tagged and identified by the sequencer and subsequent bioinformatics. For all molecules in a particular region, counts of molecules where both Watson and Crick sides were recovered (“Pairs”) versus those where only one half was recovered (“Singlets”) can be recorded. The number of unseen molecules can be estimated based on the number of Pairs and Singlets detected.

[0007] An aspect of the present disclosure provides a method for detecting and/or quantifying rare deoxyribonucleic acid (DNA) in a heterogeneous population of original DNA fragments, comprising tagging the original DNA fragments in a single reaction using a library of a plurality of different tags such that greater than 30% of the fragments are tagged at both ends, wherein each of the tags comprises a molecular barcode. The single reaction can be in a single

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reaction vessel. Greater than 50% of the fragments can be tagged at both ends. The plurality of different tags can be no more than any of 100, 500, 1000, 10,000 or 100,000 different tags.

[0008] Another aspect provides a set of library adaptors that can be used to tag the molecules of interest (e.g., by ligation, hybridization, etc.). The set of library adaptors can comprise plurality of polynucleotide molecules with molecular barcodes, wherein the plurality of polynucleotide molecules are less than or equal to 80 nucleotide bases in length, wherein the molecular barcodes are at least 4 nucleotide bases in length, and wherein (a) the molecular barcodes are different from one another and have an edit distance of at least 1 between one another; (b) the molecular barcodes are located at least one nucleotide base away from a terminal end of their respective polynucleotide molecules; (c) optionally, at least one terminal base is identical in all of the polynucleotide molecules; and (d) none of the polynucleotide molecules contains a complete sequencer motif.

[0009] In some embodiments, the library adaptors (or adapters) are identical to one another but for the molecular barcodes. In some embodiments, each of the plurality of library adaptors comprises at least one double-stranded portion and at least one single-stranded portion (e.g., a non-complementary portion or an overhang). In some embodiments, the double-stranded portion has a molecular barcode selected from a collection of different molecular barcodes. In some embodiments, the given molecular barcode is a randomer. In some embodiments, each of the library adaptors further comprises a strand-identification barcode on the at least one single-stranded portion. In some embodiments, the strand-identification barcode includes at least 4 nucleotide bases. In some embodiments, the single-stranded portion has a partial sequencer motif. In some embodiments, the library adaptors do not include a complete sequencer motif.

[0010] In some embodiments, none of the library adaptors contains a sequence for hybridizing to a flow cell or forming a hairpin for sequencing.

[0011] In some embodiments, all of the library adaptors have a terminal end with nucleotide(s) that are the same. In some embodiments, the identical terminal nucleotide(s) are over two or more nucleotide bases in length.

[0012] In some embodiments, each of the library adapters is Y-shaped, bubble shaped or hairpin shaped. In some embodiments, none of the library adapters contains a sample identification motif. In some embodiments, each of the library adapters comprises a sequence

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that is selectively hybridizable to a universal primer. In some embodiments, each of the library adapters comprises a molecular barcode that is at least 5, 6, 7, 8, 9 and 10 nucleotide bases in length. In some embodiments, each of the library adapters is from 10 nucleotide bases to 80 in length, or 30 to 70 nucleotide bases in length, or 40 to 60 nucleotide bases in length. In some embodiments, at least 1, 2, 3, or 4 terminal bases are identical in all of the library adapters. In some embodiments, at least 4 terminal bases are identical in all of the library adapters.

[0013] In some embodiments, the edit distance of the molecular barcodes of the library adapters is a Hamming distance. In some embodiments, the edit distance is at least 1, 2, 3, 4 or 5. In some embodiments, the edit distance is with respect to individual bases of the plurality of polynucleotide molecules. In some embodiments, the molecular barcodes are located at least 10 nucleotide base away from a terminal end of an adapter. In some embodiments, the plurality of library adapters includes at least 2, 4, 6, 8, 10, 20, 30, 40 or 50 different molecular barcodes, or from 2-100, 4-80, 6-60 or 8-40 different molecular barcodes. In any of the embodiments herein, there are more polynucleotides (e.g., cfDNA fragments) to be tagged than there are different molecular barcodes such that the tagging is not unique.

[0014] In some embodiments, the terminal end of an adaptor is configured for ligation (e.g., to a target nucleic acid molecule). In some embodiments, the terminal end of an adaptor is a blunt end.

[0015] In some embodiments, the adaptors are purified and isolated. In some embodiments, the library comprises one or more non-naturally occurring bases.

[0016] In some embodiments, the polynucleotide molecules comprise a primer sequence positioned 5' with respect to the molecular barcodes.

[0017] In some embodiments, the set of library adapters consists essentially of the plurality of polynucleotide molecules.

[0018] In another aspect, a method comprises (a) tagging a collection of polynucleotides with a plurality of polynucleotide molecules from a library of adapters to create a collection of tagged polynucleotides; and (b) amplifying the collection of tagged polynucleotides in the presence of sequencing adaptors, wherein the sequencing adaptors have primers with nucleotide sequences that are selectively hybridizable to complementary sequences in the plurality of polynucleotide molecules. The library of adapters may be as described above or elsewhere

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herein. In some embodiments, each of the sequencer adaptors further comprises an index tag, which can be a sample identification motif.

[0019] Another aspect, provides a method for detecting and/or quantifying rare DNA in a heterogeneous population of original DNA fragments, wherein the rare DNA has a concentration that is less than 1%, the method comprising (a) tagging the original DNA fragments in a single reaction such that greater than 30% of the original DNA fragments are tagged at both ends with library adaptors that comprise molecular barcodes, thereby providing tagged DNA fragments; (b) performing high-fidelity amplification on the tagged DNA fragments; (c) optionally, selectively enriching a subset of the tagged DNA fragments; (d) sequencing one or both strands of the tagged, amplified and optionally selectively enriched DNA fragments to obtain sequence reads comprising nucleotide sequences of the molecular barcodes and at least a portion of the original DNA fragments; (e) from the sequence reads, determining consensus reads that are representative of single-strands of the original DNA fragments; and (f) quantifying the consensus reads to detect and/or quantify the rare DNA at a specificity that is greater than 99.9%.

[0020] In some embodiments, (e) comprises comparing sequence reads having the same or similar molecular barcodes and the same or similar end of fragment sequences. In some embodiments, the comparing further comprises performing a phylogenetic analysis on the sequence reads having the same or similar molecular barcodes. In some embodiments, the molecular barcodes include a barcode having an edit distance of up to 3. In some embodiments, the end of fragment sequence includes fragment sequences having an edit distance of up to 3.

[0021] In some embodiments, the method further comprises sorting sequence reads into paired reads and unpaired reads, and quantifying a number of paired reads and unpaired reads that map to each of one or more genetic loci.

[0022] In some embodiments, the tagging occurs by having an excess amount of library adaptors as compared to original DNA fragments. In some embodiments, the excess is at least a 5-fold excess. In some embodiments, the tagging comprises using a ligase. In some embodiments, the tagging comprises attachment to blunt ends.

[0023] In some embodiments, the method further comprises binning the sequence reads according to the molecular barcodes and sequence information from at least one end of each of the original DNA fragments to create bins of single stranded reads. In some embodiments, the

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method further comprises, in each bin, determining a sequence of a given original DNA fragment among the original DNA fragments by analyzing sequence reads. In some embodiments, the method further comprises detecting and/or quantifying the rare DNA by comparing a number of times each base occurs at each position of a genome represented by the tagged, amplified, and optionally enriched DNA fragments.

[0024] In some embodiments, the library adaptors do not contain complete sequencer motifs. In some embodiments, the method further comprises selectively enriching a subset of the tagged DNA fragments. In some embodiments, the method further comprises, after enriching, amplifying the enriched tagged DNA fragments in the presence of sequencing adaptors comprising primers. In some embodiments, (a) provides tagged DNA fragments having from 2 to 1000 different combinations of molecular barcodes.

[0025] In some embodiments, the DNA fragments are tagged with polynucleotide molecules from a library of adaptors as described above or elsewhere herein.

[0026] In another aspect, a method for processing and/or analyzing a nucleic acid sample of a subject comprises (a) exposing polynucleotide fragments from the nucleic acid sample to a set of library adaptors to generate tagged polynucleotide fragments; and (b) subjecting the tagged polynucleotide fragments to nucleic acid amplification reactions under conditions that yield amplified polynucleotide fragments as amplification products of the tagged polynucleotide fragments. The set of library adaptors comprises a plurality of polynucleotide molecules with molecular barcodes, wherein the plurality of polynucleotide molecules are less than or equal to 80 nucleotide bases in length, wherein the molecular barcodes are at least 4 nucleotide bases in length, and wherein (1) the molecular barcodes are different from one another and have an edit distance of at least 1 between one another; (2) the molecular barcodes are located at least one nucleotide base away from a terminal end of their respective polynucleotide molecules; (3) optionally, at least one terminal base is identical in all of the polynucleotide molecules; and (4) none of the polynucleotide molecules contains a complete sequencer motif.

[0027] In some embodiments, the method further comprises determining nucleotide sequences of the amplified tagged polynucleotide fragments. In some embodiments, the nucleotide sequences of the amplified tagged polynucleotide fragments are determined without polymerase chain reaction (PCR). In some embodiments, the method further comprises

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analyzing the nucleotide sequences with a programmed computer processor to identify one or more genetic variants in the nucleotide sample of the subject. In some embodiments, the one or more genetic variants are selected from the group consisting of base change(s), insertion(s), repeat(s), deletion(s), copy number variation(s) and transversion(s). In some embodiments, the one or more genetic variants include one or more tumor associated genetic alterations.

[0028] In some embodiments, the subject has or is suspected of having a disease. In some embodiments, the disease is cancer. In some embodiments, the method further comprises collecting the nucleic acid sample from the subject. In some embodiments, the nucleic acid sample is collected from a location selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, cerebral spinal fluid and tears of the subject. In some embodiments, the nucleic acid sample is a cell-free nucleic acid sample. In some embodiments, the nucleic acid sample is collected from no more than 100 nanograms (ng) of double-stranded polynucleotide molecules of the subject.

[0029] In some embodiments, the polynucleotide fragments comprise double-stranded polynucleotide molecules. In some embodiments, in (a), the plurality of polynucleotide molecules couple to the polynucleotide fragments via blunt end ligation, sticky end ligation, molecular inversion probes, PCR, ligation-based PCR, multiplex PCR, single stranded ligation, and single stranded circularization. In some embodiments, exposing the polynucleotide fragments of the nucleic acid sample to the plurality of polynucleotide molecules yields the tagged polynucleotide fragments with a conversion efficiency of at least 10%. In some embodiments, any of at least 5%, 6%, 7%, 8%, 9%, 10%, 20%, or 25% of the tagged polynucleotide fragments share a common polynucleotide molecule or sequence. In some embodiments, the method further comprises generating the polynucleotide fragments from the nucleic acid sample.

[0030] In some embodiments, the subjecting comprises amplifying the tagged polynucleotide fragments from sequences corresponding to genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4,

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SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.

[0031] In another aspect, a method comprises (a) generating a plurality of sequence reads from a plurality of polynucleotide molecules, wherein the plurality of polynucleotide molecules cover genomic loci of a target genome, wherein the genomic loci correspond to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1; (b) grouping with a computer processor the plurality of sequence reads into families, wherein each family comprises sequence reads from one of the template polynucleotides; (c) for each of the families, merging sequence reads to generate a consensus sequence; (d) calling the consensus sequence at a given genomic locus among the genomic loci; and (e) detecting at the given genomic locus any of genetic variants among the calls, frequency of a genetic alteration among the calls, total number of calls, and total number of alterations among the calls.

[0032] In some embodiments, each family comprises sequence reads from only one of the template polynucleotides. In some embodiments, the given genomic locus comprises at least one nucleic acid base. In some embodiments, the given genomic locus comprises a plurality of nucleic acid bases. In some embodiments, the calling comprises calling at least one nucleic acid base at the given genomic locus. In some embodiments, the calling comprises calling a plurality of nucleic acid bases at the given genomic locus. In some embodiments, the calling comprises any one of phylogenetic analysis, voting, weighing, assigning a probability to each read at the locus in a family and calling the base with the highest probability.

[0033] In some embodiments, the method further comprises performing (d)-(e) at an additional genomic locus among the genomic loci. In some embodiments, the method further

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comprises determining a variation in copy number at one of the given genomic locus and additional genomic locus based on counts at the given genomic locus and additional genomic locus.

[0034] In some embodiments, the grouping comprises classifying the plurality of sequence reads into families by identifying (i) different molecular barcodes coupled to the plurality of polynucleotide molecules and (ii) similarities between the plurality of sequence reads, wherein each family includes a plurality of nucleic acid sequences that are associated with a different combination of molecular barcodes and similar or identical sequence reads. Different molecular barcodes have different sequences.

[0035] In some embodiments, the consensus sequence is generated by evaluating a quantitative measure or a statistical significance level for each of the sequence reads. In some embodiments, the quantitative measure comprises use of a binomial distribution, exponential distribution, beta distribution, or empirical distribution. In some embodiments, the method further comprises mapping the consensus sequence to the target genome. In some embodiments, the plurality of genes includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50 or all of the plurality of genes selected from the group.

[0036] Another aspect of the present disclosure provides a method, comprising (a) providing template polynucleotide molecules and a set of library adaptors in a single reaction vessel, wherein the library adaptors are polynucleotide molecules that have different molecular barcodes (e.g., from 2 to 1,000 different molecular barcodes), and wherein none of the library adaptors contains a complete sequencer motif; (b) in the single reaction vessel, coupling the library adaptors to the template polynucleotide molecules at an efficiency of at least 10%, thereby tagging each template polynucleotide with a tagging combination that is among a plurality of different tagging combinations (e.g., 4 to 1,000,000 different tagging combinations), to produce tagged polynucleotide molecules; (c) subjecting the tagged polynucleotide molecules to an amplification reaction under conditions that yield amplified polynucleotide molecules as amplification products of the tagged polynucleotide molecules; and (d) sequencing the amplified polynucleotide molecules.

[0037] In some embodiments, the template polynucleotide molecules are blunt ended or sticky-ended. In some embodiments, the library adaptors are identical but for the molecular

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barcodes. In some embodiments, each of the library adaptors has a double stranded portion and at least one single-stranded portion. In some embodiments, the double-stranded portion has a molecular barcode among the molecular barcodes. In some embodiments, each of the library adaptors further comprises a strand-identification barcode on the at least one single-stranded portion. In some embodiments, the single-stranded portion has a partial sequencer motif. In some embodiments, the library adaptors have a sequence of terminal nucleotides that are the same. In some embodiments, the template polynucleotide molecules are double-stranded. In some embodiments, the library adaptors couple to both ends of the template polynucleotide molecules.

[0038] In some embodiments, subjecting the tagged polynucleotide molecules to the amplification reaction comprises non-specifically amplifying the tagged polynucleotide molecules.

[0039] In some embodiments, the amplification reaction comprises use of a priming site to amplify each of the tagged polynucleotide molecules. In some embodiments, the priming site is a primer. In some embodiments, the primer is a universal primer. In some embodiments, the priming site is a nick.

[0040] In some embodiments, the method further comprises, prior to (e), (i) separating polynucleotide molecules comprising one or more given sequences from the amplified polynucleotide molecules, to produce enriched polynucleotide molecules; and (ii) amplifying the enriched polynucleotide molecules with sequencing adaptors.

[0041] In some embodiments, the efficiency is at least 30%, 40%, or 50%. In some embodiments, the method further comprises identifying genetic variants upon sequencing the amplified polynucleotide molecules. In some embodiments, the sequencing comprises (i) subjecting the amplified polynucleotide molecules to an additional amplification reaction under conditions that yield additional amplified polynucleotide molecules as amplification products of the amplified polynucleotide molecules, and (ii) sequencing the additional amplified polynucleotide molecules. In some embodiments, the additional amplification is performed in the presence of sequencing adaptors.

[0042] In some embodiments, (b) and (c) are performed without aliquoting the tagged polynucleotide molecules. In some embodiments, the tagging is non-unique tagging.

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[0043] Another aspect, provides a system for analyzing a target nucleic acid molecule of a subject, comprising a communication interface that receives nucleic acid sequence reads for a plurality of polynucleotide molecules that cover genomic loci of a target genome; computer memory that stores the nucleic acid sequence reads for the plurality of polynucleotide molecules received by the communication interface; and a computer processor operatively coupled to the communication interface and the memory and programmed to (i) group the plurality of sequence reads into families, wherein each family comprises sequence reads from one of the template polynucleotides, (ii) for each of the families, merge sequence reads to generate a consensus sequence, (iii) call the consensus sequence at a given genomic locus among the genomic loci, and (iv) detect at the given genomic locus any of genetic variants among the calls, frequency of a genetic alteration among the calls, total number of calls; and total number of alterations among the calls, wherein the genomic loci correspond to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.

[0044] In another aspect, a set of oligonucleotide molecules that selectively hybridize to at least 5 genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.

[0045] In some embodiments, the oligonucleotide molecules are from 10-200 bases in length. In some embodiments, the oligonucleotide molecules selectively hybridize to exon

SUBSTITUTE SPECIFICATION

regions of the at least 5 genes. In some embodiments, the oligonucleotide molecules selectively hybridize to at least 30 exons in the at least 5 genes. In some embodiments, multiple oligonucleotide molecules selectively hybridize to each of the at least 30 exons. In some embodiments, the oligonucleotide molecules that hybridize to each exon have sequences that overlap with at least 1 other oligonucleotide molecule.

[0046] In another aspect, a kit comprises a first container containing a plurality of library adaptors each having a different molecular barcode; and a second container containing a plurality of sequencing adaptors, each sequencing adaptor comprising at least a portion of a sequencer motif and optionally a sample barcode. The library adaptors can be as described above or elsewhere herein.

[0047] In some embodiments, the sequencing adaptor comprises the sample barcode. In some embodiments, the library adaptors are blunt ended and Y-shaped, and are less than or equal to 80 nucleic acid bases in length. In some embodiments, the sequencing adaptor is up to 70 bases from end to end.

[0048] In another aspect, a method for detecting sequence variants in a cell free DNA sample, comprising detecting rare DNA at a concentration less than 1% with a specificity that is greater than 99.9%.

[0049] In another aspect, a method comprises detecting genetic variants in a sample comprising DNA with a detection limit of at least 1% and specificity greater than 99.9%. In some embodiments, the method further comprises converting cDNA (e.g. cfDNA) into adaptor tagged DNA with a conversion efficiency of at least 30%, 40%, or 50% and reducing sequencing noise (or distortion) by eliminating false positive sequence reads.

[0050] Another aspect provides a method, comprising (a) providing a sample comprising a set of double-stranded polynucleotide molecules, each double-stranded polynucleotide molecule including first and second complementary strands; (b) tagging the double-stranded polynucleotide molecules with a set of duplex tags, wherein each duplex tag differently tags the first and second complementary strands of a double-stranded polynucleotide molecule in the set; (c) sequencing at least some of the tagged strands to produce a set of sequence reads; (d) reducing and/or tracking redundancy in the set of sequence reads; (e) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads

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generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads; (f) determining quantitative measures of (i) the paired reads and (ii) the unpaired reads that map to each of one or more genetic loci; and (g) estimating with a programmed computer processor a quantitative measure of total double-stranded polynucleotide molecules in the set that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads mapping to each locus.

[0051] In some embodiments, the method further comprises (h) detecting copy number variation in the sample by determining a normalized total quantitative measure determined in step (g) at each of the one or more genetic loci and determining copy number variation based on the normalized measure. In some embodiments, the sample comprises double-stranded polynucleotide molecules sourced substantially from cell-free nucleic acids. In some embodiments, the duplex tags are not sequencing adaptors.

[0052] In some embodiments, reducing redundancy in the set of sequence reads comprises collapsing sequence reads produced from amplified products of an original polynucleotide molecule in the sample back to the original polynucleotide molecule. In some embodiments, the method further comprises determining a consensus sequence for the original polynucleotide molecule. In some embodiments, the method further comprises identifying polynucleotide molecules at one or more genetic loci comprising a sequence variant. In some embodiments, the method further comprises determining a quantitative measure of paired reads that map to a locus, wherein both strands of the pair comprise a sequence variant. In some embodiments, the method further comprises determining a quantitative measure of paired molecules in which only one member of the pair bears a sequence variant and/or determining a quantitative measure of unpaired molecules bearing a sequence variant. In some embodiments, the sequence variant is selected from the group consisting of a single nucleotide variant, an indel, a transversion, a translocation, an inversion, a deletion, a chromosomal structure alteration, a gene fusion, a chromosome fusion, a gene truncation, a gene amplification, a gene duplication and a chromosomal lesion.

SUBSTITUTE SPECIFICATION

[0053] Another aspect provides a system comprising a computer readable medium comprising machine-executable code that, upon execution by a computer processor, implements a method comprising (a) receiving into memory a set of sequence reads of polynucleotides tagged with duplex tags; (b) reducing and/or tracking redundancy in the set of sequence reads; (c) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads; (d) determining quantitative measures of (i) the paired reads and (ii) the unpaired reads that map to each of one or more genetic loci; and (e) estimating a quantitative measure of total double-stranded polynucleotide molecules in the set that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads mapping to each locus.

[0054] Another aspect provides a method, comprising (a) providing a sample comprising a set of double-stranded polynucleotide molecules, each double-stranded polynucleotide molecule including first and second complementary strands; (b) tagging the double-stranded polynucleotide molecules with a set of duplex tags, wherein each duplex tag differently tags the first and second complementary strands of a double-stranded polynucleotide molecule in the set; (c) sequencing at least some of the tagged strands to produce a set of sequence reads; (d) reducing and/or tracking redundancy in the set of sequence reads; (e) sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads; and (f) determining quantitative measures of at least two of (i) the paired reads, (ii) the unpaired reads that map to each of one or more genetic loci, (iii) read depth of the paired reads and (iv) read depth of unpaired reads.

SUBSTITUTE SPECIFICATION

[0055] In some embodiments, (f) comprises determining quantitative measures of at least three of (i)-(iv). In some embodiments, (f) comprises determining quantitative measures of all of (i)-(iv). In some embodiments, the method further comprises (g) estimating with a programmed computer processor a quantitative measure of total double-stranded polynucleotide molecules in the set that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads and their read depths mapping to each locus.

[0056] In another aspect, a method comprises (a) tagging control parent polynucleotides with a first tag set to produce tagged control parent polynucleotides, wherein the first tag set comprises a plurality of tags, wherein each tag in the first tag set comprises a same control tag and an identifying tag, and wherein the tag set comprises a plurality of different identifying tags; (b) tagging test parent polynucleotides with a second tag set to produce tagged test parent polynucleotides, wherein the second tag set comprises a plurality of tags, wherein each tag in the second tag set comprises a same test tag that is distinguishable from the control tag and an identifying tag, and wherein the second tag set comprises a plurality of different identifying tags; (c) mixing tagged control parent polynucleotides with tagged test parent polynucleotides to form a pool; (d) amplifying tagged parent polynucleotides in the pool to form a pool of amplified, tagged polynucleotides; (e) sequencing amplified, tagged polynucleotides in the amplified pool to produce a plurality of sequence reads; (f) grouping sequence reads into families, each family comprising sequence reads generated from a same parent polynucleotide, which grouping is optionally based on information from an identifying tag and from start/end sequences of the parent polynucleotides, and, optionally, determining a consensus sequence for each of a plurality of parent polynucleotides from the plurality of sequence reads in a group; (g) classifying each family or consensus sequence as a control parent polynucleotide or as a test parent polynucleotide based on having a test tag or a control tag; (h) determining a quantitative measure of control parent polynucleotides and control test polynucleotides mapping to each of at least two genetic loci; and (i) determining copy number variation in the test parent polynucleotides at at least one locus based on relative quantity of test parent polynucleotides and control parent polynucleotides mapping to the at least one locus.

[0057] In another aspect, a method comprises (a) generating a plurality of sequence reads from a plurality of template polynucleotides, each polynucleotide mapped to a genomic locus;

SUBSTITUTE SPECIFICATION

(b) grouping the sequence reads into families, each family comprising sequence reads generated from one of the template polynucleotides; (c) calling a base (or sequence) at the genomic locus for each of the families; (d) detecting at the genomic locus any of genomic alterations among the calls, frequency of a genetic alteration among the calls, total number of calls and total number of alterations among the calls.

[0058] In some embodiments, calling comprises any of phylogenetic analysis, voting, weighing, assigning a probability to each read at the locus in a family, and calling the base with the highest probability. In some embodiments, the method is performed at two loci, comprising determining CNV at one of the loci based on counts at each of the loci.

[0059] Another aspect provides a method for determining a quantitative measure indicative of a number of individual double-stranded DNA fragments in a sample comprising (a) determining a quantitative measure of individual DNA molecules for which both strands are detected; (b) determining a quantitative measure of individual DNA molecules for which only one of the DNA strands are detected; (c) inferring from (a) and (b) above a quantitative measure of individual DNA molecules for which neither strand was detected; and (d) using (a)-(c) determining the quantitative measure indicative of a number of individual double-stranded DNA fragments in the sample.

[0060] In some embodiments, the method further comprises detecting copy number variation in the sample by determining a normalized quantitative measure determined in step (d) at each of one or more genetic loci and determining copy number variation based on the normalized measure. In some embodiments, the sample comprises double-stranded polynucleotide molecules sourced substantially from cell-free nucleic acids.

[0061] In some embodiments, determining the quantitative measure of individual DNA molecules comprises tagging the DNA molecules with a set of duplex tags, wherein each duplex tag differently tags complementary strands of a double-stranded DNA molecule in the sample to provide tagged strands. In some embodiments, the method further comprises sequencing at least some of the tagged strands to produce a set of sequence reads. In some embodiments, the method further comprises sorting sequence reads into paired reads and unpaired reads, wherein (i) each paired read corresponds to sequence reads generated from a first tagged strand and a second differently tagged complementary strand derived from a double-stranded polynucleotide

SUBSTITUTE SPECIFICATION

molecule in the set, and (ii) each unpaired read represents a first tagged strand having no second differently tag complementary strand derived from a double-stranded polynucleotide molecule represented among the sequence reads in the set of sequence reads. In some embodiments, the method further comprises determining quantitative measures of (i) the paired reads and (ii) the unpaired reads that map to each of one or more genetic loci to determine a quantitative measure of total double-stranded DNA molecules in the sample that map to each of the one or more genetic loci based on the quantitative measure of paired reads and unpaired reads mapping to each locus.

[0062] In another aspect, a method for reducing distortion in a sequencing assay, comprises (a) tagging control parent polynucleotides with a first tag set to produce tagged control parent polynucleotides; (b) tagging test parent polynucleotides with a second tag set to produce tagged test parent polynucleotides; (c) mixing tagged control parent polynucleotides with tagged test parent polynucleotides to form a pool; (d) determining quantities of tagged control parent polynucleotides and tagged test parent polynucleotides; and (e) using the quantities of tagged control parent polynucleotides to reduce distortion in the quantities of tagged test parent polynucleotides.

[0063] In some embodiments, the first tag set comprises a plurality of tags, wherein each tag in the first tag set comprises a same control tag and an identifying tag, and wherein the first tag set comprises a plurality of different identifying tags. In some embodiments, the second tag set comprises a plurality of tags, wherein each tag in the second tag set comprises a same test tag and an identifying tag, wherein the test tag is distinguishable from the control tag, and wherein the second tag set comprises a plurality of different identifying tags. In some embodiments, (d) comprises amplifying tagged parent polynucleotides in the pool to form a pool of amplified, tagged polynucleotides, and sequencing amplified, tagged polynucleotides in the amplified pool to produce a plurality of sequence reads. In some embodiments, the method further comprises grouping sequence reads into families, each family comprising sequence reads generated from a same parent polynucleotide, which grouping is optionally based on information from an identifying tag and from start/end sequences of the parent polynucleotides, and, optionally, determining a consensus sequence for each of a plurality of parent polynucleotides from the plurality of sequence reads in a group.

SUBSTITUTE SPECIFICATION

[0064] In some embodiments, (d) comprises determining copy number variation in the test parent polynucleotides at greater than or equal to one locus based on relative quantity of test parent polynucleotides and control parent polynucleotides mapping to the locus.

[0065] Another aspect provides a method comprising (a) ligating adaptors to double-stranded DNA polynucleotides, wherein ligating is performed in a single reaction vessel, and wherein the adaptors comprise molecular barcodes, to produce a tagged library comprising an insert from the double-stranded DNA polynucleotides, and having between 4 and 1 million different tags; (b) generating a plurality of sequence reads for each of the double-stranded DNA polynucleotides in the tagged library; (c) grouping sequence reads into families, each family comprising sequence reads generated from a single DNA polynucleotide among the double-stranded DNA polynucleotides, based on information in a tag and information at an end of the insert; and (d) calling bases at each position in the double-stranded DNA molecule based on bases at the position in members of a family. In some embodiments, (b) comprises amplifying each of the double-stranded DNA polynucleotide molecules in the tagged library to generate amplification products, and sequencing the amplification products. In some embodiments, the method further comprises sequencing the double-stranded DNA polynucleotide molecules a plurality of times. In some embodiments, (b) comprises sequencing the entire insert. In some embodiments, (c) further comprises collapsing sequence reads in each family to generate a consensus sequence. In some embodiments, (d) comprises calling a plurality of sequential bases from at least a subset of the sequence reads to identify single nucleotide variations (SNV) in the double-stranded DNA molecule.

[0066] Another aspect provides a method of detecting disease cell heterogeneity from a sample comprising polynucleotides from somatic cells and disease cells. The method comprises quantifying polynucleotides in the sample bearing a nucleotide sequence variant at each of a plurality of genetic loci; determining copy number variation (CNV) at each of the plurality of genetic loci, wherein the CNV indicates a genetic dose of a locus in the disease cell polynucleotides; determining with a programmed computer processor a relative measure of quantity of polynucleotides bearing a sequence variant at a locus per the genetic dose at the locus for each of a plurality of the loci; and comparing the relative measures at each of the plurality of loci, wherein different relative measures is indicative of tumor heterogeneity.

SUBSTITUTE SPECIFICATION

[0067] In another aspect, a method comprises subjecting a subject to one or more pulsed therapy cycles, each pulsed therapy cycle comprising (a) a first period during which a drug is administered at a first amount; and (b) a second period during which the drug is administered at a second, reduced amount, wherein (i) the first period is characterized by a tumor burden detected above a first clinical level; and (ii) the second period is characterized by a tumor burden detected below a second clinical level.

[0068] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0069] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0001] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “figure” and “FIG.” herein), of which:

[0070] **FIG. 1** is a flowchart representation of a method of the present disclosure for determining copy number variation (CNV);

[0071] **FIG. 2** depicts mapping of pairs and singlets to Locus A and Locus B in a genome;

[0072] **FIG. 3** shows a reference sequence encoding a genetic Locus A;

[0073] **FIGs. 4A-C** shows amplification, sequencing, redundancy reduction and pairing of complementary molecules;

SUBSTITUTE SPECIFICATION

[0074] FIG. 5 shows increased confidence in detecting sequence variants by pairing reads from Watson and Crick strands;

[0075] FIG. 6 shows a computer system that is programmed or otherwise configured to implement various methods of the present disclosure;

[0076] FIG. 7 is schematic representation of a system for analyzing a sample comprising nucleic acids from a user, including a sequencer; bioinformatic software and internet connection for report analysis by, for example, a hand held device or a desk top computer;

[0077] FIG. 8 is a flowchart representation of a method of this invention for determining CNV using pooled test and control pools; and

[0078] FIGs. 9A-9C schematically illustrate a method for tagging a polynucleotide molecule with a library adaptor and subsequently a sequencing adaptor.

DETAILED DESCRIPTION

[0079] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0080] The term “genetic variant,” as used herein, generally refers to an alteration, variant or polymorphism in a nucleic acid sample or genome of a subject. Such alteration, variant or polymorphism can be with respect to a reference genome, which may be a reference genome of the subject or other individual. Single nucleotide polymorphisms (SNPs) are a form of polymorphisms. In some examples, one or more polymorphisms comprise one or more single nucleotide variations (SNVs), insertions, deletions, repeats, small insertions, small deletions, small repeats, structural variant junctions, variable length tandem repeats, and/or flanking sequences,. Copy number variants (CNVs), transversions and other rearrangements are also forms of genetic variation. A genomic alternation may be a base change, insertion, deletion, repeat, copy number variation, or transversion.

[0081] The term “polynucleotide,” as used herein, generally refers to a molecule comprising one or more nucleic acid subunits. A polynucleotide can include one or more subunits selected from adenosine (A), cytosine (C), guanine (G), thymine (T) and uracil (U), or variants thereof. A

SUBSTITUTE SPECIFICATION

nucleotide can include A, C, G, T or U, or variants thereof. A nucleotide can include any subunit that can be incorporated into a growing nucleic acid strand. Such subunit can be an A, C, G, T, or U, or any other subunit that is specific to one or more complementary A, C, G, T or U, or complementary to a purine (i.e., A or G, or variant thereof) or a pyrimidine (i.e., C, T or U, or variant thereof). A subunit can enable individual nucleic acid bases or groups of bases (*e.g.*, AA, TA, AT, GC, CG, CT, TC, GT, TG, AC, CA, or uracil-counterparts thereof) to be resolved. In some examples, a polynucleotide is deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), or derivatives thereof. A polynucleotide can be single-stranded or double stranded.

[0082] The term “subject,” as used herein, generally refers to an animal, such as a mammalian species (*e.g.*, human) or avian (*e.g.*, bird) species, or other organism, such as a plant. More specifically, the subject can be a vertebrate, a mammal, a mouse, a primate, a simian or a human. Animals include, but are not limited to, farm animals, sport animals, and pets. A subject can be a healthy individual, an individual that has or is suspected of having a disease or a pre-disposition to the disease, or an individual that is in need of therapy or suspected of needing therapy. A subject can be a patient.

[0083] The term “genome” generally refers to an entirety of an organism’s hereditary information. A genome can be encoded either in DNA or in RNA. A genome can comprise coding regions that code for proteins as well as non-coding regions. A genome can include the sequence of all chromosomes together in an organism. For example, the human genome has a total of 46 chromosomes. The sequence of all of these together constitutes a human genome.

[0084] The terms “adaptor(s)”, “adapter(s)” and “tag(s)” are used synonymously throughout this specification. An adaptor or tag can be coupled to a polynucleotide sequence to be “tagged” by any approach including ligation, hybridization, or other approaches.

[0085] The term “library adaptor” or “library adapter” as used herein, generally refers to a molecule (*e.g.*, polynucleotide) whose identity (*e.g.*, sequence) can be used to differentiate polynucleotides in a biological sample (also “sample” herein).

[0086] The term “sequencing adaptor,” as used herein, generally refers to a molecule (*e.g.*, polynucleotide) that is adapted to permit a sequencing instrument to sequence a target polynucleotide, such as by interacting with the target polynucleotide to enable sequencing. The sequencing adaptor permits the target polynucleotide to be sequenced by the sequencing

SUBSTITUTE SPECIFICATION

instrument. In an example, the sequencing adaptor comprises a nucleotide sequence that hybridizes or binds to a capture polynucleotide attached to a solid support of a sequencing system, such as a flow cell. In another example, the sequencing adaptor comprises a nucleotide sequence that hybridizes or binds to a polynucleotide to generate a hairpin loop, which permits the target polynucleotide to be sequenced by a sequencing system. The sequencing adaptor can include a sequencer motif, which can be a nucleotide sequence that is complementary to a flow cell sequence of other molecule (e.g., polynucleotide) and usable by the sequencing system to sequence the target polynucleotide. The sequencer motif can also include a primer sequence for use in sequencing, such as sequencing by synthesis. The sequencer motif can include the sequence(s) needed to couple a library adaptor to a sequencing system and sequence the target polynucleotide.

[0087] As used herein the terms “at least”, “at most” or “about”, when preceding a series, refers to each member of the series, unless otherwise identified.

[0088] The term “about” and its grammatical equivalents in relation to a reference numerical value can include a range of values up to plus or minus 10% from that value. For example, the amount “about 10” can include amounts from 9 to 11. In other embodiments, the term “about” in relation to a reference numerical value can include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[0089] The term “at least” and its grammatical equivalents in relation to a reference numerical value can include the reference numerical value and greater than that value. For example, the amount “at least 10” can include the value 10 and any numerical value above 10, such as 11, 100, and 1,000.

[0090] The term “at most” and its grammatical equivalents in relation to a reference numerical value can include the reference numerical value and less than that value. For example, the amount “at most 10” can include the value 10 and any numerical value under 10, such as 9, 8, 5, 1, 0.5, and 0.1.

[0091] **1. Methods for processing and/or analyzing a nucleic acid sample**

[0092] An aspect of the present disclosure provides methods for determining a genomic alternation in a nucleic acid sample of a subject. **FIG. 1** shows a method of determining copy

SUBSTITUTE SPECIFICATION

number variation (CNV). The method can be implemented to determine other genomic alternations, such as SNVs.

[0093] A. Polynucleotide Isolation

[0094] Methods disclosed herein can comprise isolating one or more polynucleotides. A polynucleotide can comprise any type of nucleic acid, for example, a sequence of genomic nucleic acid, or an artificial sequence (*e.g.*, a sequence not found in genomic nucleic acid). For example, an artificial sequence can contain non-natural nucleotides. Also, a polynucleotide can comprise both genomic nucleic acid and an artificial sequence, in any portion. For example, a polynucleotide can comprise 1 to 99% of genomic nucleic acid and 99% to 1% of artificial sequence, where the total adds up to 100%. Thus, fractions of percentages are also contemplated. For example, a ratio of 99.1% to 0.9% is contemplated.

[0095] A polynucleotide can comprise any type of nucleic acids, such as DNA and/or RNA. For example, if a polynucleotide is DNA, it can be genomic DNA, complementary DNA (cDNA), or any other deoxyribonucleic acid. A polynucleotide can also be cell-free DNA (cfDNA). For example, the polynucleotide can be circulating DNA. The circulating DNA can comprise circulating tumor DNA (ctDNA). A polynucleotide can be double-stranded or single-stranded. Alternatively, a polynucleotide can comprise a combination of a double-stranded portion and a single-stranded portion.

[0096] Polynucleotides do not have to be cell-free. In some cases, the polynucleotides can be isolated from a sample. For example, in step (102) (**FIG. 1**), double-stranded polynucleotides are isolated from a sample. A sample can be any biological sample isolated from a subject. For example, a sample can comprise, without limitation, bodily fluid, whole blood, platelets, serum, plasma, stool, red blood cells, white blood cells or leucocytes, endothelial cells, tissue biopsies, synovial fluid, lymphatic fluid, ascites fluid, interstitial or extracellular fluid, the fluid in spaces between cells, including gingival crevicular fluid, bone marrow, cerebrospinal fluid, saliva, mucous, sputum, semen, sweat, urine, or any other bodily fluids. A bodily fluid can include saliva, blood, or serum. For example, a polynucleotide can be cell-free DNA isolated from a bodily fluid, *e.g.*, blood or serum. A sample can also be a tumor sample, which can be obtained from a subject by various approaches, including, but not limited to, venipuncture, excretion,

SUBSTITUTE SPECIFICATION

ejaculation, massage, biopsy, needle aspirate, lavage, scraping, surgical incision, or intervention or other approaches.

[0097] A sample can comprise various amount of nucleic acid that contains genome equivalents. For example, a sample of about 30 ng DNA can contain about 10,000 (10^4) haploid human genome equivalents and, in the case of cfDNA, about 200 billion (2×10^{11}) individual polynucleotide molecules. Similarly, a sample of about 100 ng of DNA can contain about 30,000 haploid human genome equivalents and, in the case of cfDNA, about 600 billion individual molecules.

[0098] A sample can comprise nucleic acids from different sources. For example, a sample can comprise germline DNA or somatic DNA. A sample can comprise nucleic acids carrying mutations. For example, a sample can comprise DNA carrying germline mutations and/or somatic mutations. A sample can also comprise DNA carrying cancer-associated mutations (*e.g.*, cancer-associated somatic mutations).

[0099] **B. Tagging**

[00100] Polynucleotides disclosed herein can be tagged. For example, in step (104) (**FIG. 1**) the double-stranded polynucleotides are tagged with duplex tags, tags that differently label the complementary strands (*i.e.*, the “Watson” and “Crick” strands) of a double-stranded molecule. In one embodiment the duplex tags are polynucleotides having complementary and non-complementary portions.

[00101] Tags can be any types of molecules attached to a polynucleotide, including, but not limited to, nucleic acids, chemical compounds, florescent probes, or radioactive probes. Tags can also be oligonucleotides (*e.g.*, DNA or RNA). Tags can comprise known sequences, unknown sequences, or both. A tag can comprise random sequences, pre-determined sequences, or both. A tag can be double-stranded or single-stranded. A double-stranded tag can be a duplex tag. A double-stranded tag can comprise two complementary strands. Alternatively, a double-stranded tag can comprise a hybridized portion and a non-hybridized portion. The double-stranded tag can be Y-shaped, *e.g.*, the hybridized portion is at one end of the tag and the non-hybridized portion is at the opposite end of the tag. One such example are the “Y adapters” used in Illumina sequencing. Other examples include hairpin shaped adapters or bubble shaped

SUBSTITUTE SPECIFICATION

adapters. Bubble shaped adapters have non-complementary sequences flanked on both sides by complementary sequences.

[00102] Tagging disclosed herein can be performed using any method. A polynucleotide can be tagged with an adaptor by hybridization. For example, the adaptor can have a nucleotide sequence that is complementary to at least a portion of a sequence of the polynucleotide. As an alternative, a polynucleotide can be tagged with an adaptor by ligation.

[00103] For example, tagging can comprise using one or more enzymes. The enzyme can be a ligase. The ligase can be a DNA ligase. For example, the DNA ligase can be a T4 DNA ligase, *E. coli* DNA ligase, and/or mammalian ligase. The mammalian ligase can be DNA ligase I, DNA ligase III, or DNA ligase IV. The ligase can also be a thermostable ligase. Tags can be ligated to a blunt-end of a polynucleotide (blunt-end ligation). Alternatively, tags can be ligated to a sticky end of a polynucleotide (sticky-end ligation). Efficiency of ligation can be increased by optimizing various conditions. Efficiency of ligation can be increased by optimizing the reaction time of ligation. For example, the reaction time of ligation can be less than 12 hours, *e.g.*, less than 1, less than 2, less than 3, less than 4, less than 5, less than 6, less than 7, less than 8, less than 9, less than 10, less than 11, less than 12, less than 13, less than 14, less than 15, less than 16, less than 17, less than 18, less than 19, or less than 20 hours. In a particular example, reaction time of ligation is less than 20 hours. Efficiency of ligation can be increased by optimizing the ligase concentration in the reaction. For example, the ligase concentration can be at least 10, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 400, at least 500, or at least 600 unit/microliter. Efficiency can also be optimized by adding or varying the concentration of an enzyme suitable for ligation, enzyme cofactors or other additives, and/or optimizing a temperature of a solution having the enzyme. Efficiency can also be optimized by varying the addition order of various components of the reaction. The end of tag sequence can comprise dinucleotide to increase ligation efficiency. When the tag comprises a non-complementary portion (*e.g.*, Y-shaped adaptor), the sequence on the complementary portion of the tag adaptor can comprise one or more selected sequences that promote ligation efficiency. Preferably such sequences are located at the terminal end of the tag. Such sequences can comprise 1, 2, 3, 4, 5, or 6 terminal bases. Reaction solution with high viscosity (*e.g.*, a low Reynolds number) can also be used to increase ligation efficiency. For example, solution can

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have a Reynolds number less than 3000, less than 2000, less than 1000, less than 900, less than 800, less than 700, less than 600, less than 500, less than 400, less than 300, less than 200, less than 100, less than 50, less than 25, or less than 10. It is also contemplated that roughly unified distribution of fragments (*e.g.*, tight standard deviation) can be used to increase ligation efficiency. For example, the variation in fragment sizes can vary by less than 20%, less than 15%, less than 10%, less than 5%, or less than 1%. Tagging can also comprise primer extension, for example, by polymerase chain reaction (PCR). Tagging can also comprise any of ligation-based PCR, multiplex PCR, single strand ligation, or single strand circularization.

[00104] In some instances, the tags herein comprise molecular barcodes. Such molecular barcodes can be used to differentiate polynucleotides in a sample. Preferably molecular barcodes are different from one another. For example, molecular barcodes can have a difference between them that can be characterized by a predetermined edit distance or a Hamming distance. In some instances, the molecular barcodes herein have a minimum edit distance of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. To further improve efficiency of conversion (*e.g.*, tagging) of untagged molecular to tagged molecules, one preferably utilizes short tags. For example, in some embodiments, a library adapter tag can be up to 65, 60, 55, 50, 45, 40, or 35 nucleotide bases in length. A collection of such short library barcodes preferably includes a number of different molecular barcodes, *e.g.*, at least 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20 different barcodes with a minimum edit distance of 1, 2, 3 or more.

[00105] Thus, a collection of molecules can include one or more tags. In some instances, some molecules in a collection can include an identifying tag (“identifier”) such as a molecular barcode that is not shared by any other molecule in the collection. For example, in some instances of a collection of molecules, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the molecules in the collection

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can include an identifier or molecular barcode that is not shared by any other molecule in the collection. As used herein, a collection of molecules is considered to be “uniquely tagged” if each of at least 95% of the molecules in the collection bears an identifier that is not shared by any other molecule in the collection (“unique tag” or “unique identifier”). A collection of molecules is considered to be “non-uniquely tagged” if each of at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least or about 50% of the molecules in the collection bears an identifying tag or molecular barcode that is shared by at least one other molecule in the collection (“non-unique tag” or “non-unique identifier”). Accordingly, in a non-uniquely tagged population no more than 1% of the molecules are uniquely tagged. For example, in a non-uniquely tagged population, no more than 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the molecules can be uniquely tagged.

[00106] A number of different tags can be used based on the estimated number of molecules in a sample. In some tagging methods, the number of different tags can be at least the same as the estimated number of molecules in the sample. In other tagging methods, the number of different tags can be at least two, three, four, five, six, seven, eight, nine, ten, one hundred or one thousand times as many as the estimated number of molecules in the sample. In unique tagging, at least two times (or more) as many different tags can be used as the estimated number of molecules in the sample.

[00107] The molecules in the sample may be non-uniquely tagged. In such instances a fewer number of tags or molecular barcodes is used than the number of molecules in the sample to be tagged. For example, no more than 100, 50, 40, 30, 20 or 10 unique tags or molecular barcodes are used to tag a complex sample such as a cell free DNA sample with many more different fragments.

[00108] The polynucleotide to be tagged can be fragmented, such as either naturally or using other approaches, such as, for example, shearing. The polynucleotides can be fragmented by certain methods, including but not limited to, mechanical shearing, passing the sample through a syringe, sonication, heat treatment (*e.g.*, for 30 minutes at 90°C), and/or nuclease treatment (*e.g.*, using DNase, RNase, endonuclease, exonuclease, and/or restriction enzyme).

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[00109] The polynucleotides fragments (prior to tagging) can comprise sequences of any length. For example, polynucleotide fragments (prior to tagging) can comprise at least 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000 or more nucleotides in length. The polynucleotide fragment are preferably about the average length of cell-free DNA. For example, the polynucleotide fragments can comprise about 160 bases in length. The polynucleotide fragment can also be fragmented from a larger fragment into smaller fragments about 160 bases in length.

[00110] Polynucleotides tagged can comprise sequences associated with cancer. The cancer-associated sequences can comprise single nucleotide variation (SNV), copy number variation (CNV), insertions, deletions, and/or rearrangements.

[00111] The polynucleotides can comprise sequences associated with cancer, such as acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, Kaposi Sarcoma, anal cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma, malignant fibrous histiocytoma, brain stem glioma, brain cancer, craniopharyngioma, ependymoblastoma, ependymoma, medulloblastoma, medulloepithelioma, pineal parenchymal tumor, breast cancer, bronchial tumor, Burkitt lymphoma, Non-Hodgkin lymphoma, carcinoid tumor, cervical cancer, chordoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), colon cancer, colorectal cancer, cutaneous T-cell lymphoma, ductal carcinoma in situ, endometrial cancer, esophageal cancer, Ewing Sarcoma, eye cancer, intraocular melanoma, retinoblastoma, fibrous histiocytoma, gallbladder cancer, gastric cancer, glioma, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, kidney cancer, laryngeal cancer, lip cancer, oral cavity cancer, lung cancer, non-small cell carcinoma, small cell carcinoma, melanoma, mouth cancer, myelodysplastic syndromes, multiple myeloma, medulloblastoma, nasal cavity cancer, paranasal sinus cancer, neuroblastoma, nasopharyngeal cancer, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary tumor, plasma

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cell neoplasm, prostate cancer, rectal cancer, renal cell cancer, rhabdomyosarcoma, salivary gland cancer, Sezary syndrome, skin cancer, nonmelanoma, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, testicular cancer, throat cancer, thymoma, thyroid cancer, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, and/or Wilms Tumor.

[00112] A haploid human genome equivalent has about 3 picograms of DNA. A sample of about 1 microgram of DNA contains about 300,000 haploid human genome equivalents.

Improvements in sequencing can be achieved as long as at least some of the duplicate or cognate polynucleotides bear unique identifiers with respect to each other, that is, bear different tags. However, in certain embodiments, the number of tags used is selected so that there is at least a 95% chance that all duplicate molecules starting at any one position bear unique identifiers. For example, in a sample comprising about 10,000 haploid human genome equivalents of fragmented genomic DNA, *e.g.*, cfDNA, z is expected to be between 2 and 8. Such a population can be tagged with between about 10 and 100 different identifiers, for example, about 2 identifiers, about 4 identifiers, about 9 identifiers, about 16 identifiers, about 25 identifiers, about 36 different identifiers, about 49 different identifiers, about 64 different identifiers, about 81 different identifiers, or about 100 different identifiers.

[00113] Nucleic acid barcodes having identifiable sequences including molecular barcodes, can be used for tagging. For example, a plurality of DNA barcodes can comprise various numbers of sequences of nucleotides. A plurality of DNA barcodes having 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more identifiable sequences of nucleotides can be used. When attached to only one end of a polynucleotide, the plurality of DNA barcodes can produce 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more different identifiers. Alternatively, when attached to both ends of a polynucleotide, the plurality DNA barcodes can produce 4, 9, 16, 25, 36, 49, 64, 81, 100, 121, 144, 169, 196, 225, 256, 289, 324, 361, 400 or more different identifiers (which is the 2 of when the DNA barcode is attached to only 1 end of a polynucleotide). In one example, a plurality of DNA barcodes having 6, 7, 8, 9 or 10 identifiable sequences of nucleotides can be used. When attached to both ends of a polynucleotide, they produce 36, 49, 64, 81 or 100 possible different identifiers, respectively. In

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a particular example, the plurality of DNA barcodes can comprise 8 identifiable sequences of nucleotides. When attached to only one end of a polynucleotide, the plurality of DNA barcodes can produce 8 different identifiers. Alternatively, when attached to both ends of a polynucleotide, the plurality of DNA barcodes can produce 64 different identifiers. Samples tagged in such a way can be those with a range of about 10 ng to any of about 100 ng, about 1 µg, about 10 µg of fragmented polynucleotides, *e.g.*, genomic DNA, *e.g.*, cfDNA.

[00114] A polynucleotide can be uniquely identified in various ways. A polynucleotide can be uniquely identified by a unique DNA barcode. For example, any two polynucleotides in a sample are attached two different DNA barcodes. Alternatively, a polynucleotide can be uniquely identified by the combination of a DNA barcode and one or more endogenous sequences of the polynucleotide. For example, any two polynucleotides in a sample can be attached the same DNA barcode, but the two polynucleotides can still be identified by different endogenous sequences. The endogenous sequence can be on an end of a polynucleotide. For example, the endogenous sequence can be adjacent (*e.g.*, base in between) to the attached DNA barcode. In some instances the endogenous sequence can be at least 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 bases in length. Preferably, the endogenous sequence is a terminal sequence of the fragment/polynucleotides to be analyzed. The endogenous sequence may be the length of the sequence. For example, a plurality of DNA barcodes comprising 8 different DNA barcodes can be attached to both ends of each polynucleotide in a sample. Each polynucleotide in the sample can be identified by the combination of the DNA barcodes and about 10 base pair endogenous sequence on an end of the polynucleotide. Without being bound by theory, the endogenous sequence of a polynucleotide can also be the entire polynucleotide sequence.

[00115] Also disclosed herein are compositions of tagged polynucleotides. The tagged polynucleotide can be single-stranded. Alternatively, the tagged polynucleotide can be double-stranded (*e.g.*, duplex-tagged polynucleotides). Accordingly, this invention also provides compositions of duplex-tagged polynucleotides. The polynucleotides can comprise any types of nucleic acids (DNA and/or RNA). The polynucleotides comprise any types of DNA disclosed herein. For example, the polynucleotides can comprise DNA, *e.g.*, fragmented DNA or cfDNA. A set of polynucleotides in the composition that map to a mappable base position in a genome can be non-uniquely tagged, that is, the number of different identifiers can be at least 2 and fewer

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than the number of polynucleotides that map to the mappable base position. The number of different identifiers can also be at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and fewer than the number of polynucleotides that map to the mappable base position.

[00116] In some instances, as a composition goes from about 1 ng to about 10 µg or higher, a larger set of different molecular barcodes can be used. For example, between 5 and 100 different library adaptors can be used to tag polynucleotides in a cfDNA sample.

[00117] The systems and methods disclosed herein may be used in applications that involve the assignment of molecular barcodes. The molecular barcodes can be assigned to any types of polynucleotides disclosed in this invention. For example, the molecular barcodes can be assigned to cell-free polynucleotides (*e.g.*, cfDNAs). Often, an identifier disclosed herein can be a barcode oligonucleotide that is used to tag the polynucleotide. The barcode identifier may be a nucleic acid oligonucleotide (*e.g.*, a DNA oligonucleotide). The barcode identifier can be single-stranded. Alternatively, the barcode identifier can be double-stranded. The barcode identifier can be attached to polynucleotides using any method disclosed herein. For example, the barcode identifier can be attached to the polynucleotide by ligation using an enzyme. The barcode identifier can also be incorporated into the polynucleotide through PCR. In other cases, the reaction may comprise addition of a metal isotope, either directly to the analyte or by a probe labeled with the isotope. Generally, assignment of unique or non-unique identifiers or molecular barcodes in reactions of this disclosure may follow methods and systems described by, for example, U.S. patent applications 2001/0053519, 2003/0152490, 2011/0160078 and U.S. Patent No. 6,582,908, each of which is entirely incorporated herein by reference.

[00118] Identifiers or molecular barcodes used herein may be completely endogenous whereby circular ligation of individual fragments may be performed followed by random shearing or targeted amplification. In this case, the combination of a new start and stop point of the molecule and the original intramolecular ligation point can form a specific identifier.

[00119] Identifiers or molecular barcodes used herein can comprise any types of oligonucleotides. In some cases, identifiers may be predetermined, random, or semi-random sequence oligonucleotides. Identifiers can be barcodes. For example, a plurality of barcodes may be used such that barcodes are not necessarily unique to one another in the plurality.

SUBSTITUTE SPECIFICATION

Alternatively, a plurality of barcodes may be used such that each barcode is unique to any other barcode in the plurality. The barcodes can comprise specific sequences (*e.g.*, predetermined sequences) that can be individually tracked. Further, barcodes may be attached (*e.g.*, by ligation) to individual molecules such that the combination of the barcode and the sequence it may be ligated to creates a specific sequence that may be individually tracked. As described herein, detection of barcodes in combination with sequence data of beginning (start) and/or end (stop) portions of sequence reads can allow assignment of a unique identity to a particular molecule. The length or number of base pairs of an individual sequence read may also be used to assign a unique identity to such a molecule. As described herein, fragments from a single strand of nucleic acid having been assigned a unique identity, may thereby permit subsequent identification of fragments from the parent strand. In this way the polynucleotides in the sample can be uniquely or substantially uniquely tagged. A duplex tag can include a degenerate or semi-degenerate nucleotide sequence, *e.g.*, a random degenerate sequence. The nucleotide sequence can comprise any number of nucleotides. For example, the nucleotide sequence can comprise 1 (if using a non-natural nucleotide), 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more nucleotides. In a particular example, the sequence can comprise 7 nucleotides. In another example, the sequence can comprise 8 nucleotides. The sequence can also comprise 9 nucleotides. The sequence can comprise 10 nucleotides.

[00120] A barcode can comprise contiguous or non-contiguous sequences. A barcode that comprises at least 1, 2, 3, 4, 5 or more nucleotides is a contiguous sequence or non-contiguous sequence. If the 4 nucleotides are uninterrupted by any other nucleotide. For example, if a barcode comprises the sequence TTGC, a barcode is contiguous if the barcode is TTGC. On the other hand, a barcode is non-contiguous if the barcode is TTXGC, where X is a nucleic acid base.

[00121] An identifier or molecular barcode can have an n-mer sequence which may be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more nucleotides in length. A tag herein can comprise any range of nucleotides in length. For example, the sequence can be between 2 to 100, 10 to 90, 20 to 80, 30 to 70, 40 to 60, or about 50 nucleotides in length.

SUBSTITUTE SPECIFICATION

[00122] The tag can comprise a double-stranded fixed reference sequence downstream of the identifier or molecular barcode. Alternatively, the tag can comprise a double-stranded fixed reference sequence upstream or downstream of the identifier or molecular barcode. Each strand of a double-stranded fixed reference sequence can be, for example, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 nucleotides in length.

[00123] C. Adaptors

[00124] A library of polynucleotide molecules can be synthesized for use in sequencing. For example, a library of polynucleotides comprising a plurality of polynucleotide molecules that are each less than or equal to 100, 90, 80, 70, 60, 50, 45, 40, or 35 nucleic acid (or nucleotide) bases in length can be made. A plurality of polynucleotide molecules can be each less than or equal to 35 nucleic acid bases in length. A plurality of polynucleotide molecules can be each less than or equal to 30 nucleic acid bases in length. A plurality of polynucleotide molecules can also be less than or equal to 250, 200, 150, 100, or 50 nucleic acid bases. Additionally, the plurality of polynucleotide molecules can also be less than or equal to 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleic acid bases.

[00125] A library of polynucleotides comprising a plurality of polynucleotide molecules can also have distinct (with respect to each other) molecular barcode sequences (or molecular barcodes) with respect to at least 4 nucleic acid bases. A molecular barcode (also “barcode” or “identifier” herein) sequence is a nucleotide sequence that distinguishes one polynucleotide from another. In other embodiments, the polynucleotide molecules can also have different barcode sequences with respect to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more nucleic acid bases.

[00126] A library of polynucleotides comprising a plurality of polynucleotide molecules can also have a plurality of different barcode sequences. For example, a plurality of polynucleotide molecules can have at least 4 different molecular barcode sequences. In some cases, the plurality

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of polynucleotide molecules has from 2-100, 4-50, 4-30, 4-20, or 4-10 different molecular barcode sequences. The plurality of polynucleotides molecules can also have other ranges of different barcode sequences such as, 1-4, 2-5, 3-6, 4-7, 5-8, 6-9, 7-10, 8-11, 9-12, 10-13, 11-14, 12-15, 13-16, 14-17, 15-18, 16-19, 17-20, 18-21, 19-22, 20-23, 21-24, or 22-25 different barcode sequences. In other cases, a plurality of polynucleotide molecules can have at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 more different barcode sequences. In a particular example, the plurality library adapters comprise at least 8 different sequences.

[00127] The location of the different barcode sequences can vary within the plurality of polynucleotides. For example, the different barcode sequences can be within 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, or 2 nucleic acid bases from a terminal end of a respective one of the plurality of polynucleotide molecules. In an example, a plurality of polynucleotide molecules has distinct barcode sequences that are within 10 nucleic acid bases from the terminal end. In another example, a plurality of polynucleotide molecules has distinct barcode sequences that are within 5 or 1 nucleic acid bases from the terminal end. In other instances, the distinct barcode sequences can be at the terminal end of a respective one of the plurality of polynucleotide molecules. Other variations include that the distinct molecular barcode sequences can be within 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, or more nucleic acid bases from a terminal end of a respective one of the plurality of polynucleotide molecules.

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[00128] The terminal end of the plurality of polynucleotide molecules can be adapted for ligation to a target nucleic acid molecule. For example, the terminal end can be a blunt end. In some other cases, the terminal end is adapted for hybridization to a complementary sequence of a target nucleic acid molecule.

[00129] A library of polynucleotides comprising a plurality of polynucleotide molecules can also have an edit distance of at least 1. In some cases, the edit distance is with respect to individual bases of the plurality of polynucleotide molecules. In other cases, the plurality of polynucleotide molecules can have an edit distance of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more. The edit distance can be a Hamming distance.

[00130] In some cases, the plurality of polynucleotides does not contain sequencing adaptors. A sequence adaptor can be a polynucleotide that comprises a sequence that hybridizes to one or more sequencing adaptors or primers. A sequencing adaptor can further comprise a sequence hybridizing to a solid support, *e.g.*, a flow cell sequence. The term “flow cell sequence” and its grammatical equivalents as used herein, refers to a sequence that permits hybridization to a substrate, for example, by way of a primer attached to the substrate. The substrate can be bead or a planar surface. In some embodiments, a flow cell sequence can allow a polynucleotide to attach to a flow cell or surface (*e.g.*, surface of a bead, for example, an Illumina flow cell).

[00131] When a plurality of polynucleotide molecules does not contain sequencing adaptors or primers, each polynucleotide molecule of the plurality does not contain a nucleic acid sequence or other moiety that is adapted to permit sequencing of a target nucleic acid molecule with a given sequencing approach, such as Illumina, SOLiD, Pacific Biosciences, GeneReader, Oxford Nanopore, Complete Genomics, Gnu-Bio, Ion Torrent, Oxford Nanopore or Genia. In some examples, when a plurality of polynucleotide molecules does not contain sequencing adaptors or primers, the plurality of polynucleotide molecules does not contain flow cell sequences. For example, the plurality of polynucleotide molecules cannot bind to flow cells, such as used in Illumina flow cell sequencers. However, these flow cell sequences, if desired, can be added to the plurality of polynucleotide molecules by methods such as PCR amplification or ligation. At this point, Illumina flow cell sequencers can be used. Alternatively, when the

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plurality of polynucleotide molecules does not contain sequencing adaptors or primers, the plurality of polynucleotide molecules does not contain hairpin shaped adaptors or adaptors for generating hairpin loops in a target nucleic acid molecule, such as Pacific Bioscience SMRTbell™ adaptors. However, these hairpin shaped adaptors, if desired, can be added to the plurality of polynucleotide molecules by methods such as PCR amplification or ligation. The plurality of polynucleotide molecules can be circular or linear.

[00132] A plurality of polynucleotide molecules can be double stranded. In some cases, the plurality of polynucleotide molecules can be single stranded, or can comprise hybridized and non-hybridized regions. A plurality of polynucleotide molecules can be non-naturally occurring polynucleotide molecules.

[00133] Adaptors can be polynucleotide molecules. The polynucleotide molecules can be Y-shaped, bubble-shaped or hairpin-shaped. A hairpin adaptor may contain a restriction site(s) or a Uracil containing base. Adaptors can comprise a complementary portion and a non-complementary portion. The non-complementary portion can have an edit distance (*e.g.*, Hamming distance). For example, the edit distance can be at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30. The complementary portion of the adaptor can comprise sequences that are selected to enable and/or promote ligation to a polynucleotide, *e.g.*, a sequence to enable and/or promote ligation to a polynucleotide at a high yield.

[00134] A plurality of polynucleotide molecules as disclosed herein can be purified. In some cases, a plurality of polynucleotide molecules as disclosed herein can be isolated polynucleotide molecules. In other cases, a plurality of polynucleotide molecules as disclosed herein can be purified and isolated polynucleotide molecules.

[00135] In certain aspects, each of the plurality of polynucleotide molecules is Y-shaped or hairpin-shaped. Each of the plurality of polynucleotide molecules can comprise a different barcode. The different barcode can be a randomer in the complementary portion (*e.g.*, double stranded portion) of the Y-shaped or hairpin-shaped adaptor. Alternatively, the different barcode can be in one strand of the non-complementary portion (*e.g.*, one of the Y-shaped arms). As

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discussed above, the different barcode can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more (or any length as described throughout) nucleic acid bases, *e.g.*, 7 bases. The barcode can be contiguous or non-contiguous sequences, as described above. The plurality of polynucleotide molecules is from 10 nucleic acid bases to 35 nucleic acid bases (or any length as described above) in length. Further, the plurality of polynucleotide molecules can comprise an edit distance (as described above), that is a Hamming distance. A plurality of polynucleotide molecules can have distinct barcode sequences that are within 10 nucleic acid bases from the terminal end.

[00136] In another aspect, a plurality of polynucleotide molecules can be sequencing adaptors. A sequencing adaptor can comprise a sequence hybridizing to one or more sequencing primers. A sequencing adaptor can further comprise a sequence hybridizing to a solid support, *e.g.*, a flow cell sequence. For example, a sequencing adaptor can be a flow cell adaptor. The sequencing adaptors can be attached to one or both ends of a polynucleotide fragment. In another example, a sequencing adaptor can be hairpin shaped. For example, the hairpin shaped adaptor can comprise a complementary double-stranded portion and a loop portion, where the double-stranded portion can be attached (*e.g.*, ligated) to a double-stranded polynucleotide. Hairpin shaped sequencing adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times. A sequencing adaptor can be up to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more bases from end to end. For example, a sequencing adaptor can be up to 70 bases from end to end. The sequencing adaptor can comprise 20-30, 20-40, 30-50, 30-60, 40-60, 40-70, 50-60, 50-70, bases from end to end. In a particular example, the sequencing adaptor can comprise 20-30 bases from end to end. In another example, the sequencing adaptor can comprise 50-60 bases from end to end. A sequencing adaptor can comprise one or more barcodes. For example, a sequencing adaptor can comprise a sample barcode. The sample barcode can comprise a pre-determined sequence. The sample barcodes can be used to identify the source of the polynucleotides. The sample barcode can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,

SUBSTITUTE SPECIFICATION

or more (or any length as described throughout) nucleic acid bases, *e.g.*, at least 8 bases. The barcode can be contiguous or non-contiguous sequences, as described above.

[00137] The plurality of polynucleotide molecules as described herein can be used as adaptors. Adaptors can comprise one or more identifiers. An adaptor can comprise an identifier with a random sequence. Alternatively, an adaptor can comprise an identifier with pre-determined sequences. Some adaptors can comprise an identifier with a random sequence and another identifier with a pre-determined sequence. The adaptors comprising identifiers can be double-stranded or single-stranded adaptors. The adaptors comprising identifiers can be Y-shaped adaptors. A Y-shaped adaptor can comprise one or more identifiers with a random sequence. The one or more identifiers can be on the hybrid portion and/or non-hybridized portion of the Y-shaped adaptor. A Y-shaped adaptor can comprise one or more identifiers with a pre-determined sequence. The one or more identifiers with pre-determined sequence can be on the hybridized portion and/or non-hybridized portion of the Y-shaped adaptor. A Y-shaped adaptor can comprise one or more identifiers with a random sequence and one or more identifiers with a pre-determined sequence. For example, the one or more identifiers with a random sequence can be on the hybridized portion of the Y-shaped adaptor and/or the non-hybridized portion of the Y-shaped adaptor. The one or more identifiers with a pre-determined sequence can be on the hybridized portion of the Y-shaped adaptor and/or the non-hybridized portion of the Y-shaped adaptor. In a particular example, a Y-shaped adaptor can comprise an identifier with a random sequence on its hybridized portion and an identifier with a pre-determined sequence on its non-hybridized portion. The identifiers can be in any length disclosed herein. For example, a Y-shaped adaptor can comprise an identifier with a random sequence of 7 nucleotides on its hybridized portion and an identifier with a pre-determined sequence of 8 nucleotides on its non-hybridized portion.

[00138] An adaptor can include a double-stranded portion with a molecular barcode and at least one or two single-stranded portion. For example, the adaptor can be Y-shaped and include a double-stranded portion and two single-stranded portions. The single-stranded portions can include sequences that are not complementary to one another.

[00139] The adaptor can include a terminal end that has a sequence that is selected to permit the adaptor to be efficiently (*e.g.*, at an efficiency of at least about 20%, 30%, 40%, 50%) ligated

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or otherwise coupled to a polynucleotide. In some examples, terminal nucleotides in a double-stranded portion of an adaptor are selected from a combination of purines and pyrimidines to provide for efficient ligation.

[00140] In some examples, a set of library adaptors comprises a plurality of polynucleotide molecules (library adaptors) with molecular barcodes. The library adaptors are less than or equal to 80, 70, 60, 50, 45, or 40 nucleotide bases in length. The molecular barcodes can be at least 4 nucleotide bases in length, but may be from 4 to 20 nucleotide bases in length. The molecular barcodes can be different from one another and have an edit distance of at least 1, 2, 3, 4, or 5 between one another. The molecular barcodes are located at least 1, 2, 3, 4, 5, 10, or 20 nucleotide bases away from a terminal end of their respective library adaptors. In some cases, the at least one terminal base is identical in all of the library adaptors.

[00141] The library adaptors can be identical but for the molecular barcodes. For example, the library adaptors can have identical sequences but differ only with respect to nucleotide sequences of the molecular barcodes.

[00142] Each of the library adaptors can have a double stranded portion and at least one single-stranded portion. By “single stranded portion” is meant an area of non-complementarity or an overhang. In some cases, each of the library adaptors has a double-stranded portion and two single-stranded portions. The double-stranded portion can have a molecular barcode. In some cases, the molecular barcode is a randomer. Each of the library adaptors can further include a strand-identification barcode on a single-stranded portion. The strand-identification barcode can include at least 4 nucleotide bases, in some cases from 4 to 20 nucleotide bases.

[00143] In some examples, each of the library adaptors has a double-stranded portion with a molecular barcode and two single-stranded portions. The single-stranded portions may not hybridize to one another. The single-stranded portions may not be completely complementary to one another.

[00144] The library adaptors can have a sequence of terminal nucleotides in a double-stranded portion that are the same. The sequence of terminal nucleotides can be at least 2, 3, 4, 5 or 6 nucleotide bases in length. For example, one strand of a double-stranded portion of the library adaptor can have the sequence ACTT, TCGC, or TACC at the terminal end, while the other strand can have a complementary sequence. In some cases, such a sequence is selected to

SUBSTITUTE SPECIFICATION

optimize the efficiency at which the library adaptors ligate to target polynucleotides. Such sequences can be selected to optimize a binding interaction between the ends of the library adaptors and the target polynucleotides.

[00145] In some cases, none of the library adaptors contains a sample identification motif (or sample molecular barcode). Such sample identification motif can be provided via sequencing adaptors. A sample identification motif can include a sequencer of at least 4, 5, 6, 7, 8, 9, 10, 20, 30, or 40 nucleotide bases that permits the identification of polynucleotide molecules from a given sample from polynucleotide molecules from other samples. For example, this can permit polynucleotide molecules from two subjects to be sequenced in the same pool and sequence reads for the subjects subsequently identified.

[00146] A sequencer motif includes nucleotide sequence(s) needed to couple a library adaptor to a sequencing system and sequence a target polynucleotide coupled to the library adaptor. The sequencer motif can include a sequence that is complementary to a flow cell sequence and a sequence (sequencing initiation sequence) that is selectively hybridizable to a primer (or priming sequence) for use in sequencing. For example, such sequencing initiation sequence can be complementary to a primer that is employed for use in sequence by synthesis (e.g., Illumina). Such primer can be included in a sequencing adaptor. A sequencing initiation sequence can be a primer hybridization site.

[00147] In some cases, none of the library adaptors contains a complete sequencer motif. The library adaptors can contain partial or no sequencer motifs. In some cases, the library adaptors include a sequencing initiation sequence. The library adaptors can include a sequencing initiation sequence but no flow cell sequence. The sequence initiation sequence can be complementary to a primer for sequencing. The primer can be a sequence specific primer or a universal primer. Such sequencing initiation sequences may be situated on single-stranded portions of the library adaptors. As an alternative, such sequencing initiation sequences may be priming sites (e.g., kinks or nicks) to permit a polymerase to couple to the library adaptors during sequencing.

[00148] In some cases, partial or complete sequencer motifs are provided by sequencing adaptors. A sequencing adaptor can include a sample molecular barcode and a sequencer motif. The sequencing adaptors can be provided in a set that is separate from the library adaptors. The

SUBSTITUTE SPECIFICATION

sequencing adaptors in a given set can be identical – i.e., they contain the same sample barcode and sequencer motif.

[00149] Sequencing adaptors can include sample identification motifs and sequencer motifs. Sequencer motifs can include primers that are complementary to a sequencing initiation sequence. In some cases, sequencer motifs also include flow cell sequences or other sequences that permit a polynucleotide to be configured or arranged in a manner that permits the polynucleotide to be sequenced by a sequencer.

[00150] Library adaptors and sequencing adaptors can each be partial adaptors, that is, containing part but not all of the sequences necessary to enable sequencing by a sequencing platform. Together they provide complete adaptors. For example, library adaptors can include partial or no sequencer motifs, but such sequencer motifs are provided by sequencing adaptors.

[00151] **FIGs. 9A-9C** schematically illustrate a method for tagging a target polynucleotide molecule with library adaptors. **FIG. 9A** shows a library adaptor as a partial adaptor containing a primer hybridization site on one of the strands and a molecular barcode towards another end. The primer hybridization site can be a sequencing initiation sequence for subsequent sequencing. The library adaptor is less than or equal to 80 nucleotide bases in length. In **FIG. 9B**, the library adaptors are ligated at both ends of the target polynucleotide molecule to provide a tagged target polynucleotide molecule. The tagged target polynucleotide molecule may be subjected to nucleic acid amplification to generate copies of the target. Next, in **FIG. 9C**, sequencing adaptors containing sequencer motifs are provided and hybridized to the tagged target polynucleotide molecule. The sequencing adaptors contain sample identification motifs. The sequencing adaptors can contain sequences to permit sequencing of the tagged target with a given sequencer.

[00152] **D. Sequencing**

[00153] Tagged polynucleotides can be sequenced to generate sequence reads (*e.g.*, as shown in step (106), **FIG. 1**). For example, a tagged duplex polynucleotide can be sequenced. Sequence reads can be generated from only one strand of a tagged duplex polynucleotide. Alternatively, both strands of a tagged duplex polynucleotide can generate sequence reads. The two strands of the tagged duplex polynucleotide can comprise the same tags. Alternatively, the two strands of the tagged duplex polynucleotide can comprise different tags. When the two

SUBSTITUTE SPECIFICATION

strands of the tagged duplex polynucleotide are differently tagged, sequence reads generated from one strand (*e.g.*, a Watson strand) can be distinguished from sequence reads generated from the other strands (*e.g.*, a Crick strand). Sequencing can involve generating multiple sequence reads for each molecule. This occurs, for example, as a result the amplification of individual polynucleotide strands during the sequencing process, *e.g.*, by PCR.

[00154] Methods disclosed herein can comprise amplifying of polynucleotides.

Polynucleotides amplification can result in the incorporation of nucleotides into a nucleic acid molecule or primer thereby forming a new nucleic acid molecule complementary to a template nucleic acid. The newly formed polynucleotide molecule and its template can be used as templates to synthesize additional polynucleotides. The polynucleotides being amplified can be any nucleic acids, for example, deoxyribonucleic acids, including genomic DNAs, cDNAs (complementary DNA), cfDNAs, and circulating tumor DNAs (ctDNAs). The polynucleotides being amplified can also be RNAs. As used herein, one amplification reaction may comprise many rounds of DNA replication. DNA amplification reactions can include, for example, polymerase chain reaction (PCR). One PCR reaction may comprise 2-100 “cycles” of denaturation, annealing, and synthesis of a DNA molecule. For example, 2-7, 5-10, 6-11, 7-12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, or 15-20 cycles can be performed during the amplification step. The condition of the PCR can be optimized based on the GC content of the sequences, including the primers.

[00155] Nucleic acid amplification techniques can be used with the assays described herein. Some amplification techniques are the PCR methodologies which can include, but are not limited to, solution PCR and *in situ* PCR. For example, amplification may comprise PCR-based amplification. Alternatively, amplification may comprise non PCR-based amplification. Amplification of the template nucleic acid may comprise use of one or more polymerases. For example, the polymerase may be a DNA polymerase or an RNA polymerase. In some cases, high fidelity amplification is performed such as with the use of high fidelity polymerase (*e.g.*, Phusion® High-Fidelity DNA Polymerase) or PCR protocols. In some cases, the polymerase may be a high fidelity polymerase. For example, the polymerase may be KAPA HiFi DNA polymerase. The polymerase may also be Phusion DNA polymerase. The polymerase may be

SUBSTITUTE SPECIFICATION

used under reaction conditions that reduce or minimize amplification biases, e.g., due to fragment length, GC content, etc.

[00156] Amplification of a single strand of a polynucleotide by PCR will generate copies both of that strand and its complement. During sequencing, both the strand and its complement will generate sequence reads. However, sequence reads generated from the complement of, for example, the Watson strand, can be identified as such because they bear the complement of the portion of the duplex tag that tagged the original Watson strand. In contrast, a sequence read generated from a Crick strand or its amplification product will bear the portion of the duplex tag that tagged the original Crick strand. In this way, a sequence read generated from an amplified product of a complement of the Watson strand can be distinguished from a complement sequence read generated from an amplification product of the Crick strand of the original molecule.

[00157] All amplified polynucleotides can be submitted to a sequencing device for sequencing. Alternatively, a sampling, or subset, of all of the amplified polynucleotides is submitted to a sequencing device for sequencing. With respect to any original double-stranded polynucleotide there can be three results with respect to sequencing. First, sequence reads can be generated from both complementary strands of the original molecule (that is, from both the Watson strand and from the Crick strand). Second, sequence reads can be generated from only one of the two complementary strands (that is, either from the Watson strand or from the Crick strand, but not both). Third, no sequence read may be generated from either of the two complementary strands. Consequently, counting unique sequence reads mapping to a genetic locus will underestimate the number of double-stranded polynucleotides in the original sample mapping to the locus. Described herein are methods of estimating the unseen and uncounted polynucleotides.

[00158] The sequencing method can be massively parallel sequencing, that is, simultaneously (or in rapid succession) sequencing any of at least 100, 1000, 10,000, 100,000, 1 million, 10 million, 100 million, or 1 billion polynucleotide molecules. Sequencing methods may include, but are not limited to: high-throughput sequencing, pyrosequencing, sequencing-by-synthesis, single-molecule sequencing, nanopore sequencing, semiconductor sequencing, sequencing-by-ligation, sequencing-by-hybridization, RNA-Seq (Illumina), Digital Gene Expression (Helicos), Next generation sequencing, Single Molecule Sequencing by Synthesis (SMSS)(Helicos),

SUBSTITUTE SPECIFICATION

massively-parallel sequencing, Clonal Single Molecule Array (Solexa), shotgun sequencing, Maxam-Gilbert or Sanger sequencing, primer walking, sequencing using PacBio, SOLiD, Ion Torrent, or Nanopore platforms and any other sequencing methods known in the art.

[00159] For example, duplex-tagged polynucleotides can be amplified, by for example PCR (*see e.g.*, **FIG. 4A** duplex-tagged polynucleotides are referred to as mm' and nn'). In **FIG. 4A**, the strand of the duplex polynucleotide including sequence m bears sequence tags w and y , while the strand of the duplex polynucleotide including sequence m' bears sequence tags x and z . Similarly, the strand of the duplex polynucleotide including sequence n bears sequence tags a and c , while the strand of the duplex polynucleotide including sequence n' bears sequence tags b and d . During amplification, each strand produces itself and its complementary sequence. However, for example, an amplification progeny of original strand m that includes the complementary sequence, m' , is distinguishable from an amplification progeny of original strand m' because the progeny from original strand m will have the sequence $5'-y'm'w'-3'$ and the progeny of the original m' strand one strand will have the sequence $5'-zm'x-3'$. **FIG. 4B** shows amplification in more detail. During amplification, errors can be introduced into the amplification progeny, represented by dots. The application progeny are sampled for sequencing, so that not all strands produce sequence reads, resulting in the sequence reads indicated. Because sequence reads can come from either of a strand or its complement, both sequences and complement sequences will be included in the set of sequence reads. It should be noted that it is possible that a polynucleotide would bear the same tag on each end. Thus, for a tag “a”, and polynucleotide “m”, a first strand could be tagged $a-m-a'$, and the complement could be tagged $a-m'-a$.

[00160] E. **Determining consensus sequence reads**

[00161] Methods disclosed herein can comprise determining consensus sequence reads in sequence reads (*e.g.*, as shown in step (108), **FIG. 1**), such as by reducing or tracking redundancy. Sequencing of amplified polynucleotides can produce reads of the several amplification products from the same original polynucleotide, referred to as “redundant reads”. By identifying redundant reads, unique molecules in the original sample can be determined. If the molecules in a sample are uniquely tagged, then reads generated from amplification of a

SUBSTITUTE SPECIFICATION

single unique original molecule can be identified based on their distinct barcode. Ignoring barcodes, reads from unique original molecules can be determined based on sequences at the beginning and end of a read, optionally in combination with the length of the read. In certain cases, however, a sample may be expected to have a plurality of original molecules having the same start stop sequences and the same length. Without barcoding, these molecules are difficult to distinguish from one another. However, if a collection of polynucleotides is non-uniquely tagged (that is, an original molecule shares the same identifier with at least one other original molecule), combining information from a barcode with start/stop sequence and/or polynucleotide length significantly increases the probability that any sequence read can be traced back to an original polynucleotide. This is because, in part, even without unique tagging, it is unlikely that any two original polynucleotides having the same start/stop sequence and length also will be tagged with the same identifier.

[00162] F. Collapsing

[00163] Collapsing allows for reduction in noise (*i.e.*, background) that is generated at each step of the process. Methods disclosed herein can comprise collapsing, *e.g.*, generating a consensus sequence by comparing multiple sequence reads. For example, sequence reads generated from a single original polynucleotide can be used to generate a consensus sequence of that original polynucleotide. Iterative rounds of amplification can introduce errors into progeny polynucleotides. Also, sequencing typically may not be performed with perfect fidelity so sequencing errors are introduced at this stage as well. However, comparison of sequence reads of molecules derived from a single original molecule, including those that have sequence variants, can be analyzed so as to determine the original, or “consensus” sequence. This can be done phylogenetically. Consensus sequences can be generated from families of sequence reads by any of a variety of methods. Such methods include, for example, linear or non-linear methods of building consensus sequences (such as voting (*e.g.*, biased voting), averaging, statistical, maximum a posteriori or maximum likelihood detection, dynamic programming, Bayesian, hidden Markov or support vector machine methods, etc.) derived from digital communication theory, information theory, or bioinformatics. For example, if all or most of the sequence reads tracking back to an original molecule bear the same sequence variant, that variant probably existed in the original molecule. On the other hand, if a sequence variant exists in a subset of

SUBSTITUTE SPECIFICATION

redundant sequence reads, that variant may have been introduced during amplification/sequencing and represents an artifact not existing in the original. Furthermore, if only sequence reads derived from the Watson or Crick strand of an original polynucleotide contain the variant, the variant may have been introduced through single-sided DNA damage, first-cycle PCR error or through contaminating polynucleotides that were amplified from a different sample.

[00164] After fragments are amplified and the sequences of amplified fragments are read and aligned, the fragments are subjected to base calling, *e.g.*, determining for each locus the most likely nucleotide. However, variations in the number of amplified fragments and unseen amplified fragments (*e.g.*, those without being read their sequences; reasons could be too many such as amplification errors, sequencing reading errors, too long, too short, being chopped, etc.) may introduce errors in base calling. If there are too many unseen amplified fragments with respect to the seen amplified fragments (amplified fragments actually being read), the reliability of base calling may be diminished.

[00165] Therefore, disclosed herein is a method to correct for the number of unseen fragments in base calling. For example, when base calling for locus A (an arbitrary locus), it is first assumed that there are N amplified fragments. The sequence readouts can come from two types of fragments: double-strand fragments and single-strand fragments. Therefore, we assign N_1 , N_2 , and N_3 as the numbers of double-strands, single-strands, and unseen fragments, respectively. Thus, $N=N_1+N_2+N_3$ (N_1 and N_2 are known from the sequence readouts, and N and N_3 are unknown). If the formula is solved for N (or N_3), then N_3 (or N) will be inferred.

[00166] Probability is used to estimate N . For example, we assign “ p ” to be the probability of having detected (or having read) a nucleotide of locus A in a sequence readout of a single-strand.

[00167] For sequence readouts from double-strands, the nucleotide call from a double-strand amplified fragment has a probability of $p * p=p^2$, seeing all N_1 double-strands has the following equation: $N_1=N * (p^2)$.

[00168] For sequence readouts from a single-strand. Assuming that one of the 2 strands is seen, and the other is unseen, the probability of seeing one strand is “ p ”, but the probability of missing the other strand is $(1-p)$. Furthermore, by not distinguishing the single strand sourcing from 5-primer and sourcing from 3-primer, there is a factor of 2. Therefore, the nucleotide call

SUBSTITUTE SPECIFICATION

from a single-strand amplified fragment has a probability $2 \times p \times (1-p)$. Thus, seeing all N_2 single-strands has the following equation: $N_2 = N \times 2 \times p \times (1-p)$.

[00169] “p” is also unknown. To solve p, the ratio of N_1 to N_2 is used to solve for “p”:

$$R = \frac{N_1}{N_2} = \frac{Np^2}{2Np(1-p)} = \frac{p^2}{2p(1-p)} = \frac{p}{2(1-p)}$$

Once “p” is found, N can be found. After N is found, can be found $N_3 = N - N_1 - N_2$.

[00170] Besides the ratio of paired versus unpaired strands (which is a measure post-collapsing), there is useful information in the pre-collapsing read depth at each locus. This information can be used to further improve the call for total molecule count and/or increase confidence of calling variants.

[00171] For example, **FIG. 4C** demonstrates sequence reads corrected for complementary sequences. Sequences generated from an original Watson strand or an original Crick strand can be differentiated on the basis of their duplex tags. Sequences generated from the same original strand can be grouped. Examination of the sequences can allow one to infer the sequence of the original strand (the “consensus sequence”). In this case, for example, the sequence variant in the nn' molecule is included in the consensus sequence because it included in every sequence read while other variants are seen to be stray errors. After collapsing sequences, original polynucleotide pairs can be identified based on their complementary sequences and duplex tags.

[00172] **FIG. 5** demonstrates increased confidence in detecting sequence variants by pairing reads from Watson and Crick strands. Sequence nn' can include a sequence variant indicated by a dot. In some cases, sequence pp' does not include a sequence variant. Amplification, sequencing, redundancy reduction and pairing can result in both Watson and Crick strands of the same original molecule including the sequence variant. In contrast, as a result of errors introduced during amplification and sampling during sequencing, the consensus sequence of the Watson strand p can contain a sequence variant, while the consensus sequence of the Crick strand p' does not. It is less likely that amplification and sequencing will introduce the same variant into both strands (nn' sequence) of a duplex than onto one strand (pp' sequence). Therefore, the variant in the pp' sequence is more likely to be an artifact, and the variant in the nn' sequence is more likely to exist in the original molecule.

SUBSTITUTE SPECIFICATION

[00173] Methods disclosed herein can be used to correct errors resulted from experiments, *e.g.*, PCR, amplification, and/or sequencing. For example, such a method can comprises attaching one or more double stranded adaptors to both ends of a double stranded polynucleotide, thereby providing a tagged double stranded polynucleotide; amplifying the double stranded tagged polynucleotide; sequencing both strands of the tagged polynucleotide; comparing the sequence of one strand with its complement to determine any errors introduced during sequencing; and correcting errors in the sequence based on (d). The adaptors used in this method can be any adaptors disclosed herein, *e.g.*, Y-shaped adaptors. The adaptor can comprise any barcodes (*e.g.*, distinct barcodes) disclosed herein.

[00174] **G. Mapping**

[00175] Sequence reads or consensus sequences can be mapped to one or more selected genetic loci (*e.g.*, as shown step (110), **FIG. 1**). A genetic locus can be, for example, a specific nucleotide position in the genome, a sequence of nucleotides (for example, an open reading frame), a fragment of a chromosome, a whole chromosome, or an entire genome. A genetic locus can be a polymorphic locus. Polymorphic locus can be a locus at which sequence variation exists in the population and/or exists in a subject and/or a sample. A polymorphic locus can be generated by two or more distinct sequences coexisting at the same location of the genome. The distinct sequences can differ from one another by one or more nucleotide substitutions, a deletion/insertion, and/or a duplication of any number of nucleotides, generally a relatively small number of nucleotides, such as less than 50, 45, 40, 35, 30, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide(s), among others. A polymorphic locus can be created by a single nucleotide position that varies within the population, *e.g.* a single nucleotide variation (SNV) or a single nucleotide polymorphism (SNP).

[00176] A reference genome for mapping can include the genome of any species of interest. Human genome sequences useful as references can include the hg19 assembly or any previous or available hg assembly. Such sequences can be interrogated using the genome browser available at genome.ucsc.edu/index.html. Other species genomes include, for example PanTro2 (chimp) and mm9 (mouse).

[00177] In methods disclosed herein, collapsing can be performed before or after mapping. In some aspects, collapsing can be performed before mapping. For example, sequence reads can be

SUBSTITUTE SPECIFICATION

grouped into families based on their tags and one or more endogenous sequences, without regard to where the reads map in the genome. Then, the members of a family can be collapsed into a consensus sequence. The consensus sequence can be generated using any collapsing method disclosed herein. Then the consensus sequence can be mapped to locations in the genome. Reads mapped to a locus can be quantified (*e.g.*, counted). Percentage of reads carrying a mutation at a locus can also be determined. Alternatively, collapsing can be performed after mapping. For example, all reads can first be mapped to the genome. Then the reads can be grouped into families based on their tags and one or more endogenous sequences. Since the reads have been mapped to the genome, consensus bases can be determined for each family at each locus. In other aspects, consensus sequence can be generated for one strand of a DNA molecule (*e.g.*, for a Watson strand or a Crick strand). Mapping can be performed before or after the consensus sequence for one strand of the DNA molecule is determined. Numbers of Doublets and Singlets can be determined. These numbers can be used to calculate unseen molecules. For example, the unseen molecules can be calculated using the following equation: $N = D + S + U$; $D = Np(2)$, $S = N2pq$, where $p = 1 - q$, where p is the probability of seeing; q is the probability of missing a strand.

[00178] H. Grouping

[00179] Methods disclosed herein can also comprise grouping sequence reads. Sequence reads can be grouped based on various types of sequences, *e.g.*, sequences of an oligonucleotide tag (*e.g.*, a barcode), sequence of a polynucleotide fragments, or any combinations. For example, as shown in step (112) (**FIG. 1**), sequence reads can be grouped as follows: Sequence reads generated from a “Watson” strand and those generated from a “Crick” strand of a double-stranded polynucleotide in the sample are identifiable based on the duplex tags that they bear. In this way, a sequence read or consensus sequence from a Watson strand of a duplex polynucleotide can be paired with a sequence read or consensus sequence from its complementary Crick strand. Paired sequence reads are referred to as a “Pair”.

[00180] Sequence reads for which no sequence read corresponding to a complementary strand can be found among the sequence reads are termed “Singlets”.

[00181] Double-stranded polynucleotides for which a sequence read for neither of the two complementary strands has been generated are referred to as “Unseen” molecules.

SUBSTITUTE SPECIFICATION

[00182] I. Quantifying

[00183] Methods disclosed herein also comprise quantifying sequence reads. For example, as shown in step (114) (**FIG. 1**), Pairs and Singlets mapping to a selected genetic locus, or to each of a plurality of selected genetic loci, are quantified, *e.g.*, counted.

[00184] The quantifying can comprise estimating number of polynucleotides in the sample (*e.g.*, Pairs polynucleotides, Singlets polynucleotides, or Unseen polynucleotides. For example, as shown in step (116) (**FIG. 1**), the number of double-stranded polynucleotides in the sample for which no sequence reads were generated (“Unseen” polynucleotides) is estimated. The probability that a double strand polynucleotide generates no sequence reads can be determined based on the relative number of Pairs and Singlets at any locus. Using this probability, the number of Unseen polynucleotide can be estimated.

[00185] In step (118) an estimate for the total number of double-stranded polynucleotides in a sample mapping to a selected locus is the sum of the number of Pairs, the number of Singlets and the number of Unseen molecules mapping to the locus.

[00186] The number of Unseen original molecules in a sample can be estimated based on the relative number of Pairs and Singlets (**FIG. 2**). Referring to **FIG. 2**, as an example, counts for a particular genomic locus, Locus A, are recorded, where 1000 molecules are paired and 1000 molecules are unpaired. Assuming a uniform probability, p , for an individual Watson or Crick strand to make it through the process subsequent to conversion, one can calculate the proportion of molecules that fail to make it through the process (Unseen) as follows: Let R = ratio of paired to unpaired molecules = 1, so $R=1=p^2/(2p(1-p))$. This implies that $p=2/3$ and that the quantity of lost molecules is equal to $(1-p)^2 = 1/9$. Thus in this example, approximately 11% of converted molecules are lost and never detected. Consider another genomic locus, Locus B, in the same sample where 1440 molecules are paired and 720 are unpaired. Using the same method, we can infer the number of molecules that are lost, is only 4%. Comparing the two areas, it may be assumed that Locus A had 2000 unique molecules as compared to 2160 molecules in Locus B – a difference of almost 8%. However, by correctly adding in the lost molecules in each region, we infer there are $2000/(8/9)=2250$ molecules in Locus A and $2160/.96=2250$ molecules in Locus B. Hence, the counts in both regions are actually equal. This correction and thus much higher sensitivity can be achievable by converting the original double-stranded nucleic acid

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molecules and bioinformatically keeping track of all those that are paired and unpaired at the end of the process. Similarly, the same procedure can be used to infer true copy number variations in regions that appear to have similar counts of observed unique molecules. By taking the number of unseen molecules into consideration in the two or more regions, the copy number variation becomes apparent.

[00187] In addition to using binomial distribution, other methods of estimating numbers of unseen molecules include exponential, beta, gamma or empirical distributions based on the redundancy of sequence reads observed. In the latter case, the distribution of read counts for paired and unpaired molecules can be derived from such redundancy to infer the underlying distribution of original polynucleotide molecules at a particular locus. This can often lead to a better estimation of the number of unseen molecules.

[00188] J. CNV Detection

[00189] Methods disclosed herein also comprise detecting CNV. For example, as shown in step (120) (**FIG. 1**), once the total number of polynucleotides mapping to a locus is determined, this number can be used in standard methods of determining CNV at the locus. A quantitative measure can be normalized against a standard. The standard can be an amount of any polynucleotides. In one method, a quantitative measure at a test locus can be standardized against a quantitative measure of polynucleotides mapping to a control locus in the genome, such as gene of known copy number. Quantitative measures can be compared against the amount of nucleic acid in any sample disclosed herein. For example, in another method, the quantitative measure can be compared against the amount of nucleic acid in the original sample. For example, if the original sample contained 10,000 haploid gene equivalents, the quantitative measure can be compared against an expected measure for diploidy. In another method, the quantitative measure can be normalized against a measure from a control sample, and normalized measures at different loci can be compared.

[00190] In some cases, in which copy number variation analysis is desired, sequence data may be: 1) aligned with a reference genome; 2) filtered and mapped; 3) partitioned into windows or bins of sequence; 4) coverage reads counted for each window; 5) coverage reads can then be normalized using a stochastic or statistical modeling algorithm; 6) and an output file can be generated reflecting discrete copy number states at various positions in the genome. In other

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cases, in which rare mutation analysis is desired, sequence data may be 1) aligned with a reference genome; 2) filtered and mapped; 3) frequency of variant bases calculated based on coverage reads for that specific base; 4) variant base frequency normalized using a stochastic, statistical or probabilistic modeling algorithm; 5) and an output file can be generated reflecting mutation states at various positions in the genome.

[00191] After the sequence read coverage ratios have been determined, a stochastic modeling algorithm can be optionally applied to convert the normalized ratios for each window region into discrete copy number states. In some cases, this algorithm may comprise a Hidden Markov Model. In other cases, the stochastic model may comprise dynamic programming, support vector machine, Bayesian modeling, probabilistic modeling, trellis decoding, Viterbi decoding, expectation maximization, Kalman filtering methodologies, or neural networks.

[00192] Methods disclosed herein can comprise detecting SNVs, CNVs, insertions, deletions, and/or rearrangements at a specific region in a genome. The specific genomic region can comprise a sequence in a gene, such as ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, or NTRK1.

[00193] In some cases, the method uses a panel which comprises exons of one or more genes. The panel can comprise introns of one or more genes as well. The panel can also comprise exons and introns of one or more genes. The one or more genes can be those disclosed above. The panel can comprise about 80,000 bases which cover a panel of genes. The panel can comprise about 1000, 2000, 3000, 4000, 5000, 10000, 15000, 20000, 25000, 30000, 35000, 40000, 45000, 50000, 55000, 60000, 65000, 70000, 75000, 80000, 85000, 90000, 95000, 100000, 105000, 110000, 115000, 120000, 125000, or more bases.

[00194] In some aspects, copy number of a gene can be reflected in the frequency of a genetic form of the gene in a sample. For example, in a healthy individual, no copy number variation is

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reflected in a variant in a gene in one chromosome (*e.g.*, heterozygosity) being detected in about 50% of detected molecules in a sample. Also, in a healthy individual, duplication of a gene bearing a variant can be reflected in the variant being detected in about 66% of detected molecules in a sample. Accordingly, if the tumor burden in a DNA sample is 10%, the frequency of a somatic mutation in a gene in one chromosome of cancer cells, without CNV, can be about 5%. The converse can be true in the case of aneuploidy.

[00195] The methods disclosed herein can be used to determine whether a sequence variant is more likely present in the germ line level or resulted from a somatic cell mutation, *e.g.*, in a cancer cell. For example, a sequence variant in a gene detected at levels arguably consistent with heterozygosity in the germ line is more likely the product of a somatic mutation if CNV is also detected in that gene. In some cases, to the extent we expect that a gene duplication in the germ line bears a variant consistent with genetic dose (*e.g.*, 66% for trisomy at a locus), detection gene amplification with a sequence variant dose that deviates significantly from this expected amount indicates that the CNV is more likely present as a result of somatic cell mutation.

[00196] The methods disclosed herein can also be used to infer tumor heterogeneity in a situation in which sequence variants in two genes are detected at different frequencies. For example, tumor heterogeneity can be inferred when two genes are detected at different frequencies but their copy numbers are relatively equal. Alternatively, tumor homogeneity can be inferred when the difference in frequency between two sequence variants is consistent with difference in copy number for the two genes. Thus, for example, if an EGFR variant is detected at 11% and a KRAS variant is detected at 5%, and no CNV is detected at these genes, the difference in frequency likely reflects tumor heterogeneity (*e.g.*, all tumor cells carry an EGFR mutant and half the tumor cells also carry a KRAS mutant). Alternatively, if the EGFR gene carrying the mutant is detected at 2-times normal copy number, one interpretation is a homogenous population of tumor cells, each cell carrying a mutant in the EGFR and KRAS genes, but in which the KRAS gene is duplicated.

[00197] In response to chemotherapy, a dominant tumor form can eventually give way through Darwinian selection to cancer cells carrying mutants that render the cancer unresponsive to the therapy regimen. Appearance of these resistance mutants can be delayed through methods of this invention. In one embodiment of this method, a subject is subjected to one or more

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pulsed therapy cycles, each pulsed therapy cycle comprising a first period during which a drug is administered at a first amount and a second cycle during which the drug is administered at a second, reduced amount. The first period can be characterized by a tumor burden detected above a first clinical level. The second period can be characterized by a tumor burden detected below a second clinical level. First and second clinical levels can be different in different pulsed therapy cycles. For example, the first clinical level can be lower in succeeding cycles. A plurality of cycles can include at least 2, 3, 4, 5, 6, 7, 8 or more cycles. For example, the BRAF mutant V600E may be detected in polynucleotides of a disease cell at an amount indicating a tumor burden of 5% in cfDNA. Chemotherapy can commence with dabrafenib. Subsequent testing can show that the amount of the BRAF mutant in the cfDNA falls below 0.5% or to undetectable levels. At this point, dabrafenib therapy can stop or be significantly curtailed. Further subsequent testing may find that DNA bearing the BRAF mutation has risen to 2.5% of polynucleotides in cfDNA. At this point, dabrafenib therapy can be re-started, *e.g.*, at the same level as the initial treatment. Subsequent testing may find that DNA bearing the BRAF mutation has decreased to 0.5% of polynucleotides in cfDNA. Again, dabrafenib therapy can be stopped or reduced. The cycle can be repeated a number of times.

[00198] A therapeutic intervention can also be changed upon detection of the rise of a mutant form resistant to an original drug. For example, cancers with the EGFR mutation L858R respond to therapy with erlotinib. However, cancers with the EGFR mutation T790M are resistant to erlotinib. However, they are responsive to ruxolitinib. A method of this invention involves monitoring changes in tumor profile and changing a therapeutic intervention when a genetic variant associated with drug resistance rises to a predetermined clinical level.

[00199] Methods disclosed in this invention can comprise a method of detecting disease cell heterogeneity from a sample comprising polynucleotides from somatic cells and disease cells, the method comprising: a) quantifying polynucleotides in the sample bearing a sequence variant at each of a plurality of genetic loci; b) determining CNV at each of the plurality of genetic loci; different relative amounts of disease molecules at a locus, wherein the CNV indicates a genetic dose of a locus in the disease cell polynucleotides; c) determining a relative measure of quantity of polynucleotides bearing a sequence variant at a locus per genetic dose at the locus for each of a plurality of the loci; and d) comparing the relative measures at each of the plurality of loci,

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wherein different relative measures indicates tumor heterogeneity. In the methods disclosed herein, the genetic dose can be determined on a total molecule basis. For example, if there are 1X total molecules at a first locus, and 1.2X molecules mapped to a second locus, then the genetic dose is 1.2. Variants at this locus can be divided by 1.2. In some aspects, the method disclosed herein can be used to detect any disease cell heterogeneity, *e.g.*, tumor cell heterogeneity. The methods can be used to detect disease cell heterogeneity from a sample comprising any types of polynucleotides, *e.g.*, cfDNA, genomic DNA, cDNA, or ctDNA. In the methods, the quantifying can comprise, for example, determining the number or relative amount of the polynucleotides. Determining CNV can comprise mapping and normalizing different relative amounts of total molecules to a locus.

[00200] In another aspect, in response to chemotherapy, a dominant tumor form can eventually give way through Darwinian selection to cancer cells carrying mutants that render the cancer unresponsive to the therapy regimen. Appearance of these resistance mutants can be delayed through methods disclosed throughout. The methods disclosed herein can comprise a method comprising: a) subjecting a subject to one or more pulsed therapy cycles, each pulsed therapy cycle comprising (i) a first period during which a drug is administered at a first amount and (ii) a second period during which the drug is administered at a second, reduced amount; wherein (A) the first period is characterized by a tumor burden detected above a first clinical level; and (B) the second period is characterized by a tumor burden detected below a second clinical level.

[00201] K. Sequence Variant Detection

[00202] Systems and methods disclosed herein can be used to detect sequence variants, *e.g.*, SNVs. For example, a sequence variant can be detected from consensus sequences from multiple sequence reads, for example, from at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56, at least 57, at

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least 58, at least 59, at least 60, at least 61, at least 62, at least 63, at least 64, at least 65, at least 66, at least 67, at least 68, at least 69, at least 70, at least 71, at least 72, at least 73, at least 74, at least 75, at least 76, at least 77, at least 78, at least 79, at least 80, at least 81, at least 82, at least 83, at least 84, at least 85, at least 86, at least 87, at least 88, at least 89, at least 90, at least 91, at least 92, at least 93, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 2000, at least 3000, at least 4000, at least 5000, at least 6000, at least 7000, at least 8000, at least 9000, at least 10000 or more sequence reads. A consensus sequence can be from sequence reads of a single strand polynucleotide. A consensus sequence can also be from sequence reads of one strand of a double-stranded polynucleotide (*e.g.*, pairing reads). In an exemplary method, pairing reads allows one to identify with increased confidence the existence of a sequence variant in a molecule. For example, if both strands of a Pair include the same variant, one can be reasonably sure that the variant existed in the original molecule, as the chance that the same variant is introduced into both strands during amplification/sequencing is rare. In contrast, if only one strand of a Pair includes the sequence variant, this is more likely to be an artifact. Similarly, the confidence that a Singlet bearing a sequence variant existed in the original molecule is less than the confidence if the variant exists in a Duplex, as there is higher probability that the variant can be introduced once than twice during amplification/sequencing.

[00203] Other methods of copy number variation detection and the sequence variant detection are described in PCT/US2013/058061, which is entirely incorporated herein by reference.

[00204] Sequence reads can be collapsed to generate a consensus sequence, which can be mapped to a reference sequence to identify genetic variants, such as CNV or SNV. As an alternative, the sequence reads are mapped prior to or even without mapping. In such a case, the sequence reads can be individually mapped to the reference to identify a CNV or SNV.

[00205] **FIG. 3** shows a reference sequence encoding a genetic Locus A. The polynucleotides in **FIG. 3** may be Y-shaped or have other shapes, such as hairpin.

[00206] In some cases, an SNV or multiple-nucleotide variant (MNV) can be determined across multiple sequence reads at a given locus (*e.g.*, nucleotide base) by aligning sequence reads that correspond to that locus. Next, a plurality of sequential nucleotide bases from at least a

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subset of the sequence reads are mapped to the reference to a SNV or MNV in a polynucleotide molecule or portion thereof that corresponds to the reads. The plurality of sequential nucleotide bases can span an actual, inferred or suspected location of the SNV or MNV. The plurality of sequential nucleotide bases can span at least 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide bases.

[00207] L. Detecting/Quantifying Nucleic Acids

[00208] The methods described throughout can be used to tag nucleic acids fragments, such as deoxyribonucleic acid (DNA), at extremely high efficiency. This efficient tagging allows a person to efficiently and accurately detect rare DNA in heterogenous populations of original DNA fragments (such as in cfDNA). A rare polynucleotide (e.g., rare DNA) can be a polynucleotide that comprises a genetic variant occurring in a population of polynucleotides at a frequency of less than 10%, 5%, 4%, 3%, 2%, 1%, or 0.1%. A rare DNA can be a polynucleotide with a detectable property at a concentration less than 50%, 25%, 10%, 5%, 1%, or 0.1%

[00209] Tagging can occur in a single reaction. In some cases, two or more reactions can be performed and pooled together. Tagging each original DNA fragments in a single reaction can result in tagging such that greater than 50% (e.g., 60%, 70%, 80%, 90%, 95%, or 99%) of the original DNA fragments are tagged at both ends with tags that comprise molecular barcodes, thereby providing tagged DNA fragments. Tagging can also result in greater than 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the original DNA fragments tagged at both ends with tags that comprise molecular barcodes. Tagging can also result in 100% of the original DNA fragments tagged at both ends with tags that comprise molecular barcodes. Tagging can also result in single end tagging.

[00210] Tagging can also occur by using an excess amount of tags as compared to the original DNA fragments. For example, the excess can be at least 5-fold excess. In other cases, the excess can be at least 1.25, 1.5, 1.75, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more fold excess. Tagging can comprise attachment to blunt ends or sticky ends. Tagging can also be

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performed by hybridization PCR. Tagging can also be performed in low reaction volumes, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 pico- and/or microliters.

[00211] The method can also include performing high fidelity amplification on the tagged DNA fragments. Any high fidelity DNA polymerases can be used. For example, the polymerase may be KAPA HiFi DNA polymerase or Phusion DNA polymerase.

[00212] Further, the method can comprise selectively enriching a subset of the tagged DNA fragments. For example, selective enrichment can be performed by hybridization or amplification techniques. The selective enrichment can be performed using a solid support (*e.g.*, beads). The solid support (*e.g.*, beads) can comprise probes (*e.g.*, oligonucleotides specifically hybridizing to certain sequences. For example, the probes can hybridize with certain genomic regions, *e.g.*, genes. In some cases, the genomic regions, *e.g.*, genes, can be regions associated with diseases, *e.g.*, cancer. After enrichment, the selected fragmented can be attached any sequencing adaptor disclosed in this invention. For example, a sequence adaptor can comprise a flow cell sequence, a sample barcode, or both. In another example, a sequence adaptor can be a hairpin shaped adaptor and/or comprises a sample barcode. Further, the resulting fragments can be amplified and sequenced. In some cases, the adaptor does not comprise a sequencing primer region.

[00213] The method can include sequencing one or both strands of the DNA fragments. In one case, both strands of the DNA fragment are independently sequenced. The tagged, amplified, and/or selectively enriched DNA fragments are sequenced to obtain sequence reads that comprise sequence information of the molecular barcodes and at least a portion of the original DNA fragments.

[00214] The method can include reducing or tracking redundancy (as described above) in the sequence reads to determine consensus reads that are representative of single-strands of the original DNA fragments. For example, to reduce or track redundancy, the method can include comparing sequence reads having the same or similar molecular barcodes and the same or

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similar end of fragment sequences. The method can comprise performing a phylogenetic analysis on the sequence reads having the same or similar molecular barcodes. The molecular barcodes can have a barcode with varying edit distances (including any edit distances as described throughout), for example, an edit distance of up to 3. The end of the fragment sequences can include fragment sequences having an edit distance with varying distances (including any edit distances as described throughout), for example, an edit distance of up to 3.

[00215] The method can comprise binning the sequence reads according to the molecular barcodes and sequence information. For example, binning the sequence reads according to the molecular barcodes and sequence information can be performed from at least one end of each of the original DNA fragments to create bins of single stranded reads. The method can further comprise in each bin, determining a sequence of a given original DNA fragment among the original DNA fragments by analyzing sequence reads.

[00216] In some cases, sequence reads in each bin can be collapsed to a consensus sequence and subsequently mapped to a genome. As an alternative, sequence reads can be mapped to a genome prior to binning and subsequently collapsed to a consensus sequence.

[00217] The method can also comprise sorting sequence reads into paired reads and unpaired reads. After sorting, the number of paired reads and unpaired reads that map to each of one or more genetic loci can be quantified.

[00218] The method can include quantifying the consensus reads to detect and/or quantify the rare DNA, which are described throughout. The method can comprise detecting and/or quantifying the rare DNA by comparing a number of times each base occurs at each position of a genome represented by the tagged, amplified, and/or enriched DNA fragments.

[00219] The method can comprise tagging the original DNA fragments in a single reaction using a library of tags. The library can include at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 50, at least 100, at least 500, at least 1000, at least 5000, at least 10000, or any number of tags as disclosed throughout. For example, the library of tags can include at least 8 tags. The library of tags can include 8 tags (which can generate 64 different possible combinations). The method can be conducted such

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that a high percentage of fragments, *e.g.*, greater than 50% (or any percentages as described throughout) are tagged at both ends, wherein each of the tags comprises a molecular barcode.

[00220] M. Processing and/or Analyzing Nucleic Acids

[00221] The methods described throughout can be used for processing and/or analyzing a nucleic acid sample of a subject. The method can comprising exposing polynucleotide fragments of the nucleic acid sample to a plurality of polynucleotide molecules to yield tagged polynucleotide fragments. The plurality of polynucleotide molecules that can be used are described throughout the application.

[00222] For example, the plurality of polynucleotide molecules can be each less than or equal to 40 nucleic acid bases in length and have distinct barcode sequences with respect to at least 4 nucleic acid bases and an edit distance of at least 1, wherein each of the distinct barcode sequences is within 20 nucleic acid bases from a terminal end of a respective one of the plurality of polynucleotide molecules, and wherein the plurality of polynucleotide molecules are not sequencing adaptors.

[00223] The tagged polynucleotide fragments can be subjected to nucleic acid amplification reactions under conditions that yield amplified polynucleotide fragments as amplification products of the tagged polynucleotide fragments. After amplification, the nucleotide sequence of the amplified tagged polynucleotide fragments is determined. In some cases, the nucleotide sequences of the amplified tagged polynucleotide fragments are determined without the use of polymerase chain reaction (PCR).

[00224] The method can comprise analyzing the nucleotide sequences with a programmed computer processor to identify one or more genetic variants in the nucleotide sample of the subject. Any genetic alterations can be identified, including but not limited to, base change(s), insertion(s), repeat(s), deletion(s), copy number variation(s), epigenetic modification(s), nucleosome binding site(s), copy number change(s) due to origin(s) of replication, and transversion(s). Other genetic alterations can include, but are not limited to, one or more tumor associated genetic alterations.

[00225] The subject of the methods can be suspected of having a disease. For example, the subject can be suspected of having cancer. The method can comprise collecting a nucleic acid sample from a subject. The nucleic acid sample can be collected from blood, plasma, serum,

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urine, saliva, mucosal excretions, sputum, stool, cerebral spinal fluid, skin, hair, sweat, and/or tears. The nucleic acid sample can be a cell-free nucleic acid sample. In some cases, the nucleic acid sample is collected from no more than 100 nanograms (ng) of double-stranded polynucleotide molecules of the subject.

[00226] The polynucleotide fragments can comprise double-stranded polynucleotide molecules. In some cases, the plurality of polynucleotide molecules are coupled to the polynucleotide fragments via blunt end ligation, sticky end ligation, molecular inversion probes, polymerase chain reaction (PCR), ligation-based PCR, multiplex PCR, single strand ligation, or single strand circularization.

[00227] The method as described herein results in high efficiency tagging of nucleic acids. For example, exposing the polynucleotide fragments of the nucleic acid sample to the plurality of polynucleotide molecules yields the tagged polynucleotide fragments with a conversion efficiency of at least 30%, *e.g.*, of at least 50% (*e.g.*, 60%, 70%, 80%, 90%, 95%, or 99%).

Conversion efficiency of at least 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% can be achieved.

[00228] The method can result in a tagged polynucleotide fragment that share common polynucleotide molecules. For example, any of at least 5%, 6%, 7%, 8%, 9%, 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of the tagged polynucleotide fragments share a common polynucleotide molecule. The method can comprise generating the polynucleotide fragments from the nucleic acid sample.

[00229] In some cases, the subjecting of the method comprises amplifying the tagged polynucleotide fragments in the presence primers corresponding to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4,

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CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. Additionally, any combination of these genes can be amplified. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, or all 54 of these genes can be amplified.

[00230] The methods described herein can comprise generating a plurality of sequence reads from a plurality of polynucleotide molecules. The plurality of polynucleotide molecules can cover genomic loci of a target genome. For example, the genomic loci can correspond to a plurality of genes as listed above. Further, the genomic loci can be any combination of these genes. Any given genomic locus can comprise at least two nucleic acid bases. Any given genomic locus can also comprise a plurality of nucleic acid bases, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more nucleic acid bases.

[00231] The method can comprise grouping with a computer processor the plurality of sequence reads into families. Each of the family can comprises sequence reads from one of the template polynucleotides. Each family can comprise sequence reads from only one of the template polynucleotides. For each of the family, the sequence reads can be merged to generate a consensus sequence. The grouping can comprise classifying the plurality of sequence reads into families by identifying (i) distinct molecular barcodes coupled to the plurality of polynucleotide molecules and (ii) similarities between the plurality of sequence reads, wherein each family includes a plurality of nucleic acid sequences that are associated with a distinct combination of molecular barcodes and similar or identical sequence reads.

[00232] Once merged, a consensus sequence can be called at a given genomic locus among the genomic loci. At any given genomic loci, any of the following can be determined: i) genetic variants among the calls; ii) frequency of a genetic alteration among the calls; iii) total number of calls; and iv) total number of alterations among the calls. The calling can comprise calling at least one nucleic acid base at the given genomic locus. The calling can also comprise calling a plurality of nucleic acid bases at the given genomic locus. In some cases, the calling can comprise phylogenetic analysis, voting (*e.g.*, biased voting), weighing, assigning a probability to

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each read at the locus in a family, or calling the base with the highest probability. The consensus sequence can be generated by evaluating a quantitative measure or a statistical significance level for each of the sequence reads. If a quantitative measure is performed, the method can comprise use of a binomial distribution, exponential distribution, beta distribution, or empirical distribution. However, frequency of the base at the particular location can also be used for calling, for example, if 51% or more of the reads is a “A” at the location, then the base may be called an “A” at that particular location. The method can further comprise mapping a consensus sequence to a target genome.

[00233] The method can further comprising performing consensus calling at an additional genomic locus among the genomic loci. The method can comprise determining a variation in copy number at one of the given genomic locus and additional genomic locus based on counts at the given genomic locus and additional genomic locus.

[00234] The methods described herein can comprise providing template polynucleotide molecules and a library of adaptor polynucleotide molecules in a reaction vessel. The adaptor polynucleotide molecules can have from 2 to 1,000 different barcode sequences and in some cases are not sequencing adaptors. Other variations of adaptor polynucleotide molecules are described throughout, which can also be used in the methods.

[00235] The polynucleotide molecules of the adaptors can have the same sample tag. The adaptor polynucleotide molecules can be coupled to both ends of the template polynucleotide molecules. The method can comprise coupling the adaptor polynucleotide molecules to the template polynucleotide molecules at an efficiency of at least 30%, *e.g.*, of at least 50% (*e.g.*, 60%, 70%, 80%, 90%, 95%, or 99%), thereby tagging each template polynucleotide with a tagging combination that is among 4 to 1,000,000 different tagging combinations, to produce tagged polynucleotide molecules. In some cases, the reaction can occur in a single reaction vessel. Coupling efficiency can also be at least 30%, 35%, 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. Tagging can be non-unique tagging.

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[00236] The tagged polynucleotide molecules can then be subject to an amplification reaction under conditions that will yield amplified polynucleotide molecules as amplification products of the tagged polynucleotide molecules. The template polynucleotide molecules can be double-stranded. Further, the template polynucleotide molecules can be blunt ended. In some cases, the amplification reaction comprises non-specifically amplifying the tagged polynucleotide molecules. The amplification reaction can also comprises using a priming site to amplify each of the tagged polynucleotide molecules. The priming site can be a primer, *e.g.*, a universal primer. The priming site can also be a nick.

[00237] The method can also comprise sequencing the amplified polynucleotide molecules. The sequencing can comprise (i) subjecting the amplified polynucleotide molecules to an additional amplification reaction under conditions that yield additional amplified polynucleotide molecules as amplification products of the amplified polynucleotide molecules, and/or (ii) sequencing the additional amplified polynucleotide molecules. The additional amplification can be performed in the presence of primers comprising flow cells sequences, which will produce polynucleotide molecules that are capable of binding to a flow cell. The additional amplification can also be performed in the presence of primers comprising sequences for hairpin shaped adaptors. The hairpin shaped adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times. The method can further comprise identifying genetic variants upon sequencing the amplified polynucleotide molecules.

[00238] The method can further comprising separating polynucleotide molecules comprising one or more given sequences from the amplified polynucleotide molecules, to produce enriched polynucleotide molecules. The method can also comprise amplifying the enriched polynucleotide molecules with primers comprising the flow cell sequences. This amplification with primers comprising flow cell sequences will produce polynucleotide molecules that are capable of binding to a flow cell. The amplification can also be performed in the presence of primers comprising sequences for hairpin shaped adaptors. The hairpin shaped adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times.

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[00239] Flow cell sequences or hairpin shaped adaptors can be added by non-amplification methods such as through ligation of such sequences. Other techniques such as hybridization methods can be used, *e.g.*, nucleotide overhangs.

[00240] The method can be performed without aliquoting the tagged polynucleotide molecules. For example, once the tagged polynucleotide molecule is made, the amplification and sequencing can occur in the same tube without any further preparation.

[00241] The methods described herein can be useful in detecting single nucleotide variations (SNV), copy number variations (CNV), insertions, deletions, and/or rearrangements. In some cases, the SNVs, CNVs, insertions, deletions, and/or rearrangements, can be associated with disease, for example, cancer.

[00242] **N. Monitoring a Patient's Status**

[00243] Methods disclosed herein can also be used to monitor a patient's disease status. The disease of a subject can be monitored over time to determine a progression of the disease (*e.g.*, regression). Markers indicative of the disease can be monitored in a biological sample of the subject, such as a cell-free DNA sample.

[00244] For example, monitoring a subject's cancer status can comprise (a) determining an amount of one or more SNVs or copy numbers of a plurality of genes (*e.g.*, in an exon), (b) repeating such determination at different points in time, and (c) determining if there is a difference in the number of SNVs, level of SNVs, number or level of genomic rearrangements, or copy numbers between (a) and (b). The genes can be selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. The genes can be selected from any 5, 10, 15, 20, 30, 40, 50, or all of the genes in this group.

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[00245] O. Sensitivity and Specificity

[00246] Methods disclosed herein can be used to detect cancer polynucleotides in a sample, and cancer in a subject, with high measures of agreement, *e.g.*, high sensitivity and/or specificity. For example, such methods can detect cancer polynucleotides (*e.g.*, rare DNA) in a sample at a concentration that is less than 5%, 1%, 0.5%, 0.1%, 0.05%, or 0.01%, at a specificity of at least 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or 99.99999%. Such polynucleotides may be indicative of cancer or other disease. Further, such methods can detect cancer polynucleotides in a sample with a positive predictive value of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, or 99.9999%.

[00247] Subjects identified as positive in a test that are in reality positive are referred as true positives (TP). Subjects identified as positive in a test that are in reality negative are referred as false positives (FP). Subjects identified as negative in a test that are in reality negative are referred as true negatives (TN). Subjects identified as negative in a test that are in reality positive are referred as false negatives (FN). Sensitivity is the percentage of actual positives identified in a test as positive. This includes, for example, instances in which one should have found a cancer genetic variant and did. ($\text{Sensitivity} = \text{TP}/(\text{TP}+\text{FN})$.) Specificity is the percentage of actual negatives identified in a test as negative. This includes, for example, instances in which one should have found no cancer genetic variant and did not. Specificity can be calculated using the following equation: $\text{Specificity} = \text{TN}/(\text{TN}+\text{FP})$. Positive predictive value (PPV) can be measured by the percentage of subjects who test positive that are true positives. PPV can be calculated using the following equation: $\text{PPV} = \text{TP}/(\text{TP}+\text{FP})$. Positive predictive value can be increased by increasing sensitivity (*e.g.*, chance of an actual positive being detected) and/or specificity (*e.g.*, chance of not mistaking an actual negative for a positive).

[00248] Low conversion rates of polynucleotides into adaptor-tagged polynucleotides can compromise sensitivity as it decreases the chance of converting, and therefore detecting, rare polynucleotide targets. Noise in a test can compromise specificity as it increases the number of false positives detected in a test. Both low conversion rate and noise compromise positive predictive value as they decrease the percentage of true positives and increase the percentage of false positives.

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[00249] The methods disclosed herein can achieve high levels of agreement, *e.g.*, sensitivity and specificity, leading to high positive predictive values. Methods of increasing sensitivity include high efficiency conversion of polynucleotides into adaptor-tagged polynucleotides in a sample. Methods of increasing specificity include reducing sequencing errors, for example, by molecular tracking.

[00250] Methods of the present disclosure can be used to detect genetic variation in non-uniquely tagged initial starting genetic material (*e.g.*, rare DNA) at a concentration that is less than 5%, 1%, 0.5%, 0.1%, 0.05%, or 0.01%, at a specificity of at least 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or 99.99999%. In some aspects, the methods can further comprise converting polynucleotides in the initial starting material at an efficiency of at least at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%. Sequence reads of tagged polynucleotides can be subsequently tracked to generate consensus sequences for polynucleotides with an error rate of no more than 2%, 1%, 0.1%, or 0.01%.

[00251] **2. Pooling Methods**

[00252] Disclosed herein are methods of detecting copy number variation and/or sequence variants at one or more genetic loci in a test sample. One embodiment is shown in **FIG. 8**. Typically, detecting copy number variation involves determining a quantitative measure (*e.g.*, an absolute or relative number) of polynucleotides mapping to a genetic locus of interest in a genome of a test sample, and comparing that number to a quantitative measure of polynucleotides mapping to that locus in a control sample. In certain methods, the quantitative measure is determined by comparing the number of molecules in the test sample that map to a locus of interest with a number of molecules in the test sample mapping to a reference sequence, *e.g.*, a sequence expected to be present at wild type ploidy number. In some examples, the reference sequence is HG19, build 37, or build 38. The comparison could involve, for example, determining a ratio. Then, this measure is compared with a similar measure determined in a control sample. So, for example, if a test sample has a ratio of 1.5:1 for locus of interest versus reference locus, and a control sample has a ratio of 1:1 for the same loci, one may conclude that the test sample exhibits polyploidy at the locus of interest.

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[00253] When the test sample and the control sample are analyzed separately, the work flow can introduce distortions between final numbers in the control and test samples.

[00254] In one method disclosed herein (*e.g.*, flow chart 800), polynucleotides are provided from a test and a control sample (802). Polynucleotides in a test sample and those in a control sample are tagged with tags that identify the polynucleotides as originating from the test or control sample (a source tag). (804.) The tag can be, for example, a polynucleotide sequence or barcode that unambiguously identifies the source.

[00255] The polynucleotides in each of the control and test samples also can be tagged with identifier tags that will be carried by all amplification progeny of a polynucleotide. Information from start and end sequences of a polynucleotide and identifier tags can identify sequence reads from polynucleotides amplified from an original parent molecule. Each molecule can be uniquely tagged compared with other molecules in the sample. Alternatively, each molecule need not be uniquely tagged compared with other molecules in the sample. That is, the number of different identifier sequences can be fewer than that the number of molecules in sample. By combining identifier information with start/stop sequence information, the probability of confusing two molecules having the same start/stop sequence is significantly diminished.

[00256] Number of different identifiers used to tag a nucleic acid (*e.g.*, cfDNA) can dependent on the number of different haploid genome equivalents. Different identifiers can be used to tag at least 2, least 10, least 100, least 200, least 300, least 400, least 500, least 600, least 700, least 800, least 900, least 1,000, least 2,000, least 3,000, least 4,000, least 5,000, least 6,000, least 7,000, least 8,000, least 9,000, least 10,000 or more different haploid genome equivalents. Accordingly, the number of different identifiers used to tag a nucleic acid sample, *e.g.*, cell-free DNA from 500 to 10,000 different haploid genome equivalents and be between any of 1, 2, 3, 4 and 5 and no more than 100, 90, 80, 70, 60, 50, 40 or 30. For example, the number of different identifier used to tag a nucleic acid sample from 500 to 10,000 different haploid genome equivalents can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or less.

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[00257] Polynucleotides can be tagged by ligation of adaptors comprising the tags or identifiers before amplification. Ligation can be performed using an enzyme, *e.g.*, a ligase. For example, tagging can be performed using a DNA ligase. The DNA ligase can be a T4 DNA ligase, *E. coli* DNA ligase, and/or mammalian ligase. The mammalian ligase can be DNA ligase I, DNA ligase III, or DNA ligase IV. The ligase may also be a thermostable ligase. Tags can be ligated to a blunt-end of a polynucleotide (blunt-end ligation). Alternatively, tags can be ligated to a sticky end of a polynucleotide (sticky-end ligation). The polynucleotides can be tagged by blunt end ligation using adaptors (*e.g.*, adaptors having forked ends). High efficiency of ligation can be achieved using high excess of adaptors (*e.g.*, more than 1.5X, more than 2X, more than 3X, more than 4X, more than 5X, more than 6X, more than 7X, more than 8X, more than 9X, more than 10X, more than 11X, more than 12X, more than 13X, more than 14X, more than 15X, more than 20X, more than 25X, more than 30X, more than 35X, more than 40X, more than 45X, more than 50X, more than 55X, more than 60X, more than 65X, more than 70X, more than 75X, more than 80X, more than 85X, more than 90X, more than 95X, or more than 100).

[00258] Once tagged with tags that identify source of the polynucleotides, polynucleotides from different sources (*e.g.*, different samples) can be pooled. After pooling, polynucleotides from different sources (*e.g.*, different samples) can be distinguished by any measurement using the tags, including any process of quantitative measurement. For example, as shown in (806) (**FIG. 8**), polynucleotides from the control sample and the test sample can be pooled. The pooled molecules can be subject to the sequencing (808) and bioinformatic work flow. Both will be subject to the same variations in the process and, therefore, any differential bias is reduced. Because molecules originating from control and test samples are differently tagged, they can be distinguished in any process of quantitative measurement.

[00259] The relative amount of control and test sample pooled can be varied. The amount of control sample can be same as the amount of test sample. The amount of control sample can also be larger than the amount of test sample. Alternatively, the amount of control sample can be smaller than the amount of test sample. The smaller the relative amount of one sample to the total, the fewer identifying tags needed in the original tagging process. A number can be selected to reduce to acceptable levels the probability that two parent molecules having the same start/end sequences will bear the same identifying tag. This probability can be less than 10%,

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less than 1%, less than 0.1% or less than 0.01%. The probability can be less than 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%.

[00260] Methods disclosed herein can also comprise grouping sequence reads. For example, bioinformatic workflow can include grouping sequence reads produced from progeny of a single parent molecule, as shown in (810) (**FIG. 8**). This can involve any of the redundancy reduction methods described herein. Molecules sourced from test and control samples can be differentiated based on source tags they carry (812). Molecules mapping to a target locus are quantified for both test-sourced and control-sourced molecules (812). This can include the normalization methods discussed herein, *e.g.*, in which numbers at a target locus are normalized against numbers at a reference locus.

[00261] Normalized (or raw) quantities at a target locus from test and control samples are compared to determine presence of copy number variation (814).

[00262] **3. Computer Control Systems**

[00263] The present disclosure provides computer control systems that are programmed to implement methods of the disclosure. **FIG. 6** shows a computer system 1501 that is programmed or otherwise configured to implement the methods of the present disclosure. The computer system 1501 can regulate various aspects sample preparation, sequencing and/or analysis. In some examples, the computer system 1501 is configured to perform sample preparation and sample analysis, including nucleic acid sequencing. The computer system 1501 can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device.

[00264] The computer system 1501 includes a central processing unit (CPU, also “processor” and “computer processor” herein) 1505, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system 1501 also includes memory or memory location 1510 (*e.g.*, random-access memory, read-only memory, flash memory), electronic storage unit 1515 (*e.g.*, hard disk), communication interface 1520 (*e.g.*, network adapter) for communicating with one or more other systems, and peripheral devices 1525, such as cache, other memory, data storage and/or electronic display adapters. The memory 1510, storage unit 1515, interface 1520 and peripheral devices 1525 are in

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communication with the CPU 1505 through a communication bus (solid lines), such as a motherboard. The storage unit 1515 can be a data storage unit (or data repository) for storing data. The computer system 1501 can be operatively coupled to a computer network (“network”) 1530 with the aid of the communication interface 1520. The network 1530 can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network 1530 in some cases is a telecommunication and/or data network. The network 1530 can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network 1530, in some cases with the aid of the computer system 1501, can implement a peer-to-peer network, which may enable devices coupled to the computer system 1501 to behave as a client or a server.

[00265] The CPU 1505 can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such as the memory 1510. The instructions can be directed to the CPU 1505, which can subsequently program or otherwise configure the CPU 1505 to implement methods of the present disclosure. Examples of operations performed by the CPU 1505 can include fetch, decode, execute, and writeback.

[00266] The CPU 1505 can be part of a circuit, such as an integrated circuit. One or more other components of the system 1501 can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00267] The storage unit 1515 can store files, such as drivers, libraries and saved programs. The storage unit 1515 can store user data, *e.g.*, user preferences and user programs. The computer system 1501 in some cases can include one or more additional data storage units that are external to the computer system 1501, such as located on a remote server that is in communication with the computer system 1501 through an intranet or the Internet.

[00268] The computer system 1501 can communicate with one or more remote computer systems through the network 1530. For instance, the computer system 1501 can communicate with a remote computer system of a user (*e.g.*, an operator). Examples of remote computer systems include personal computers (*e.g.*, portable PC), slate or tablet PC’s (*e.g.*, Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (*e.g.*, Apple® iPhone, Android-enabled

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device, Blackberry®), or personal digital assistants. The user can access the computer system 1501 via the network 1530.

[00269] Methods as described herein can be implemented by way of machine (*e.g.*, computer processor) executable code stored on an electronic storage location of the computer system 1501, such as, for example, on the memory 1510 or electronic storage unit 1515. The machine executable or machine readable code can be provided in the form of software. During use, the code can be executed by the processor 1505. In some cases, the code can be retrieved from the storage unit 1515 and stored on the memory 1510 for ready access by the processor 1505. In some situations, the electronic storage unit 1515 can be precluded, and machine-executable instructions are stored on memory 1510.

[00270] The code can be pre-compiled and configured for use with a machine have a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[00271] Aspects of the systems and methods provided herein, such as the computer system 1501, can be embodied in programming. Various aspects of the technology may be thought of as “products” or “articles of manufacture” typically in the form of machine (or processor) executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such memory (*e.g.*, read-only memory, random-access memory, flash memory) or a hard disk. “Storage” type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry

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such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible “storage” media, terms such as computer or machine “readable medium” refer to any medium that participates in providing instructions to a processor for execution.

[00272] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[00273] The computer system 1501 can include or be in communication with an electronic display 1535 that comprises a user interface (UI) 1540. The UI can allow a user to set various conditions for the methods described herein, for example, PCR or sequencing conditions. Examples of UI's include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00274] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the

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central processing unit 1505. The algorithm can, for example, process the reads to generate a consequence sequence.

[00275] FIG. 7 schematically illustrates another system for analyzing a sample comprising nucleic acids from a subject. The system includes a sequencer, bioinformatic software and internet connection for report analysis by, for example, a hand held device or a desktop computer

[00276] Disclosed herein is a system for analyzing a target nucleic acid molecule of a subject, comprising: a communication interface that receives nucleic acid sequence reads for a plurality of polynucleotide molecules that cover genomic loci of a target genome; computer memory that stores the nucleic acid sequence reads for the plurality of polynucleotide molecules received by the communication interface; and a computer processor operatively coupled to the communication interface and the memory and programmed to (i) group the plurality of sequence reads into families, wherein each family comprises sequence reads from one of the template polynucleotides, (ii) for each of the families, merge sequence reads to generate a consensus sequence, (iii) call the consensus sequence at a given genomic locus among the genomic loci, and (iv) detect at the given genomic locus any of genetic variants among the calls, frequency of a genetic alteration among the calls, total number of calls; and total number of alterations among the calls, wherein the genomic loci correspond to a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. The different variations of each component of the system are described throughout the disclosure within the methods and compositions. These individual components and variations thereof, are also applicable in this system.

[00277] **4. Kits**

[00278] Kits comprising the compositions as described herein. The kits can be useful in performing the methods as described herein. Disclosed herein is a kit comprising a plurality of

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oligonucleotide probes that selectively hybridize to least 5, 6, 7, 8, 9, 10, 20, 30, 40 or all genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1. The number genes to which the oligonucleotide probes can selectively hybridize can vary. For example, the number of genes can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, or 54. The kit can include a container that includes the plurality of oligonucleotide probes and instructions for performing any of the methods described herein.

[00279] The oligonucleotide probes can selectively hybridize to exon regions of the genes, *e.g.*, of the at least 5 genes. In some cases, the oligonucleotide probes can selectively hybridize to at least 30 exons of the genes, *e.g.*, of the at least 5 genes. In some cases, the multiple probes can selectively hybridize to each of the at least 30 exons. The probes that hybridize to each exon can have sequences that overlap with at least 1 other probe. In some embodiments, the oligoprobes can selectively hybridize to non-coding regions of genes disclosed herein, for example, intronic regions of the genes. The oligoprobes can also selectively hybridize to regions of genes comprising both exonic and intronic regions of the genes disclosed herein.

[00280] Any number of exons can be targeted by the oligonucleotide probes. For example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, , 295, 300, 400, 500, 600, 700, 800, 900, 1,000, or more, exons can be targeted.

[00281] The kit can comprise at least 4, 5, 6, 7, or 8 different library adaptors having distinct molecular barcodes and identical sample barcodes. The library adaptors may not be sequencing

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adaptors. For example, the library adaptors do not include flow cell sequences or sequences that permit the formation of hairpin loops for sequencing. The different variations and combinations of molecular barcodes and sample barcodes are described throughout, and are applicable to the kit. Further, in some cases, the adaptors are not sequencing adaptors. Additionally, the adaptors provided with the kit can also comprise sequencing adaptors. A sequencing adaptor can comprise a sequence hybridizing to one or more sequencing primers. A sequencing adaptor can further comprise a sequence hybridizing to a solid support, *e.g.*, a flow cell sequence. For example, a sequencing adaptor can be a flow cell adaptor. The sequencing adaptors can be attached to one or both ends of a polynucleotide fragment. In some cases, the kit can comprise at least 8 different library adaptors having distinct molecular barcodes and identical sample barcodes. The library adaptors may not be sequencing adaptors. The kit can further include a sequencing adaptor having a first sequence that selectively hybridizes to the library adaptors and a second sequence that selectively hybridizes to a flow cell sequence. In another example, a sequencing adaptor can be hairpin shaped. For example, the hairpin shaped adaptor can comprise a complementary double stranded portion and a loop portion, where the double stranded portion can be attached (*e.g.*, ligated) to a double-stranded polynucleotide. Hairpin shaped sequencing adaptors can be attached to both ends of a polynucleotide fragment to generate a circular molecule, which can be sequenced multiple times. A sequencing adaptor can be up to 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more bases from end to end. The sequencing adaptor can comprise 20-30, 20-40, 30-50, 30-60, 40-60, 40-70, 50-60, 50-70, bases from end to end. In a particular example, the sequencing adaptor can comprise 20-30 bases from end to end. In another example, the sequencing adaptor can comprise 50-60 bases from end to end. A sequencing adaptor can comprise one or more barcodes. For example, a sequencing adaptor can comprise a sample barcode. The sample barcode can comprise a pre-determined sequence. The sample barcodes can be used to identify the source of the polynucleotides. The sample barcode can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

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21, 22, 23, 24, 25, or more (or any length as described throughout) nucleic acid bases, *e.g.*, at least 8 bases. The barcode can be contiguous or non-contiguous sequences, as described above.

[00282] The library adaptors can be blunt ended and Y-shaped and can be less than or equal to 40 nucleic acid bases in length. Other variations of the can be found throughout and are applicable to the kit.

EXAMPLES

[00283] **Example 1. Methods for copy number variation detection.**

[00284] Blood collection

[00285] 10-30 mL Blood samples are collected at room temperature. The samples are centrifuged to remove cells. Plasma is collected after centrifugation.

[00286] cfDNA extraction

[00287] The sample is subjected to proteinase K digestion. DNA is precipitated with isopropanol. DNA is captured on a DNA purification column (*e.g.*, a QIAamp DNA Blood Mini Kit) and eluted in 100 µl solution. DNAs below 500 bp are selected with Ampure SPRI magnetic bead capture (PEG/salt). The resulting production is suspended in 30 µl H₂O. Size distribution is checked (major peak = 166 nucleotides; minor peak = 330 nucleotides) and quantified. 5 ng of extracted DNA contain approximately 1700 haploid genome equivalents (“HGE”). The general correlation between the amount of DNA and HGE is as follow: 3 pg DNA = 1 HGE; 3 ng DNA = 1K HGE; 3 µg DNA = 1M HGE; 10 pg DNA = 3 HE; 10 ng DNA = 3K HGE; 10 µg DNA = 3M HGE.

[00288] “Single Molecule” library prep

[00289] High-efficiency DNA tagging (>80%) is performed by blunt-end repair and ligation with 8 different octomers (*i.e.*, 64 combinations) with overloaded hairpin adaptors. 2.5 ng DNA (*i.e.* approximately 800 HGE) is used as the starting material. Each hairpin adaptor comprises a random sequence on its non-complementary portion. Both ends of each DNA fragment are attached with hairpin adaptors. Each tagged fragment can be identified by the random sequence on the hairpin adaptors and a 10 p endogenous sequence on the fragment.

[00290] Tagged DNA is amplified by 10 cycles of PCR to produce about 1-7 µg DNAs that contain approximately 500 copies of each of the 800 HGE in the starting material.

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[00291] Buffer optimization, polymerase optimization and cycle reduction may be performed to optimize the PCR reactions. Amplification bias, *e.g.*, non-specific bias, GC bias, and/or size bias are also reduced by optimization. Noise(s) (*e.g.*, polymerase-introduced errors) are reduced by using high-fidelity polymerases.

[00292] The Library may be prepared using Verniata or Sequenom methods.

[00293] Sequences may be enriched as follow: DNAs with regions of interest (ROI) are captured using biotin-labeled bead with probe to ROIs. The ROIs are amplified with 12 cycles of PCR to generate a 2000 times amplification. The resulting DNA is then denatured and diluted to 8 pM and loaded into an Illumina sequencer.

[00294] Massively parallel sequencing

[00295] 0.1 to 1% of the sample (approximately 100pg) are used for sequencing.

[00296] Digital bioinformatics

[00297] Sequence reads are grouped into families, with about 10 sequence reads in each family. Families are collapsed into consensus sequences by voting (*e.g.*, biased voting) each position in a family. A base is called for consensus sequence if 8 or 9 members agree. A base is not called for consensus sequence if no more than 60% of the members agree.

[00298] The resulting consensus sequences are mapped to a reference genome. Each base in a consensus sequence is covered by about 3000 different families. A quality score for each sequence is calculated and sequences are filtered based on the their quality scores.

[00299] Sequence variation is detected by counting distribution of bases at each locus. If 98% of the reads have the same base (homozygous) and 2% have a different base, the locus is likely to have a sequence variant, presumably from cancer DNA.

[00300] CNV is detected by counting the total number of sequences (bases) mapping to a locus and comparing with a control locus. To increase CNV detection, CNV analysis is performed specific regions, including regions on ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A,

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BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, or NTRK1 genes.

[00301] Example 2. Method for Correcting Base Calling by Determining the Total Number Unseen Molecules in a Sample

[00302] After fragments are amplified and the sequences of amplified fragments are read and aligned, the fragments are subjected to base calling. Variations in the number of amplified fragments and unseen amplified fragments can introduce errors in base calling. These variations are corrected by calculating the number of unseen amplified fragments.

[00303] When base calling for locus A (an arbitrary locus), it is first assumed that there are N amplified fragments. The sequence readouts can come from two types of fragments: double-strand fragments and single-strand fragments. The following is a theoretical example of calculating the total number of unseen molecules in a sample.

[00304] N is the total number of molecules in the sample.

Assuming 1000 is the number of duplexes detected.

Assuming 500 is the number of single-stranded molecule detected.

P is the probability of seeing a strand.

Q is the probability of not detecting a strand.

[00305] Since $Q = 1 - P$.

$$1000 = NP(2).$$

$$500 = N2PQ.$$

$$1000 / P(2) = N.$$

$$500 \div 2 PQ = N.$$

$$1000 / P(2) = 500 \div 2PQ.$$

$$1000 * 2 PQ = 500 P(2).$$

$$2000 PQ = 500 P(2).$$

$$2000 Q = 500 P.$$

$$2000 (1-P) = 500P$$

$$2000 - 2000 P = 500P.$$

$$2000 = 500P + 2000 P.$$

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$$2000 = 2500 P.$$

$$2000 \div 2500 = P.$$

$$0.8 = P.$$

$$1000 / P(2) = N.$$

$$1000 \div 0.64 = N.$$

$$1562 = N.$$

$$\text{Number of unseen fragments} = 62.$$

[00306] Example 3. Identification of genetic variants in cancer-associated somatic variants in a patient.

[00307] An assay is used to analyze a panel of genes to identify genetic variants in cancer-associated somatic variants with high sensitivity.

[00308] Cell-free DNA is extracted from plasma of a patient and amplified by PCR. Genetic variants are analyzed by massively parallel sequencing of the amplified target genes. For one set of genes, all exons are sequenced as such sequencing coverage had shown to have clinically utility (Table 1). For another set of genes, sequencing coverage included those exons with a previously reported somatic mutation (Table 2). The minimum detectable mutant allele (limit of detection) is dependent on the patient's sample cell-free DNA concentration, which varied from less than 10 to over 1,000 genomic equivalents per mL of peripheral blood. Amplification may not be detected in samples with lower amounts of cell-free DNA and/or low-level gene copy amplification. Certain sample or variant characteristics resulted in reduced analytic sensitivity, such as low sample quality or improper collection.

[00309] The percentage of genetic variants found in cell-free DNA circulating in blood is related to the unique tumor biology of this patient. Factors that affected the amount/percentages of detected genetic variants in circulating cell-free DNA in blood include tumor growth, turnover, size, heterogeneity, vascularization, disease progression or treatment. Table 3 annotates the percentage, or allele frequency, of altered circulating cell-free DNA (% cfDNA) detected in this patient. Some of the detected genetic variants are listed in descending order by % cfDNA.

[00310] Genetic variants are detected in the circulating cell-free DNA isolated from this patient's blood specimen. These genetic variants are cancer-associated somatic variants, some of

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which have been associated with either increased or reduced clinical response to specific treatment. “Minor Alterations” are defined as those alterations detected at less than 10% the allele frequency of “Major Alterations”. The detected allele frequencies of these alterations (Table 3) and associated treatments for this patient are annotated.

[00311] All genes listed in Tables 1 and 2 are analyzed as part of the Guardant360™ test. Amplification is not detected for *ERBB2*, *EGFR*, or *MET* in the circulating cell-free DNA isolated from this patient’s blood specimen.

[00312] Patient test results comprising the genetic variants are listed in Table 4.

Table 1. Genes in which all exons are sequenced

GENES IN WHICH ALL EXONS ARE SEQUENCED			
ALK	< 0.1%	APC	< 0.1%
AR	< 0.1%	BRAF	< 0.1%
CDKN2A	< 0.1%	EGFR	< 0.1%
ERBB2	< 0.1%	FBXW7	< 0.1%
KRAS	< 0.1%	MET	< 0.1%
MYC	< 0.1%	NOTCH1	< 0.1%
NRAS	< 0.1%	PIK3CA	< 0.1%
PTEN	< 0.1%	PROC	< 0.1%
RB1	< 0.1%	TP53	< 0.1%

LOD: Limit of Detection. The minimum detectable mutant allele frequency for this specimen in which 80% of somatic variants is detected.

Table 2. Genes in which exons with a previously reported somatic mutation are sequenced

GENES IN WHICH EXONS WITH A PREVIOUSLY REPORTED SOMATIC MUTATION ARE SEQUENCED			
ABL1	< 0.1%	AKT1	< 0.1%
ATM	< 0.1%	CDH1	< 0.1%
CSF1R	< 0.1%	CTNNB1	< 0.1%
ERBB4	< 0.1%	EZH2	< 0.1%
FGFR1	< 0.1%	FGFR2	< 0.1%
FGFR3	< 0.1%	FLT3	< 0.1%
GNA11	< 0.1%	GNAQ	< 0.1%
GNAS	< 0.1%	HNF1A	< 0.1%
HRAS	< 0.1%	IDH1	< 0.1%

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IDH2	< 0.1%	JAK2	< 0.1%
JAK3	< 0.1%	KDR	< 0.1%
KIT	< 0.1%	MLH1	< 0.1%
MPL	< 0.1%	NPM1	< 0.1%
PDGFRA	< 0.1%	PTPN11	< 0.1%
RET	< 0.1%	SMAD4	< 0.1%
SMARCB1	< 0.1%	SMO	< 0.1%
SRC	< 0.1%	STK11	< 0.1%
TERT	< 0.1%	VHL	< 0.1%

LOD: Limit of Detection. The minimum detectable mutant allele frequency for this specimen in which 80% of somatic variants is detected.

Table 3. Allele frequency of altered circulating cell-free DNA detected in this patient

Gene	cfDNA with alterations (%)	cfDNA without alterations (%)
BRAF V600E	8.9	91.1
NRAS Q61K	6.2	93.8
JAK V617F	1.5	98.6

Table 4. Genomic alterations detected in selected genes

Detected: 51 Genomic Alterations							
Gene	Chromosome	Position	Mutation (nt)	Mutation (AA)	Percentage	Cosmic ID	DBSNP ID
KRAS	12	25368462	C>T		100.0%		rs4362222
ALK	2	29416572	T>C	I1461V	100.0%		rs1670283
ALK	2	29444095	C>T		100.0%		rs1569156
ALK	2	29543663	T>C	Q500Q	100.0%		rs2293564
ALK	2	29940529	A>T	P234P	100.0%		rs2246745
APC	5	112176756	T>A	V1822D	100.0%		rs459552
CDKN2A	9	21968199	C>G		100.0%	COSM14251	rs11515
FGFR3	4	1807894	G>A	T651T	100.0%		rs7688609
NOTCH1	9	139410424	A>G		100.0%		rs3125006
PDGFRA	4	55141055	A>G	P567P	100.0%		rs1873778
HRAS	11	534242	A>G	H27H	100.0%	COSM249860	rs12628
EGFR	7	55214348	C>T	N158N	99.9%	COSM42978	rs2072454
TP53	17	7579472	G>C	P72R	99.8%		rs1042522
APC	5	112162854	T>C	Y486Y	55.0%		rs2229992
APC	5	112177171	G>A	P1960P	53.8%		rs465899
EGFR	7	55266417	T>C	T903T	53.6%		rs1140475
APC	5	112176325	G>A	G1678G	53.2%		rs42427
APC	5	112176559	T>G	S1756S	53.0%		rs866006
EGFR	7	55229255	G>A	R521K	53.0%		
MET	7	116397572	A>G	Q648Q	52.7%		
APC	5	112175770	G>A	T1493T	52.7%		rs41115

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EGFR	7	55249063	G>A	Q787Q	52.6%		rs1050171
NOTCH1	9	139411714	T>C		52.4%		rs11145767
EGFR	7	55238874	T>A	T629T	52.0%		rs2227984
ERBB2	17	37879588	A>G	I655V	51.6%		rs1136201
NOTCH1	9	139397707	G>A	D1698D	51.3%	COSM33747	rs10521
ALK	2	30143499	G>C	L9L	51.0%		rs4358080
APC	5	112164561	G>A	A545A	51.0%		rs351771
FLT3	13	28610183	A>G		50.8%		rs2491231
NOTCH1	9	139418260	A>G	N104N	50.5%		rs4489420
ALK	2	29444076	G>T		50.4%		rs1534545
PIK3CA	3	178917005	A>G		50.3%		rs3729674
NOTCH1	9	139412197	G>A		50.2%		rs9411208
ALK	2	29455267	A>G	G845G	50.0%	COSM148825	rs2256740
KIT	4	55593464	A>C	M541L	49.9%	COSM28026	
NOTCH1	9	139391636	G>A	D2185D	48.9%		rs2229974
PDGFRA	4	55152040	C>T	V824V	48.9%	COSM22413	rs2228230
ALK	2	29416481	T>C	K1491R	48.9%	COSM1130802	rs1881420
ALK	2	29445458	G>T	G1125G	48.6%		rs3795850
NOTCH1	9	139410177	T>C		48.5%		rs3124603
RET	10	43613843	G>T	L769L	48.2%		rs1800861
EGFR	7	55214443	G>A		48.0%		rs7801956
ALK	2	29416366	G>C	D1529E	47.2%		rs1881421
EGFR	7	55238087	C>T		45.5%		rs10258429
RET	10	43615633	C>G	S904S	44.8%		rs1800863
BRAF	7	140453136	A>T	V600E	8.9%	COSM476	
NRAS	1	115256530	G>T	Q61K	6.2%	COSM580	rs121913254
JAK2	9	5073770	G>T	V617F	1.5%	COSM12600	rs77375493

[00313] Example 4. Determining patient-specific limits of detection for genes analyzed by Guardant360™ assays.

[00314] Using the method of Example 3, Genetic alterations in cell-free DNA of a patient are detected. The sequence reads of these genes include exon and/or intron sequences.

[00315] Limits of detection of the test are shown in Table 5. The limits of detection values are dependent on cell-free DNA concentration and sequencing coverage for each gene.

Table 5. Limits of Detection of selected genes in a patient using Guardant

Complete Exon and Partial Intron Coverage					
APC	0.1%	AR *	0.2%	ARID1A	
BRAF *	0.1%	BRCA1		BRCA2	
CCND1 *		CCND2 *		CCNE1 *	
CDK4 *		CDK6 *		CDKN2A	0.1%
CDKN2B		EGFR *	< 0.1%	ERBB2 *	0.1%
FGFR1 *	< 0.1%	FGFR2 *	0.1%	HRAS	0.1%
KIT *	0.1%	KRAS *	0.1%	MET *	0.1%

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MYC *	0.1%	NF1		NRAS	0.1%
PDGFRA *	0.1%	PIK3CA*	0.1%	PTEN	0.1%
RAF1 *		TP53	0.1%		
Exons Covered with Reported Somatic Mutations					
AKT1	0.1%	ALK	< 0.1%	ARAF	
ATM	0.1%	CDH1	0.1%	CTNNB1	0.1%
ESR1		EZH2	0.1%	FBXW7	0.1%
FGFR3	0.1%	GATA3		GNA11	0.1%
GNAQ	0.1%	GNAS	0.1%	HNF1A	0.1%
IDH1	0.1%	IDH2	0.1%	JAK2	0.1%
JAK3	0.1%	MAP2K1		MAP2K2	
MLH1	0.1%	MPL	0.2%	NFE2L2	
NOTCH1	0.1%	NPM1	0.1%	PTPN11	0.1%
RET	0.1%	RHEB		RHOA	
RIT1		ROS1		SMAD4	0.1%
SMO	0.1%	SRC	< 0.1%	STK11	0.2%
TERT	0.1%	VHL	0.2%		
Fusions					
ALK	< 0.1%	RET	0.1%	ROS1	
NTRK1					

LOD: Limit of Detection. The minimum detectable mutant allele frequency for this specimen in which 80% of somatic variants is detected. * indicates CNV genes.

[00316] Example 5. Correcting Sequence Errors Comparing Watson and Crick Sequences

[00317] Double-stranded cell-free DNA is isolated from the plasma of a patient. The cell-free DNA fragments are tagged using 16 different bubble-containing adaptors, each of which comprises a distinctive barcode. The bubble-containing adaptors are attached to both ends of each cell-free DNA fragment by ligation. After ligation, each of the cell-free DNA fragment can be distinctly identified by the sequence of the distinct barcodes and two 20 bp endogenous sequences at each end of the cell-free DNA fragment.

[00318] The tagged cell-free DNA fragments are amplified by PCR. The amplified fragments are enriched using beads comprising oligonucleotide probes that specifically bind to a group of

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cancer-associated genes. Therefore, cell-free DNA fragments from the group of cancer-associated genes are selectively enriched.

[00319] Sequencing adaptors, each of which comprises a sequencing primer binding site, a sample barcode, and a cell-flow sequence, are attached to the enriched DNA molecules. The resulting molecules are amplified by PCR.

[00320] Both strands of the amplified fragments are sequenced. Because each bubble-containing adaptor comprises a non-complementary portion (*e.g.*, the bubble), the sequence of the one strand of the bubble-containing adaptor is different from the sequence of the other strand (complement). Therefore, the sequence reads of amplicons derived from the Watson strand of an original cell-free DNA can be distinguished from amplicons from the Crick strand of the original cell-free DNA by the attached bubble-containing adaptor sequences.

[00321] The sequence reads from a strand of an original cell-free DNA fragment are compared to the sequence reads from the other strand of the original cell-free DNA fragment. If a variant occurs in only the sequence reads from one strand, but not other strand, of the original cell-free DNA fragment, this variant will be identified as an error (*e.g.*, resulted from PCR and/or amplification), rather than a true genetic variant.

[00322] The sequence reads are grouped into families. Errors in the sequence reads are corrected. The consensus sequence of each family is generated by collapsing.

[00323] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations

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or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		
<p>The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.</p>			

Secrecy Order 37 CFR 5.2:

Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

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Legal Name				
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Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service				
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305		
		Application Number			
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS				
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All Inventors Must Be Listed - Additional Inventor information blocks may be generated within this form by selecting the Add button.					<input type="button" value="Add"/>

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<input type="checkbox"/> An Address is being provided for the correspondence information of this application.			
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Application Information:

Title of the Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		
Attorney Docket Number	42534-708.305	Small Entity Status Claimed	<input type="checkbox"/>
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	11	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-706.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Publication Information:

<input type="checkbox"/>	Request Early Publication (Fee required at time of Request 37 CFR 1.219)
<input type="checkbox"/>	Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	<input checked="" type="radio"/> Customer Number	<input type="radio"/> US Patent Practitioner	<input type="radio"/> Limited Recognition (37 CFR 11.9)
Customer Number	115823		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending	Remove			
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
	Continuation of	16672267	2019-11-01		
Prior Application Status	Pending	Remove			
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
16672267	Continuation of	16601168	2019-10-14		
Prior Application Status	Pending	Remove			
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
16601168	Continuation of	15892178	2018-02-08		
Prior Application Status	Patented	Remove			
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15892178	Continuation of	14861989	2015-09-22	9920366	2018-03-20

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		
Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14861989	Continuation of	PCTUS2014072383	2014-12-24
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCTUS2014072383	Claims benefit of provisional	61948509	2014-03-05
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCTUS2014072383	Claims benefit of provisional	61921456	2013-12-28
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)¹ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove			
Application Number	Country ¹	Filing Date (YYYY-MM-DD)	Access Code ¹ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Assignee
 Legal Representative under 35 U.S.C. 117
 Joint inventor

Person to whom the inventor is obligated to assign.
 Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here.

Organization Name:

Mailing Address Information For Applicant:

Address 1:

Address 2:

City: State/Province:

Country: Postal Code:

Phone Number: Fax Number:

Email Address:

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Assignee 1				
Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.				
If the Assignee or Non-Applicant Assignee is an Organization check here. <input type="checkbox"/>				
Prefix	Given Name	Middle Name	Family Name	Suffix
Mailing Address Information For Assignee including Non-Applicant Assignee:				
Address 1				
Address 2				
City		State/Province		
Country ⁱ		Postal Code		
Phone Number		Fax Number		
Email Address				
Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.				

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). However, if this Application Data Sheet is submitted with the **INITIAL** filing of the application and either box A or B is **not** checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Timothy A. Hott/		Date (YYYY-MM-DD)	2020-01-16
First Name	Timothy	Last Name	Hott	Registration Number
67740				
Additional Signature may be generated within this form by selecting the Add button.				

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-706.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
---------------------------	---

As the below named inventor, I hereby declare that:

This declaration is directed to: The attached application, orUnited States application or PCT international application number 16/601,168filed on 2019-10-14

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTORInventor: Stefanie Ann Ward MORTIMER

Date (Optional): _____

Signature: *S. Mortimer*

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-0199 and select option 2.

00228

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	AmirAli TALASAZ et al.	Confirmation No.:	3111
Serial No.:	16/714,579	Art Unit:	1636
Filing Date:	December 13, 2019	Examiner:	To be assigned
Title:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:

- (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

(2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491 in an international application;

-- OR --

(3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

(4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

B. *37 CFR § 1.97(c)*. Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b)*, above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

a statement as specified in § 1.97 (e) provided concurrently herewith;

-- OR --

a fee of \$240.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. *37 CFR § 1.97 (d)*. Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in § 1.97 (e);

-- AND --

ii. a fee of \$240.00 as set forth in § 1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. *37 CFR § 1.97 (e)*. Statement.

A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (c)*;

-- AND/OR --

A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (d)*;

-- AND/OR --

A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under *37 C.F.R. § 1.97(e) (1)* as provided for under *MPEP 609.04(b) V*.

E. *Statement Under 37 C.F.R. § 1.704(d)*. Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure

statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. 37 CFR §1.98 (a) (2). The content of the Information Disclosure Statement is as follows:

Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. 37 CFR §1.98(a)(3). The Information Disclosure Statement includes non-English patents and/or references.

Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. 37 CFR §1.98(d). Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: 16/672,267

Information Disclosure Statement(s) filed on: 12/06/19

AND

The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

USSN: 16/714,579

January 16, 2020

Page 4 of 4

- I. *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of \$0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 60-2231(Docket No. GH0004US-CON2).

Respectfully submitted,

Dated: January 16, 2020

By: /Timothy A. Hott/
Timothy A. Hott, Reg. No. 67740

Customer No. 115823
GUARDANT HEALTH, INC.
505 Penobscot Drive
Redwood City, CA 94063

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

U.S.PATENTS						Remove
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	4725536	A	1988-02-16	Fritsch et al.	
	2	4942124	A	1990-07-17	Church	
	3	5124246	A	1992-06-23	Urdea et al.	
	4	5149625	A	1992-09-22	Church et al.	
	5	5200314	A	1993-04-06	Urdea	
	6	5424186	A	1995-06-13	Fodor et al.	
	7	5424413	A	1995-06-13	Hogan et al.	
	8	5445934		1995-08-01	Fodor et al.	

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

9	5604097	A	1997-02-18	Brenner
10	5635352	A	1997-06-03	Urdea et al.
11	5635400	A	1997-06-03	Brenner
12	5648245	A	1997-07-15	Fire et al.
13	5654413	A	1997-08-05	Brenner
14	5656731	A	1997-08-12	Urdea
15	5658737	A	1997-08-19	Nelson et al.
16	5714330	A	1998-02-03	Brenner et al.
17	5744305		1998-04-01	Fodor et al.
18	5759778	A	1998-06-02	Li et al.
19	5763175	A	1998-06-09	Brenner

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

20	5800992		1998-09-01	Fodor et al.	
21	5846719	A	1998-12-08	Brenner et al.	
22	5854033	A	1998-12-29	Lizardi	
23	5871928	A	1999-02-16	Fodor et al.	
24	5925525	A	1999-07-20	Fodor et al.	
25	5935793	A	1999-08-10	Wong	
26	5952170	A	1999-09-14	Stroun et al.	Entire Document
27	5968740	A	1999-10-19	Fodor et al.	
28	5981176	A	1999-11-09	Wallace	
29	5981179	A	1999-11-09	Lorinez et al.	
30	6013445	A	2000-01-11	Albrecht et al.	

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

31	6020124	A	2000-02-01	Sorenson
32	6040138	A	2000-03-21	Lockhart et al.
33	6046005	A	2000-04-04	Ju et al.
34	6060596	A	2000-05-09	Lerner et al.
35	6117631	A	2000-09-12	Nilsen
36	6124092	A	2000-09-26	O'Neill et al.
37	6138077	A	2000-10-24	Brenner
38	6140489	A	2000-10-31	Brenner
39	6172214	B1	2001-01-09	Brenner
40	6197506	B1	2001-03-06	Fodor et al.
41	6235475	B1	2001-05-22	Brenner et al.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

42	6242186	B1	2001-06-05	Salonen
43	6268152	B1	2001-07-31	Fodor et al.
44	6284460	B1	2001-09-04	Fodor et al.
45	6300077	B1	2001-10-09	Shuber et al.
46	6309822	B1	2001-10-30	Fodor et al.
47	6309823	B1	2001-10-30	Cronin et al.
48	6326148	B1	2001-12-04	Pauletti et al.
49	6355431	B1	2002-03-12	Chee et al.
50	6355432	B1	2002-03-12	Fodor et al.
51	6395491	B1	2002-05-28	Fodor et al.
52	6406848	B1	2002-06-18	Bridgham et al.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number		16714579
Filing Date		2019-12-13
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number		42534-708.305

53	6440667	B1	2002-08-27	Fodor et al.	
54	6440706	B1	2002-08-27	Vogelstein et al.	
55	6451536	B1	2002-09-17	Fodor et al.	
56	6458530	B1	2002-10-01	Morris et al.	
57	6468744	B1	2002-10-22	Cronin et al.	
58	6489114	B2	2002-12-03	Laayoun et al.	
59	6492121	B2	2002-12-10	Kurane et al.	Entire Document
60	6498012	B2	2002-12-24	Laken	Entire Document
61	6503718	B2	2003-01-07	Shuber et al.	Entire Document
62	6512105	B1	2003-01-28	Hogan et al.	
63	6514699	B1	2003-02-04	O'Neill et al.	

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number		16714579
Filing Date		2019-12-13
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number		42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number		16714579
Filing Date		2019-12-13
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number		42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number		16714579
Filing Date		2019-12-13
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number		42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number		16714579
Filing Date		2019-12-13
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number		42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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	4	2110442	EP	A1	2009-10-21	Shuber	Entire Document	
	5	3070177	EP	A1	2016-09-21	Kitano et al.	Entire Document	
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**INFORMATION DISCLOSURE
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(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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Examiner Name		
Attorney Docket Number		42534-708.305

18	2009152928	WO	A2	2009-12-23	ROTTER	Entire Document	<input checked="" type="checkbox"/>
19	2011060240	WO	A1	2011-05-19	Akmaev et al.	Entire Document	<input type="checkbox"/>
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Attorney Docket Number	42534-708.305

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Attorney Docket Number		42534-708.305

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**INFORMATION DISCLOSURE
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Examiner Name	
Attorney Docket Number	42534-708.305

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	Art Unit	
	Examiner Name	
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	Examiner Name	
	Attorney Docket Number	42534-708.305

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Attorney Docket Number		42534-708.305

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	Art Unit	
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	Attorney Docket Number	42534-708.305

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Application Number	16714579
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First Named Inventor	AmirAli TALASAZ
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Examiner Name	
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38	Guardant Health vs. FMI 1st Amended Answer to Second Amended Complaint dated May 6, 2019 (C.A. No. 17-cv-1616-LPS-CJB)
39	Guardant Health vs. FMI Invalidity Contentions dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
40	Guardant Health vs. FMI Invalidity Exhibit A-1, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
41	Guardant Health vs. FMI Invalidity Exhibit A-2, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
42	Guardant Health vs. FMI Invalidity Exhibit A-3, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
43	Guardant Health vs. FMI Invalidity Exhibit A-8, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
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47	Guardant Health vs. FMI Invalidity Exhibit B-7, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
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49	Guardant Health vs. FMI Invalidation Exhibit C-11, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
50	Guardant Health vs. FMI Invalidation Exhibit C-2, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)

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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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1	Guardant Health vs. FMI Invalidation Exhibit C-3, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
2	Guardant Health vs. FMI Invalidation Exhibit C-4, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
3	Guardant Health vs. FMI Invalidation Exhibit C-5, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
4	Guardant Health vs. FMI Invalidation Exhibit D-1, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
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8	Guardant Health vs. FMI Second Amended Complaint dated March 6, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
9	Guardant Health vs. FMI Suppl Invalidation References dated February 15, 2019 (C.A. No. 17-cv-1616-LPS-CJB)
10	Guardant Health vs. FMI Supplemental Invalidation Contentions dated March 29, 2019 (C.A. No. 17-cv-1616-LPS-CJB)
11	Guardant Health vs. PGDx Amended Answer to Second Amended Complaint dated April 30, 2019 (C.A. No. 17-cv-1623-LPS-CJB)

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12	Guardant Health vs. PGDx Invalidity Claim Chart A, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
13	Guardant Health vs. PGDx Invalidity Claim Chart B, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
14	Guardant Health vs. PGDx Invalidity Claim Chart C, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
15	Guardant Health vs. PGDx Invalidity Claim Chart D, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
16	Guardant Health vs. PGDx Invalidity Claim Chart E, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
17	Guardant Health vs. PGDx Invalidity Claim Chart F, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
18	Guardant Health vs. PGDx Invalidity Claim Chart G, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
19	Guardant Health vs. PGDx Invalidity Claim Chart H, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
20	Guardant Health vs. PGDx Invalidity Claim Chart I, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
21	Guardant Health vs. PGDx Invalidity Claim Chart J, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
22	Guardant Health vs. PGDx Invalidity Claim Chart K, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)

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26	Guardant Health vs. PGDx Second Updated Invalidation Contentions dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
27	Guardant Health vs. PGDx Updated Invalidation Contentions dated March 29, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
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34	HENSEL, et al. Simultaneous identification of bacterial virulence genes by negative selection. Science. 1995 Jul 21;269(5222):400-3.
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45	HUG, et al. Measurement of the number of molecules of a single mRNA species in a complex mRNA preparation. J Theor Biol. 2003 Apr 21;221(4):615-24.
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See attached certification statement.

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SIGNATURE

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

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Name/Print	Timothy A. Hott	Registration Number	67740

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
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1	NAWROZ, et al. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med. 1996 Sep;2(9):1035-7.
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7	Notice of allowance dated 03/21/2014 for US Application No. 12/969,581.
8	Notice of allowance dated 06/15/2017 for US Application No.15/076,565.
9	Notice of allowance dated 06/19/2014 for US Application No. 12/969,581.
10	Notice of allowance dated 08/01/2017 for US Application No. 15/492,659
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14	Notice of allowance dated 09/11/2017 for US Application No. 15/076,565
15	Notice of allowance dated 10/03/2017 for US Application No. 15/076,565.
16	Notice of allowance dated 10/25/2017 for US Application No. 14/861,989
17	Notice of allowance dated 12/28/2017 for US Application No. 14/861,989.
18	Office Action dated 02/09/2017 for U.S. Patent Application No. 15/076,565.
19	Office action dated 05/13/2019 for US Application No. 15/669,779.
20	Office action dated 05/20/2016 for US Application No. 14/855,301.
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24	Office action dated 06/12/2017 for US Application No. 15/492,659.
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37	Opposition Brief dated August 7, 2018 for Japanese Opposition No. 2018-700659 to JP Patent 6275145	×
38	Opposition Decision dated January 15, 2019 for Japanese Opposition No. 2018-700659 to JP Patent 6275145	☒
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44	Pacific Biosciences. Template Preparation and Sequencing Guide. Publication date: 2014-10-14. Pacific Biosciences website http://www.pacificbiosciences.com/support/pubmap/documentation.html .	<input type="checkbox"/>

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45	PAN, et al. Loss of heterozygosity patterns provide fingerprints for genetic heterogeneity in multistep cancer progression of tobacco smoke-induced non-small cell lung cancer. Cancer Res. 2005 Mar 1;65(5):1664-9.	<input type="checkbox"/>
46	PARK, et al. Discovery of common Asian copy number variants using integrated high-resolution array CGH and massively parallel DNA sequencing. Nat Genet. 2010 May;42(5):400-5. doi: 10.1038/ng.555. Epub 2010 Apr 4.	<input type="checkbox"/>
47	PARKINSON, N.J. et al., "Preparation of high-quality next-generation sequencing libraries from picogram quantities of target DNA," Genome Res. 2012, 22(1), 125-133	<input type="checkbox"/>
48	PEL, J. et al. "Duplex proximity sequencing (pro-seq): A method to improve DNA sequencing accuracy without the cost of molecular barcoding redundancy" bioRxiv (2017) https://doi.org/10.1101/163444	<input type="checkbox"/>
49	PERAKIS, S. et al. "Advances in Circulating Tumor DNA Analysis" Adv Clin Chem (2017) pp 1-81	<input type="checkbox"/>
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1	PINKEL, et al. Comparative Genomic Hybridization. Annual Review of Genomics and Human Genetics, 6: 331-354 (2005).
2	PLEASANCE, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature. 2010 Jan 14;463(7278):184-90. doi: 10.1038/nature08629. Epub 2009 Dec 16.
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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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**REQUEST FOR CORRECTION IN A
PATENT APPLICATION RELATING TO
INVENTORSHIP OR AN INVENTOR
NAME, OR ORDER OF NAMES, OTHER
THAN IN A REISSUE APPLICATION (37
CFR 1.48)**

Application Number	16/714,579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Practitioner Docket Number	42534-708.305

To: **Commissioner for Patents**
P.O. Box 1450
Alexandria, VA 22313-1450

Applicant hereby requests that the inventorship be corrected or changed, or that the name of the inventor or a joint inventor, or the order of the names of joint inventors, be changed, in the above-identified application. **Note:** 37 CFR 1.48 applies to any request to correct inventorship filed on or after September 16, 2012, regardless of the application filing date. **Do not submit this form after payment of the issue fee or if the application has been patented.** See 37 CFR 1.324 for correction of inventorship in a patent.

Please check the applicable box(es) below.

For a nonprovisional application:

1. This request is to correct or change the inventorship in a **nonprovisional** application (under 37 CFR 1.48(a)) and includes:

An application data sheet (ADS) in accordance with 37 CFR 1.76(c) with the corrected or updated information shown with markings (e.g., underlining for insertions, strikethrough for deletions). See the Manual of Patent Examining Procedure (MPEP) section 601.05(a) for information about filing an ADS in an application filed on/after September 16, 2012. For information about filing a Supplemental ADS in an application filed before September 16, 2012, see MPEP 601.05(b).

The processing fee set forth in 37 CFR 1.17(i).

\$ 140

An inventor is being added. An inventor's oath or declaration by any actual inventor who has not yet executed an oath or declaration is required (see 37 CFR 1.48(b)). See MPEP 602.01(a) for information about an inventor's oath or declaration for an application filed on/after September 16, 2012 (e.g., form **PTO/AIA/01**). For information about an inventor's oath or declaration for an application filed before September 16, 2012 (e.g., form **PTO/SB/01**), see MPEP 602.01(b).

This request is being filed after the first Office action on the merits has been given or mailed (see 37 CFR 1.48(c) and 1.17(d)). Check one of the following:

This request to correct or change the inventorship is due solely to the cancellation of claims in the application.

OR

The fee set forth in 37 CFR 1.17(d) is due (in addition to the fee set forth in 37 CFR 1.17(i)). \$ _____

[Page 1 of 2]

This collection of information is required by 37 CFR 1.48. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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**REQUEST FOR CORRECTION IN A PATENT APPLICATION RELATING TO INVENTORSHIP OR AN INVENTOR NAME, OR ORDER OF NAMES, OTHER THAN IN A REISSUE APPLICATION
(37 CFR 1.48)**

2. This request is to correct or update the name of the inventor or a joint inventor, or the order of names of joint inventors, in a **nonprovisional** application (under 37 CFR 1.48(f)) and includes:

An application data sheet in accordance with 37 CFR 1.76(c) identifying the complete inventive entity, including the corrected or updated name of the inventor, or the new order of names shown with markings (e.g., underlining for insertions, strikethrough for deletions). See the MPEP 601.05(a) for information about filing an ADS in an application filed on/after September 16, 2012. For information about filing a Supplemental ADS in an application filed before September 16, 2012, see MPEP 601.05(b).

The processing fee set forth in 37 CFR 1.17(i). \$ _____

For a provisional application:

This request is to change or correct the inventorship, or correct or update the name of the inventor or a joint inventor, in a **provisional** application (under 37 CFR 1.48(d)) and includes:

Attached hereto is a request, signed by a party set forth in 37 CFR 1.33(b), that identifies each inventor by his or her legal name, in the preferred order.

The processing fee set forth in 37 CFR 1.17(q). \$ _____

Fee Payment Information:

Applicant asserts small entity status. See 37 CFR 1.27.

Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously

A check in the amount of the fee is enclosed.

Payment by credit card. Form PTO-2038 is attached.

The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 602231.

Payment made via EFS-Web.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

Applicant*

attorney or agent of record

attorney or agent acting under 37 CFR 1.34

Registration number 67740

Registration number _____

Signature /Timothy A. Hott/

Typed or printed name Timothy A. Hott/

Date January 16, 2020

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. *Juristic entities must be represented by a patent practitioner (See 37 CFR 1.31, applicable to any paper filed on or after September 16, 2012 that is presented on behalf of a juristic entity, regardless of application filing date). Submit multiple forms if more than one signature is required, see below**.

** Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16714579			
Filing Date:	13-Dec-2019			
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS			
First Named Inventor/Applicant Name:	AmirAli TALASAZ			
Filer:	Timothy A Hott/Michelle Chan			
Attorney Docket Number:	42534-708.305			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
PROCESSING FEE, EXCEPT PROV. APPLS.	1830	1	140	140
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				140

Electronic Acknowledgement Receipt

EFS ID:	38320983
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	16-JAN-2020
Filing Date:	13-DEC-2019
Time Stamp:	18:13:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$140
RAM confirmation Number	E20201FI14519729
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2020-01-16_GH0004US-CON4_MPRResponse.pdf	119957 8ebf0eea334b802e1eed6048a40c7a32557ed7ce	yes	7

Multipart Description/PDF files in .zip description					
Document Description		Start	End		
Applicant Arguments/Remarks Made in an Amendment		7	7		
Claims		3	6		
Specification		2	2		
Applicant Response to Pre-Exam Formalities Notice		1	1		

Warnings:

Information:

2	Specification	2020-01-16_GH0004US-CON4_SubSpecAsFiled.pdf	602353 71b377b85c3415fb05c1f118321924c1e5ecd74e	no	86
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Warnings:

Information:

3	Application Data Sheet	2020-01-16_GH0004US-CON4_CorrADS.pdf	8731640 4cd1f564808d4bcea6a770b6d208c352f46994d5	no	8
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Warnings:

Information:

This is not an USPTO supplied ADS fillable form

4	Oath or Declaration filed	2020-01-16_GH0004US-CON4_Mortimer_Dec.pdf	2445236 0eab8fa5008def7dbe9e9b96fe4e3d692f9b93ce	no	1
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Warnings:

Information:

5	Transmittal Letter	2020-01-16_GH0004US- CON4_IDSTrans.pdf	110207	no	4
			8686984f7acd7923805cf404dc0431d962 cb0f		
Warnings:					
Information:					
6	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US- CON4_SB08_1_OF_8.pdf	1067952	no	39
			94374daedf9fdf1f6a42b5272d0a2f7dd830 ee53		
Warnings:					
Information:					
7	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US- CON4_SB08_2_OF_8.pdf	1057321	no	10
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Warnings:					
Information:					
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8	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US- CON4_SB08_3_OF_8.pdf	1055741	no	8
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Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
9	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US- CON4_SB08_4_OF_8.pdf	1056441	no	8
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Warnings:					
Information:					
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10	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US-CON4_SB08_5_OF_8.pdf	1057801	no	8
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11	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US-CON4_SB08_6_OF_8.pdf	1055470	no	8
			e6264ff99a50d73447596ab55dc1c148b1d0f6eb		

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Information:

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12	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US-CON4_SB08_7_OF_8.pdf	1057629	no	8
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Warnings:

Information:

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13	Information Disclosure Statement (IDS) Form (SB08)	2020-01-16_GH0004US-CON4_SB08_8_OF_8.pdf	1056623	no	7
			6b258cb0505d19afdb122a2f2d1ccba23f1a2c59		

Warnings:

Information:

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14	Request under Rule 48 correcting inventorship	2020-01-16_GH0004US-CON4_ReqCorrInv.pdf	158837	no	3
			2831af886f55e33abd163e9812cfc14e3cb8b4e1		

Warnings:

Information:					
15	Fee Worksheet (SB06)	fee-info.pdf	30438	no	2
			f7eebc714c69667e732fb656f4933557b63c03d2		
Warnings:					
Information:					
Total Files Size (in bytes):				20663646	
<p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

REMARKS

Claims

Claims 1-30 were previously pending, but are hereby cancelled without disclaimer or prejudice. Claims 31-60 are newly added. Support for the new claims may be found throughout the application as filed. No new matter is added by these amendments. Thus, claims 31-60 are now pending and ready for examination.

Substitute Specification

A substitute specification is filed herewith that includes a line-spacing correction to page 79. No new matter is added by this amendment. Accordingly, Applicant submits that the specification is in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125.

CONCLUSION

Applicant believes that the present application is now in condition for examination and respectfully requests that the Examiner expedite the prosecution of this application to allowance. The Commissioner is authorized to charge any underpayment, or credit any overpayment, to Deposit Account No. 60-2231 (Attorney Docket No. GH0004US-CON4).

Respectfully submitted,
GUARDANT HEALTH, INC.

Date: January 16, 2020

By: /Timothy A. Hott/

Timothy A. Hott
Registration No.: 67740

GUARDANT HEALTH, INC.
505 Penobscot Drive
Redwood City, CA 94063
Customer No. 115823

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application. The foregoing amendments are without prejudice and do not constitute an admission regarding the patentability of the amended subject matter and should not so be construed. Applicant reserves the right to pursue the subject matter of the canceled claims in this or any other appropriate patent application.

Listing of Claims:

1-30. (Cancelled).

31. (New): A method for processing polynucleotide molecules from a sample of a subject, comprising:

exposing the polynucleotide molecules from the sample to a set of library adaptors comprising a plurality of molecular barcodes to generate tagged parent polynucleotides,

wherein exposing comprises ligating a plurality of library adaptors from the set of library adaptors to some of the polynucleotide molecules from the sample using at least a 10X molar excess of library adaptors as compared to the polynucleotide molecules;

wherein exposing yields at least 20% of the polynucleotide molecules from the sample having library adaptors ligated to both ends of a molecule of the polynucleotide molecules.

32. (New): The method of claim 31, wherein the polynucleotide molecules are cell-free deoxyribonucleic acid (cfDNA) molecules.

33. (New): The method of claim 31, wherein the polynucleotide molecules are fragmented nucleic acid molecules.

34. (New): The method of claim 31, wherein the sample is selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, and tears.

35. (New): The method of claim 31, wherein the library adaptors are ligated to the polynucleotide molecules by blunt-end ligation or sticky-end ligation.

36. (New): The method of claim 31, wherein the ligating comprises using more than a 100X molar excess of library adaptors as compared to the polynucleotide molecules.

37. (New): The method of claim 31, wherein at least 40% of the polynucleotide molecules from the sample are attached to library adaptors at both ends of a molecule of the polynucleotide molecules.
38. (New): The method of claim 31, wherein the molecular barcodes in the plurality of library adaptors have 2 to 100,000 different molecular barcode sequences.
39. (New): The method of claim 31, wherein the molecular barcodes in the plurality of library adaptors have 5 to 100 different molecular barcode sequences.
40. (New): The method of claim 39, wherein the molecular barcodes have a length of 5 to 20 nucleotides.
41. (New): The method of claim 31, wherein the molecular barcodes are different from one another and have an edit distance of at least 1 between one another.
42. (New): The method of claim 31, wherein the library adaptors are Y-shaped adaptors.
43. (New): The method of claim 31, further comprising amplifying a plurality of the tagged parent polynucleotides to generate amplified progeny polynucleotides.
44. (New): The method of claim 43, further comprising selectively enriching the amplified progeny polynucleotides for a plurality of genomic regions of interest.
45. (New): The method of claim 44, wherein the plurality of genomic regions of interest comprises exon sequences.
46. (New): The method of claim 44, the wherein the genomic regions of interest comprise sequences from one or more genes selected from the group consisting of:
ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
47. (New): The method of claim 44, wherein selectively enriching the subset of amplified progeny polynucleotides for a plurality of genomic regions of interest comprises using a set of probes that hybridize to the plurality of genomic regions of interest.

48. (New): The method of claim 44, further comprising amplifying a subset of enriched amplified progeny polynucleotides.
49. (New): A method for analyzing polynucleotide molecules from a sample of a subject, comprising:
- (a) exposing the polynucleotide molecules from the sample to a set of library adaptors comprising a plurality of molecular barcodes to generate tagged parent polynucleotides,
 - wherein exposing comprises ligating a plurality of library adaptors from the set of library adaptors to some of the polynucleotide molecules from the sample using at least a 10X molar excess of library adaptors as compared to the polynucleotide molecules;
 - wherein exposing yields at least 20% of the polynucleotide molecules from the sample having library adaptors ligated to both ends of a molecule of the polynucleotide molecules;
 - (b) subjecting a plurality of the tagged parent polynucleotides to amplification reactions under conditions that yield amplified progeny polynucleotides;
 - (c) selectively enriching the amplified progeny polynucleotides for genomic regions of interests;
 - (d) determining nucleotide sequences of a plurality of enriched amplified progeny polynucleotides; and
 - (e) analyzing the nucleotide sequences with a programmed computer processor to identify one or more genetic variants in the sample of the subject.
50. (New): The method of claim 49, wherein the polynucleotide molecules are cell-free deoxyribonucleic acid (cfDNA) molecules.
51. (New): The method of claim 49, wherein the polynucleotide molecules are fragmented nucleic acid molecules.
52. (New): The method of claim 49, wherein the sample is selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, and tears.

53. (New): The method of claim 49, wherein the library adaptors are ligated to the polynucleotide molecules by blunt-end ligation or sticky-end ligation.
54. (New): The method of claim 49, wherein the ligating comprises using more than a 100X molar excess of library adaptors as compared to the polynucleotide molecules.
55. (New): The method of claim 49, wherein at least 40% of the polynucleotide molecules from the sample are attached to library adaptors at both ends of a molecule of the polynucleotide molecules.
56. (New): The method of claim 49, wherein the genomic regions of interest comprise sequences from one or more genes selected from the group consisting of:
ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
57. (New): The method of claim 49, wherein the analyzing in (e) further comprises mapping a plurality of the nucleotide sequences to a reference sequence.
58. (New): The method of claim 57, further comprising sorting the nucleotide sequences into families based on a combination of the molecular barcode sequences and the mapping positions of the nucleotide sequences that map to a reference sequence, wherein a family is representative of a tagged parent polynucleotide.
59. (New): The method of claim 58, further comprising determining a consensus sequence for each of the families, and identifying consensus sequences having a genetic variant as compared to the reference sequence.
60. (New): The method of claim 49, wherein the one or more genetic variants comprise a cancer-associated somatic mutation.

AMENDMENTS TO THE CLAIMS

Please replace the specification with the substitute specification submitted herewith.

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number
16/714,579

APPLICATION AS FILED - PART I

(Column 1) (Column 2)

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A
TOTAL CLAIMS (37 CFR 1.16(j))	30 minus 20 = *	10
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 = *	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))		

SMALL ENTITY

RATE(\$)	FEE(\$)
N/A	
N/A	
N/A	
TOTAL	

OR OTHER THAN SMALL ENTITY

RATE(\$)	FEE(\$)
N/A	300
N/A	660
N/A	760
x 100 =	1000
x 460 =	0.00
	0.00
	0.00
TOTAL	2720

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED - PART II

(Column 1) (Column 2) (Column 3)

AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total (37 CFR 1.16(i))	*	Minus	**
Independent (37 CFR 1.16(h))	*	Minus	***	=
Application Size Fee (37 CFR 1.16(s))				
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))				

SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

OR OTHER THAN SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

(Column 1) (Column 2) (Column 3)

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total (37 CFR 1.16(i))	*	Minus	**
Independent (37 CFR 1.16(h))	*	Minus	***	=
Application Size Fee (37 CFR 1.16(s))				
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))				

SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

OR OTHER THAN SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/714,579	12/13/2019	AmirAli TALASAZ	42534-708.305

CONFIRMATION NO. 3111

**37 CFR 1.48 ACKNOWLEDGEMENT
LETTER**

115823
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304



OC000000114077936

Date Mailed: 01/22/2020

NOTICE OF ACCEPTANCE OF REQUEST UNDER 37 CFR 1.48(a)

This is in response to the applicant's request under 37 CFR 1.48(a) submitted on 01/16/2020.

The request under 37 CFR 1.48(a) to correct the inventorship, to correct or update the name of an inventor, or to correct the order of names of joint inventors is accepted.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/ebekele/



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Table with 7 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY. DOCKET NO, TOT CLAIMS, IND CLAIMS. Row 1: 16/714,579, 12/13/2019, 1636, 2720, 42534-708.305, 30, 2

CONFIRMATION NO. 3111

UPDATED FILING RECEIPT

115823
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304



Date Mailed: 01/22/2020

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

AmirAli TALASAZ, Atherton, CA;
Stefanie Ann Ward MORTIMER, Morgan Hill, CA;

Applicant(s)

GUARDANT HEALTH, INC., Redwood City, CA;

Power of Attorney: The patent practitioners associated with Customer Number 115823

Domestic Priority data as claimed by applicant

This application is a CON of 16/672,267 01/07/2020
which is a CON of 16/601,168 10/14/2019
which is a CON of 15/892,178 02/08/2018
which is a CON of 14/861,989 09/22/2015 PAT 9920366
which is a CON of PCT/US2014/072383 12/24/2014
which claims benefit of 61/948,509 03/05/2014
and claims benefit of 61/921,456 12/28/2013

Foreign Applications for which priority is claimed (You may be eligible to benefit from the Patent Prosecution Highway program at the USPTO. Please see http://www.uspto.gov for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

Projected Publication Date: 04/30/2020

Non-Publication Request: No

Early Publication Request: No

Title

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

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115823
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

CONFIRMATION NO. 3111
UPDATED FILING RECEIPT



Date Mailed: 01/22/2020

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AmirAli TALASAZ, Atherton, CA;
Stefanie Ann Ward MORTIMER, Morgan Hill, CA;

Applicant(s)

GUARDANT HEALTH, INC., Redwood City, CA;

Power of Attorney: The patent practitioners associated with Customer Number 115823

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which is a CON of 16/601,168 10/14/2019
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Non-Publication Request: No

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Preliminary Class

435

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NOT GRANTED

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Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO.
16/714,579 12/13/2019 AmirAli TALASAZ 42534-708.305 3111

115823 7590 01/28/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

EXAMINER

BENZION, GARY

ART UNIT PAPER NUMBER

1637

NOTIFICATION DATE DELIVERY MODE

01/28/2020

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents@guardanthealth.com
patentdocket@wsgr.com

<i>Decision Granting Request for Prioritized Examination (Track I)</i>	Application No. 16/714,579	Applicant(s) TALASAZ et al.	
	Examiner DIANE C GOODWYN	Art Unit OPET	AIA (FITF) Status Yes

1. THE REQUEST FILED 13 December 2019 IS **GRANTED** .

The above-identified application has met the requirements for prioritized examination

- A. for an original nonprovisional application (Track I).
- B. for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

- A. filing a **petition for extension of time** to extend the time period for filing a reply;
- B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
- C. filing a **request for continued examination** ;
- D. filing a notice of appeal;
- E. filing a request for suspension of action;
- F. mailing of a notice of allowance;
- G. mailing of a final Office action;
- H. completion of examination as defined in 37 CFR 41.102; or
- I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to DIANE GOODWYN at (571) 272-6735. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/DIANE C GOODWYN/
Paralegal Specialist, OPET



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/714,579	12/13/2019	AmirAli TALASAZ	42534-708.305	3111
115823	7590	03/03/2020	EXAMINER	
Wilson Sonsini Goodrich & Rosati / Guardant Health			HORLICK, KENNETH R	
650 Page Mill Road			ART UNIT	
Palo Alto, CA 94304			PAPER NUMBER	
			1637	
			NOTIFICATION DATE	
			DELIVERY MODE	
			03/03/2020	
			ELECTRONIC	

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Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents@guardanthealth.com
patentdocket@wsg.com

Continuation of Attachment(s) 2) Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date: 1/16/20; 1/16/20; 1/16/20; 1/16/20; 1/16/20; 1/16/20; 1/16/20; 1/16/20

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

NON-PRIOR ART REJECTIONS

2. The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 46 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention. This claim is confusing because of the language in line 1 'the wherein the'. Correction is required.

3. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided

the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(I)(I) - 706.02(I)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-l.jsp.

4. Claims 31-60 is rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-33 of U.S. Patent No. 9,902,992. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims and the patented claims are related as genus-species. That is, the step of the instant claim is included within the steps of the patent claims.

5. Claims 31-60 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 9,920,366. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims and the patented claims are related as genus-species. That is, the step of the instant claims is included within the steps of the patent claims.

6. Claims 31-60 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31-53 of copending Application No. 15/892,178 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims and the copending claims are related as genus-species. That is, the step of the instant claims is included within the steps of the copending claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

7. Claims 31-60 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31-60 of copending Application No. 16/601,168 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims and the copending claims are related as genus-species. That is, the step of the instant claims is included within the steps of the copending claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

8. Claims 31-60 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31-60 of copending Application No. 16/672,267 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the instant claims and the copending claims are related as genus-species. That is, the step of the instant claims is included within the steps of the copending claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

PRIOR ART REJECTION

9. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of

the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

10. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

Claims 31-60 are rejected under 35 U.S.C. 103 as being unpatentable over Schmitt et al. (US 9,752,188; effective filing date 3/20/12) in view of Deciu et al. (US 2013/0288244; effective filing date 3/14/13).

Claims 31-48 are drawn to a method comprising: tagging polynucleotide molecules with a set of library adaptors comprising barcodes, wherein at least a 10X molar excess of library adaptors is used relative to polynucleotide molecules, and at least 20% of molecules are tagged at both ends. Claims 49-60 are drawn to such a method, additionally comprising: amplification, enrichment, determining sequence, and computer analysis.

Schmitt et al. discloses a method comprising: tagging double-stranded DNA molecules with a set of duplex tags comprising barcodes; selectively enriching (amplifying) for tagged strands that map to a genetic locus; sequencing enriched tagged strands to generate sequence reads; and grouping

sequence reads into families and collapsing sequence reads into consensus sequence reads using computer analysis. See columns 5-30, especially column 20, line 39 to column 21, line 25.

Schmitt et al. does not disclose wherein at least a 10X molar excess of library adaptors is used relative to polynucleotide molecules, and wherein at least 20% of molecules are tagged at both ends.

One of ordinary skill in the art would have been motivated to modify the method of Schmitt et al. by using conditions such that at least a 10X molar excess of library adaptors is used relative to polynucleotide molecules, and at least 20% of molecules are tagged, because this would have merely involved routine optimization of known-important reaction parameters, which as well established in U.S. patent practice does not support unobviousness (see M.P.E.P. 2144.05). This is supported by Deciu et al., which discloses the desirability to optimize ligation of adaptors to both ends of a polynucleotide in paragraph [1152]. Ligation reactions were textbook-level subject matter for which the important parameters, such as enzyme and polynucleotide component concentrations, were unarguably known. It is submitted that the further limitations of the dependent claims also fall within the category of routine optimization of known-important reaction parameters. Thus, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the application was filed to carry out the claimed methods.

CONCLUSION

11. No claims are free of the prior art.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KENNETH R HORLICK whose telephone number is (571)272-0784. The examiner can normally be reached on Mon. - Thurs. 8:30 - 6:30.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

02/27/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Notice of References Cited

Application/Control No.
16/714,579

Applicant(s)/Patent Under
Reexamination
TALASAZ et al.

Examiner
KENNETH R HORLICK

Art Unit
1637

Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20130288244-A1	10-2013	DECIU; Cosmin	G16B20/00	435/6.11
	B					
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FOREIGN PATENT DOCUMENTS

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Search Notes 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
inventor name search	02/27/2020	KH
updated parent searches in USPAT and PGPUB	02/27/2020	KH
reviewed parent applications and references therein	02/27/2020	KH

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	
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Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (02-18)

Approved for use through 11/30/2020. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

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Examiner Name		
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EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	02/26/2020
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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
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Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
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(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

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STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
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Art Unit	
Examiner Name	
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**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
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STATEMENT BY APPLICANT**
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Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
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	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.305

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Name/Print	Timothy A. Hott	Registration Number	67740

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(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

34	Office Action dated 12/09/2016 for U.S. Patent Application No. 14/861,989.	
35	Office action dated 12/21/2018 for US Application No. 15/978,848.	
36	OGINO, et al. Quantification of PCR bias caused by a single nucleotide polymorphism in SMN gene dosage analysis. J Mol Diagn. 2002 Nov;4(4):185-90.	
37	Opposition Brief dated August 7, 2018 for Japanese Opposition No. 2018-700659 to JP Patent 6275145	×
38	Opposition Decision dated January 15, 2019 for Japanese Opposition No. 2018-700659 to JP Patent 6275145	☒
39	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Foundation Medicine Inc.	<input type="checkbox"/>
40	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Grunecker Patent-und Rechtsanwälte PartG mbB	<input type="checkbox"/>
41	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Personal Genome Diagnostics, Inc.	<input type="checkbox"/>
42	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Strawman Limited	<input type="checkbox"/>
43	Opposition Form and Statement to EP3087204 filed November 14, 2018	<input type="checkbox"/>
44	Pacific Biosciences. Template Preparation and Sequencing Guide. Publication date: 2014-10-14. Pacific Biosciences website http://www.pacificbiosciences.com/support/pubmap/documentation.html .	<input type="checkbox"/>

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45	PAN, et al. Loss of heterozygosity patterns provide fingerprints for genetic heterogeneity in multistep cancer progression of tobacco smoke-induced non-small cell lung cancer. Cancer Res. 2005 Mar 1;65(5):1664-9.	<input type="checkbox"/>
46	PARK, et al. Discovery of common Asian copy number variants using integrated high-resolution array CGH and massively parallel DNA sequencing. Nat Genet. 2010 May;42(5):400-5. doi: 10.1038/ng.555. Epub 2010 Apr 4.	<input type="checkbox"/>
47	PARKINSON, N.J. et al., "Preparation of high-quality next-generation sequencing libraries from picogram quantities of target DNA," Genome Res. 2012, 22(1), 125-133	<input type="checkbox"/>
48	PEL, J. et al. "Duplex proximity sequencing (pro-seq): A method to improve DNA sequencing accuracy without the cost of molecular barcoding redundancy" bioRxiv (2017) https://doi.org/10.1101/163444	<input type="checkbox"/>
49	PERAKIS, S. et al. "Advances in Circulating Tumor DNA Analysis" Adv Clin Chem (2017) pp 1-81	<input type="checkbox"/>
50	PIHLAK, et al. Rapid genome sequencing with short universal tiling probes. Nature Biotechnology, 26: 676-684 (2008).	<input type="checkbox"/>

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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1	PINKEL, et al. Comparative Genomic Hybridization. Annual Review of Genomics and Human Genetics, 6: 331-354 (2005).
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12	RYAN, et al. A prospective study of circulating mutant KRAS2 in the serum of patients with colorectal neoplasia: strong prognostic indicator in postoperative follow up. Gut. 2003 Jan;52(1):101-8.
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23	SEHNERT, A.J. et al. "Optimal Detection of Fetal Chromosomal Abnormalities by Massively Parallel DNA Sequencing of Cell-Free Fetal DNA from Maternal Blood" Clin Chem (2011) 57(7):1042-1049
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34	SPARKS, et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn. 2012 Jan;32(1):3-9. doi: 10.1002/pd.2922. Epub 2012 Jan 6
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40	TABACK, et al. Detection of tumor-specific genetic alterations in bone marrow from early-stage breast cancer patients. Cancer Res. 2003 Apr 15;63(8):1884-7
41	TAN, et al. Genome-wide comparison of DNA hydroxymethylation in mouse embryonic stem cells and neural progenitor cells by a new comparative hMeDIP-seq method. Nucleic Acids Res. 2013 Apr;41(7):e84. doi: 10.1093/nar/gkt091. Epub 2013 Feb 13.
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43	TEER, J.K. et al. "Systematic comparison of three genomic enrichment methods for massively parallel DNA sequencing" Genome Res (2010) 20(10):1420-1431
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49	J.S. Provisional Application 61/613,413 ("Schmitt '413 provisional") (2012-03-20)
50	J.S. Provisional Application 61/625,319 ("Schmitt '319 provisional") (2012-04-17)

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STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit		
Examiner Name		
Attorney Docket Number	42534-708.305	

1	Guardant Health vs. FMI Invalidation Exhibit C-3, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
2	Guardant Health vs. FMI Invalidation Exhibit C-4, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
3	Guardant Health vs. FMI Invalidation Exhibit C-5, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
4	Guardant Health vs. FMI Invalidation Exhibit D-1, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
5	Guardant Health vs. FMI Invalidation Exhibit D-2, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
6	Guardant Health vs. FMI Invalidation Exhibit D-3, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
7	Guardant Health vs. FMI Invalidation Exhibit D-5, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
8	Guardant Health vs. FMI Second Amended Complaint dated March 6, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
9	Guardant Health vs. FMI Suppl Invalidation References dated February 15, 2019 (C.A. No. 17-cv-1616-LPS-CJB)
10	Guardant Health vs. FMI Supplemental Invalidation Contentions dated March 29, 2019 (C.A. No. 17-cv-1616-LPS-CJB)
11	Guardant Health vs. PGDx Amended Answer to Second Amended Complaint dated April 30, 2019 (C.A. No. 17-cv-1623-LPS-CJB)

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12	Guardant Health vs. PGDx Invalidation Claim Chart A, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
13	Guardant Health vs. PGDx Invalidation Claim Chart B, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
14	Guardant Health vs. PGDx Invalidation Claim Chart C, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
15	Guardant Health vs. PGDx Invalidation Claim Chart D, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
16	Guardant Health vs. PGDx Invalidation Claim Chart E, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
17	Guardant Health vs. PGDx Invalidation Claim Chart F, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
18	Guardant Health vs. PGDx Invalidation Claim Chart G, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
19	Guardant Health vs. PGDx Invalidation Claim Chart H, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
20	Guardant Health vs. PGDx Invalidation Claim Chart I, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
21	Guardant Health vs. PGDx Invalidation Claim Chart J, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
22	Guardant Health vs. PGDx Invalidation Claim Chart K, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)

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23	Guardant Health vs. PGDx Invalidation Claim Chart L, dated May 13, 2019 (C.A. No. 17-cv-1623-LPS-CJB)
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Name/Print	Timothy A. Hott	Registration Number	67740

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Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.305

38	Guardant Health vs. FMI 1st Amended Answer to Second Amended Complaint dated May 6, 2019 (C.A. No. 17-cv-1616-LPS-CJB)
39	Guardant Health vs. FMI Invalidity Contentions dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
40	Guardant Health vs. FMI Invalidity Exhibit A-1, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
41	Guardant Health vs. FMI Invalidity Exhibit A-2, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
42	Guardant Health vs. FMI Invalidity Exhibit A-3, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
43	Guardant Health vs. FMI Invalidity Exhibit A-8, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
44	Guardant Health vs. FMI Invalidity Exhibit B-1, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
45	Guardant Health vs. FMI Invalidity Exhibit B-2, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
46	Guardant Health vs. FMI Invalidity Exhibit B-3, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
47	Guardant Health vs. FMI Invalidity Exhibit B-7, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
48	Guardant Health vs. FMI Invalidity Exhibit C-1, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16714579
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49	Guardant Health vs. FMI Invalidity Exhibit C-11, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)
50	Guardant Health vs. FMI Invalidity Exhibit C-2, dated June 25, 2018 (C.A. No. 17-cv-1616-LPS-CJB)

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The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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SIGNATURE

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-16
Name/Print	Timothy A. Hott	Registration Number	67740

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CONFIRMATION NO. 3111

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
16/714,579	12/13/2019	435	1637	42534-708.305
	RULE			

APPLICANTS

GUARDANT HEALTH, INC., Redwood City, CA;

INVENTORS

AmirAli TALASAZ, Atherton, CA;
 Stefanie Ann Ward MORTIMER, Morgan Hill, CA;

**** CONTINUING DATA *******

This application is a CON of 16/672,267 01/07/2020
 which is a CON of 16/601,168 10/14/2019
 which is a CON of 15/892,178 02/08/2018
 which is a CON of 14/861,989 09/22/2015 PAT 9920366
 which is a CON of PCT/US2014/072383 12/24/2014
 which claims benefit of 61/948,509 03/05/2014
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 Palo Alto, CA 94304
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TITLE

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CONFIRMATION NO. 3111

PUBLICATION NOTICE

115823
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304



Title:METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

Publication No.US-2020-0131568-A1
Publication Date:04/30/2020

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli TALASAZ et al.	Confirmation No.: 3111
Serial Number: 16/714,579	Customer No.: 115823
Filing Date: December 13, 2019	Group Art Unit: 1637
Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: Kenneth R. HORLICK

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE TO NON-FINAL OFFICE ACTION

Sir:

This paper is in response to the Non-Final Office Action mailed on March 3, 2020. The shortened statutory period for reply expires June 3, 2020, therefore, this response is timely filed. Applicant respectfully requests consideration of the above-referenced application in view of the following amendments and remarks:

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 8 of this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application. The foregoing amendments are without prejudice and do not constitute an admission regarding the patentability of the amended subject matter and should not so be construed. Applicant reserves the right to pursue the subject matter of the canceled claims in this or any other appropriate patent application.

Listing of Claims:

1. - 30. (Cancelled).

31. (Currently amended): A method for ~~processing~~ analyzing sequencing reads of double-stranded cell-free deoxyribonucleic acid (cfDNA) polynucleotide molecules from a sample of a subject, comprising:

(a) ~~exposing~~ tagging a plurality of double-stranded cfDNA ~~the polynucleotide~~ molecules from a population of double-stranded cfDNA molecules obtained from the sample to a set of library adaptors comprising a plurality of molecular barcodes to generate tagged parent polynucleotides,

wherein ~~exposing~~ tagging comprises ligating a plurality of library adaptors from the set of library adaptors to ~~some of the~~ plurality of double-stranded cfDNA polynucleotide molecules ~~from~~ from the population sample using at least a 10X molar excess of library adaptors as compared to the cfDNA polynucleotide molecules in the population;

wherein ~~exposing~~ tagging yields at least 20% of the cfDNA polynucleotide molecules from the population sample having library adaptors ligated to both ends of a molecule of the double-stranded cfDNA polynucleotide molecules;

(b) amplifying a plurality of the tagged parent polynucleotides to produce progeny polynucleotides;

(c) sequencing at least a subset of the progeny polynucleotides to produce a set of sequencing reads; and

(d) determining, based at least on sequence information from the molecular barcodes, individual double-stranded cfDNA molecules from among the tagged parent

polynucleotides for which (1) both a Watson strand and a Crick strand are detected or (2) only a Watson strand or a Crick strand is detected from a plurality of sequencing reads from the set of sequencing reads.

32. (Cancelled).

33. (Cancelled).

34. (Previously presented): The method of claim 31, wherein the sample is selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, and tears.

35. (Currently amended): The method of claim 31, wherein the library adaptors are ligated to the double-stranded cfDNA polynucleotide molecules by blunt-end ligation or sticky-end ligation.

36. (Currently amended): The method of claim 31, wherein the ligating comprises using more than a 100X molar excess of library adaptors as compared to the double-stranded cfDNA polynucleotide molecules.

37. (Currently amended): The method of claim 31, wherein at least 40% of the double-stranded cfDNA polynucleotide molecules from the sample are attached to library adaptors at both ends of a molecule of the double-stranded cfDNA polynucleotide molecules.

38. (Previously presented): The method of claim 31, wherein the molecular barcodes in the plurality of library adaptors have 2 to 100,000 different molecular barcode sequences.

39. (Previously presented): The method of claim 31, wherein the molecular barcodes in the plurality of library adaptors have 5 to 100 different molecular barcode sequences.

40. (Previously presented): The method of claim 39, wherein the molecular barcodes have a length of 5 to 20 nucleotides.

41. (Currently amended): The method of claim 31, wherein a plurality of the molecular barcodes have are different sequences from one another and have an edit distance of at least 1 between one another.

42. (Previously presented): The method of claim 31, wherein the library adaptors are Y-shaped adaptors.

43. (Cancelled).

44. (Currently amended): The method of claim 31 ~~[[43]]~~, further comprising selectively enriching the amplified progeny polynucleotides for a plurality of genomic regions of interest.
45. (Previously presented): The method of claim 44, wherein the plurality of genomic regions of interest comprises exon sequences.
46. (Currently amended): The method of claim 44, ~~the~~ wherein the genomic regions of interest comprise sequences from one or more genes selected from the group consisting of:
ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
47. (Previously presented): The method of claim 44, wherein selectively enriching the subset of amplified progeny polynucleotides for a plurality of genomic regions of interest comprises using a set of probes that hybridize to the plurality of genomic regions of interest.
48. (Previously presented): The method of claim 44, further comprising amplifying a subset of enriched amplified progeny polynucleotides.
49. (Currently amended): A method for analyzing double-stranded cell-free deoxyribonucleic acid (cfDNA) ~~polynucleotide~~ molecules from a sample of a subject, comprising:
- (a) exposing a population of the double-stranded cfDNA ~~polynucleotide~~ molecules obtained from the sample to a set of library adaptors comprising a plurality of molecular barcodes to generate tagged parent polynucleotides,
wherein exposing comprises ligating a plurality of library adaptors from the set of library adaptors to some of the double-stranded cfDNA ~~polynucleotide~~ molecules from the sample using at least a 10X molar excess of library adaptors as compared to the double-stranded cfDNA ~~polynucleotide~~ molecules in the population;

- wherein exposing yields at least 20% of the double-stranded cfDNA polynucleotide molecules from the population sample having library adaptors ligated to both ends of a molecule of the double-stranded cfDNA polynucleotide molecules;
- (b) subjecting a plurality of the tagged parent polynucleotides to amplification reactions under conditions that yield amplified progeny polynucleotides;
- ~~(c) selectively enriching the amplified progeny polynucleotides for genomic regions of interests;~~
- (c) ~~[[d]]~~ determining nucleotide sequences of a plurality of ~~enriched~~ amplified progeny polynucleotides; and
- (d) ~~[[e]]~~ analyzing a plurality of the nucleotide sequences with a programmed computer processor, comprising:
- mapping a plurality of the nucleotide sequences to a reference sequence to generate mapped sequences,
- grouping a plurality of the mapped sequences into families based on a combination of sequence information from the molecular barcodes and start and stop positions of the mapped sequences, wherein a family is representative of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides, and
- identifying a plurality of the families as having nucleotide sequences representing either (1) both a Watson strand and a Crick strand of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides or (2) only a Watson strand or a Crick strand of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides to identify one or more genetic variants in the sample of the subject.

50. (Cancelled).

51. (Cancelled).

52. (Previously presented): The method of claim 49, wherein the sample is selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, and tears.

53. (Currently amended): The method of claim 49, wherein the library adaptors are ligated to the double-stranded cfDNA polynucleotide molecules by blunt-end ligation or sticky-end ligation.
54. (Currently amended): The method of claim 49, wherein the ligating comprises using more than a 100X molar excess of library adaptors as compared to the double-stranded cfDNA polynucleotide molecules.
55. (Currently amended): The method of claim 49, wherein at least 40% of the double-stranded cfDNA polynucleotide molecules from the sample are attached to library adaptors at both ends of a molecule of the double-stranded cfDNA polynucleotide molecules.
56. (Currently amended): The method of claim 49, further comprising enriching the amplified progeny polynucleotides for genomic regions of interest prior to sequencing, wherein the genomic regions of interest comprise sequences from one or more genes selected from the group consisting of:
ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
57. - 60. (Cancelled).
61. (New): The method of claim 49, wherein the double-stranded cfDNA molecules are non-uniquely tagged, wherein the double-stranded cfDNA molecules that map to a mappable base position of a reference sequence are tagged with a number of different molecular barcodes ranging from at least 2 and fewer than a number of double-stranded cfDNA molecules that map to the mappable base position.
62. (New): The method of claim 49, wherein nucleotide sequences generated from a Watson strand are distinguished from nucleotide sequences generated from a Crick strand of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides based at least on sequence information from the molecular barcodes.

63. (New): The method of claim 31, wherein the tagging comprises non-uniquely tagging the plurality of double-stranded cfDNA molecules, wherein the double-stranded cfDNA molecules that map to a mappable base position of a reference sequence are tagged with a number of different molecular barcodes ranging from at least 2 and fewer than a number of double-stranded cfDNA molecules that map to the mappable base position.
64. (New): The method of claim 31, wherein sequencing reads generated from a Watson strand are distinguished from sequencing reads generated from a Crick strand of a double-stranded cfDNA molecule from among the tagged parent polynucleotides based at least on sequence information from the molecular barcodes.
65. (New): The method of claim 31, further comprising mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence prior to (d).
66. (New): The method of claim 65, further comprising grouping a plurality of sequencing reads mapping to the reference sequence into families based on a combination of sequence information from the molecular barcodes and start and stop positions of the sequencing reads that map to the reference sequence, wherein a family is representative of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides.
67. (New): The method of claim 31, further comprising:
- (e) estimating a number of total tagged parent polynucleotides that map to one or more genetic loci of a reference sequence based at least on quantitative measures of individual double-stranded cfDNA molecules determined in (d) that map to the one or more genetic loci.
68. (New): The method of claim 67, wherein estimating the number of total tagged parent polynucleotides is further based on inferring, from at least the quantitative measure of the individual double-stranded cfDNA molecules determined in (d), a quantitative measure of individual double-stranded cfDNA that map to each of the one or more genetic loci for which neither a Watson strand or a Crick strand was detected.

REMARKS

Claims 31-60 were pending prior to entry of the above-referenced claim amendments. Claims 32-33, 43, 50-51, and 57-60 are hereby cancelled without disclaimer or prejudice. Claims 31, 35-37, 41, 44, 46, and 53-56 are amended to clarify the claimed subject matter, to correct for typographical errors, and/or for antecedent basis purposes. Claims 61-68 are newly added. Support for these amendments may be found throughout the application as published, for example, at least at paragraphs [0006], [00115], [00174]-[00186], and Figure 2, and the subject matter of previously pending claims 32, 50 and 58. No new matter is believed to be added by these amendments. Therefore, claims 31, 34-42, 44-49, 52-56 and 61-68 are pending and set for examination.

Rejection- 35 U.S.C. §112(b)

Claim 46 was rejected under 35 U.S.C. §112(b) as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter the inventor regards as the invention.

Applicant has amended claim 46 to remove the extra “the” in the wherein clause. Accordingly, Applicant respectfully requests that this rejection be withdrawn.

Rejection- Nonstatutory Double Patenting

Claims 31-60 were rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 1-33 of U.S. Patent No. 9,902,992. Claims 31-60 were rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 1-21 of U.S. Patent No. 9,920,366. Claims 31-60 were provisionally rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 31-53 of copending Application No. 15/892,178. Claims 31-60 were provisionally rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 31-60 of copending Application No. 16/601,168. Claims 31-60 were provisionally rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 31-60 of copending Application No. 16/672,267.

Without conceding in the basis of rejection, Applicant respectfully requests that the nonstatutory double patenting rejections be held in abeyance until allowable subject matter has been determined.

Rejection- 35 U.S.C. §103

Claims 31-60 were rejected under 35 U.S.C. §103 as allegedly being unpatentable over Schmitt et al. (U.S. 9,752,188) in view of Deciu et al. (U.S. 2013/0288244).

Without conceding in the basis of the rejection, and solely to expedite prosecution, independent claims 31 and 49 are amended herein to clarify the claimed subject matter over the cited art. Applicant submits that the asserted combination of Schmitt et al. and Deciu et al. does not meet all of the elements of independent claims 31 and 49 as amended, much less the elements of dependent claims 34-42, 44-48, and 52-56. Moreover, a person of ordinary skill in the art would recognize that there was no motivation or reasonable expectation of success in applying the method of Schmitt et al. to cfDNA. Indeed, as disclosed in Perakis et al., “Advances in Circulating Tumor DNA Analysis,” *Adv. Clin Chem* (2017) pp 1-81 (IDS submitted 1/16/20), “[p]rospects of success are limited since the [Schmitt et al.] method is relatively inefficient when limited amounts of input DNA—as it is most likely the case for cfDNA—are used” (page 30, internal citation omitted).

Therefore, independent claims 31 and 49 and dependent claims 34-42, 44-48, and 52-56 are not obvious over Schmitt et al. in view of Deciu et al. For purposes solely to expedite the prosecution of this application, claims 32-33, 43, 50-51, and 57-60 are cancelled, thereby rendering the rejection moot as to these claims.

Accordingly, Applicant respectfully requests that the §103 rejection of the above-mentioned claims over Schmitt et al. in view of Deciu et al. be withdrawn.

It shall be understood herein that any instance in which Applicant has addressed certain comments set forth by the Office shall not be construed as a concession to other comments or arguments advanced by the Office. Any circumstance in which Applicant has amended or

cancelled a claim also does not mean that Applicant concedes to the arguments or positions advanced by the Office with respect to that claim or other claims pending herein.

CONCLUSION

Applicant believes that the present application is now in condition for examination and respectfully requests that the Examiner expedite the prosecution of this application to allowance. The Commissioner is authorized to charge any underpayment, or credit any overpayment, to Deposit Account No. 60-2231 (Attorney Docket No. GH0004US-CON4).

Respectfully submitted,
GUARDANT HEALTH, INC.

Date: June 1, 2020

By: /Timothy A. Hott/

Timothy A. Hott
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Customer No. 115823

Electronic Acknowledgement Receipt

EFS ID:	39589501
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	01-JUN-2020
Filing Date:	13-DEC-2019
Time Stamp:	17:19:59
Application Type:	Utility under 35 USC 111(a)

Payment information:

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1		2020-06-01_GH0004US- CON4_OA1Response.pdf	156415 190ae07c6edd9e006e77ed39a53fc59ed43 57247	yes	10

Multipart Description/PDF files in .zip description			
Document Description		Start	End
Amendment/Req. Reconsideration-After Non-Final Reject		1	1
Claims		2	7
Applicant Arguments/Remarks Made in an Amendment		8	10

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New International Application Filed with the USPTO as a Receiving Office

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ENTITY: LARGE SMALL MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE (\$)	FEE (\$)
<input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
<input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
<input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 = *		x \$ 100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 = *		x \$ 460 =	
<input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED - PART II

	(Column 1)		(Column 2)	(Column 3)	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT	06/01/2020		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total (37 CFR 1.16(i))	*	29	Minus	** 30	= 0
	Independent (37 CFR 1.16(h))	*	2	Minus	*** 3	= 0
<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))						
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
TOTAL ADD'L FEE						0
AMENDMENT			CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	
	Total (37 CFR 1.16(i))	*		Minus	**	= 0
	Independent (37 CFR 1.16(h))	*		Minus	***	= 0
<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))						
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
TOTAL ADD'L FEE						
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.						LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".						/CORALIA BETANCOURT/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".						
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.						

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

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	1	10287631	B2	2019-05-14	Salk et al.	Entire Document	
	2	10370713	B2	2019-08-06	Salk et al.	Entire Document	
	3	10385393	B2	2019-08-20	Salk et al.	Entire Document	
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	1	20100264331	A1	2010-10-21	Sacko et al.	Entire Document	

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

2	20190271040	A1	2019-09-05	Salk et al.	Entire Document
3	20190292597	A1	2019-09-26	Salk et al.	Entire Document
4	20190338358	A1	2019-11-07	Salk et al.	Entire Document
5	20190352714	A1	2019-11-21	Salk et al.	Entire Document

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FOREIGN PATENT DOCUMENTS

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	1	CLARK, T.A. et al. "Analytical Validation of a Hybrid Capture Based Next-Generation Sequencing Clinical Assay for Genomic Profiling of Cell-Free Circulating Tumor DNA," J. Mol. Diagnostics (2018) 20(5):686-702	
	2	KAMPS-HUGHES, N. et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data" PLOS One (2018)	

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

3	LENNON, N.J. et al. "Technological considerations for genome-guided diagnosis and management of cancer" Gen Med (2016) 8:112
4	MISHRA, S. et al. "Different Facets of Copy Number Changes: Permanent, Transient, and Adaptive" Mol Cell Biol (2016) 36(7):1050-1063
5	MOENCH, S. "Genomic Profiling Using Guardant 360 Cell-Free DNA-Based Assay vs Tumor-Based Genotyping Assays in Advanced NSCLC, CANCER THERAPY ADVISOR (Feb. 28, 2019), https://www.cancertherapyadvisor.com/home/news/conferencecoverage/american-association-for-cancer-research-aacr/aacr-2019/genomic-profiling-using-guardant-360-cell-free-dna-based-assay-vs-tumor-based-genotyping-assays-in-advanced-nsclc/ (lastaccessed Nov. 30, 2019)
6	NEWMAN, A. et al. "Integrated digital error suppression for improved detection of circulating tumor DNA" Nature Biotech (2016) 34(5):547-555
7	ODEGAARD, J.I. et al. "Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies" Clin Canc Res (2018) 24(15):3539-3549
8	OU, SHI et al. "Liquid Biopsy to Identify Actionable Genomic Alterations" Am Soc Clin Onc (2018) 978
9	PAWELETZ, C.P. et al. "Bias-corrected targeted next-generation sequencing for rapid, multiplexed detection of actionable alterations in cell-free DNA from advanced lung cancer patients" Clin Canc Res (2016) 22(4):915-922
10	PHALLEN, J. et al. "Direct detection of early-stage cancers using circulating tumor DNA" Sci Trans Med (2017) Vol. 9, Issue 403, eaan2415DOI: 10.1126/scitranslmed.aan2415
11	SATHIRAPONGSASUTI, J.F. et al. "Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV" Bioinformatics (2011) 27(19):2648-2654
12	SHIROGUCHI, et al. Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes. Proc Natl Acad Sci U S A. 2012 Supplemental Information (8 pages)
13	TRAPNELL, C. et al. "How to map billions of short reads onto genomes" Nature Biotech (2009) 27(5):455-457

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

14	VAN LOO, P. et al. "Allele-specific copy number analysis of tumors" PNAS (2010) 107(39):16910-16915
15	WANG, T.T. et al. "High efficiency error suppression for accurate detection of low-frequency variants" NAR (2019) 47 (15):

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

- The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.
- A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-06-03
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16714579
Filing Date:	13-Dec-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.305

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
Total in USD (\$)				240

Electronic Acknowledgement Receipt

EFS ID:	39613483
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	03-JUN-2020
Filing Date:	13-DEC-2019
Time Stamp:	13:44:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$240
RAM confirmation Number	E202063D44332686
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	2020-06-03_GH0004US- CON4_IDSTrans.pdf	145170	no	4
			5bfbdf46bb8de26504fb05fd0f2018c4719cd1d1d		

Warnings:

Information:

2	Information Disclosure Statement (IDS) Form (SB08)	2020-06-03_GH0004US- CON4_SB08.pdf	1055136	no	6
			2051f1ba034d70cd221f8e54f0f20adaaf3c22037		

Warnings:

Information:

3	Non Patent Literature	CLARK_JMolDiag_2018_686-70 2pdf.pdf	3158287	no	17
			a0919e0dddb3ec5f7ca0f3bb3ae016a43ad3b2eae		

Warnings:

Information:

4	Non Patent Literature	KAMPS- HUGHES_PLOOne_2018.pdf	5707894	no	18
			ea4d5cf7239d69ce8d4cdfae92b25404809533e1		

Warnings:

Information:

5	Non Patent Literature	LENNON_GenMed_2016_112. pdf	689512	no	10
			67e86d1424805846207040bcbbef63ab7e600b00		

Warnings:

Information:

6	Non Patent Literature	MISHRA_MCB_2016_1050-106 3.pdf	1216913	no	14
			5f63e365b9b13c1c13c502d1372bc57258b43782		

Warnings:

Information:

7	Non Patent Literature	MOENCH_CancTherAdv_2019.pdf	640654	no	3
			cbedcb9c9350ebc56dce20b0e103f70b26863c3		
Warnings:					
Information:					
8	Non Patent Literature	NEWMAN_NatMed_2014_547-555.pdf	1377423	no	9
			69ec2aa88500b798bf011f81a5bfe5b8de41be93		
Warnings:					
Information:					
9	Non Patent Literature	ODEGAARD_ClinCancRes_2018_3539-3549.pdf	1062206	no	11
			cb51a9f4833c005db46554e71fa08573f616a364		
Warnings:					
Information:					
10	Non Patent Literature	OU_ACSO_2018_978-997.pdf	664167	no	20
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Warnings:					
Information:					
11	Non Patent Literature	PAWELETZ_ClinCancRes_2017_915-922.pdf	464079	no	16
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Information:					
12	Non Patent Literature	Phallen_SciTranslMed_2017.pdf	2398204	no	13
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Warnings:					
Information:					
13	Non Patent Literature	Sathirapongsasuti_BioInformati cs_2011.pdf	484289	no	7
			78ff01d10aa50d956f5f7a931c727355d943f9e		
Warnings:					
Information:					

14	Non Patent Literature	SHIROGUCHI_PNAS_2012_Sup plInfo.pdf	667103	no	8
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Warnings:					
Information:					
15	Non Patent Literature	TRAPNELL_NatBiotech_2009_4 55-457.pdf	183342	no	3
			8d9e4daf2d810f2b71651c6feb734337a82 b52a		
Warnings:					
Information:					
16	Non Patent Literature	VAN_LOO_PNAS_2010_16910- 16915.pdf	2450725	no	6
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Warnings:					
Information:					
17	Non Patent Literature	WANG_NAR_2019.pdf	735593	no	11
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Warnings:					
Information:					
18	Fee Worksheet (SB06)	fee-info.pdf	30478	no	2
			425b3301b3e53761cbc50fc7cc7d801e7ac 260f2		
Warnings:					
Information:					
Total Files Size (in bytes):			23131175		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli TALASAZ et al.

Serial No.: 16/714,579

Filing Date: December 13, 2019

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Confirmation No.: 3111

Art Unit: 1637

Examiner: Kenneth R.
HORLICK

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:

(1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

(2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491

in an international application;

-- OR --

- (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

- (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

- B. *37 CFR § 1.97(c)*. Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b)*, above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

- a statement as specified in § 1.97 (e) provided concurrently herewith;

-- OR --

- a fee of \$240.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

- C. *37 CFR § 1.97 (d)*. Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

- i. a statement as specified in § 1.97 (e);

-- AND --

- ii. a fee of \$240.00 as set forth in § 1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

- D. *37 CFR § 1.97 (e)*. Statement.

- A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (c)*;

-- AND/OR --

- A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (d)*;

-- AND/OR --

- A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under *37 C.F.R. § 1.97(e) (1)* as provided for under MPEP 609.04(b) V.

- E. *Statement Under 37 C.F.R. § 1.704(d)*. Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure

statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. 37 CFR §1.98 (a) (2). The content of the Information Disclosure Statement is as follows:

Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. 37 CFR §1.98(a)(3). The Information Disclosure Statement includes non-English patents and/or references.

Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. 37 CFR §1.98(d). Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

USSN: 16/714,579

June 3, 2020

Page 4 of 4

- I. *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of \$240.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 60-2231(Docket No. GH0004US-CON4).

Respectfully submitted,

Dated: June 3, 2020

By: /Timothy A. Hott/
Timothy A. Hott, Reg. No. 67740

Customer No. 115823
GUARDANT HEALTH, INC.
505 Penobscot Drive
Redwood City, CA 94063



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO.
16/714,579 12/13/2019 AmirAli TALASAZ 42534-708.305 3111

115823 7590 08/10/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

EXAMINER

HORLICK, KENNETH R

ART UNIT PAPER NUMBER

1637

NOTIFICATION DATE DELIVERY MODE

08/10/2020

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents@guardanthealth.com
patentdocket@wsgr.com

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

NEW GROUNDS OF REJECTION NECESSITATED BY THE AMENDMENT

2. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(I)(1) - 706.02(I)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

3. Claims 31, 34-42, 44-49, 52-56, and 61-68 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 2 of U.S. Patent No. 9,920,366. Although the claims at issue are not identical, they are not patentably distinct from each other because the patented claim and the instant claims are related as species-genus. That is, the steps of the instant claims, including analysis of paired reads and unpaired reads, are included in the steps of the patented claim.

4. Claims 31, 34-42, 44-49, 52-56, and 61-68 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 31, 33-41, 47-49, 51, 57-59, and 61-71 of copending Application No. 16/601,168 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the copending claims and the instant claims are related as species-genus. That is, the steps of the instant claims, including analysis of paired reads and unpaired reads, are included in the steps of the copending claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

5. Claims 31, 34-42, 44-49, 52-56, and 61-68 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 54-73 of copending Application No. 15/892,178 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the copending claims and the instant claims are related as species-genus. That is, the steps of the instant claims, including analysis of paired reads and unpaired reads, are included in the steps of the copending claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

6. Claims 31, 34-42, 44-49, 52-56, and 61-68 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 61-90 of copending Application No. 16/672,267 (reference application). Although the claims at issue are not identical, they are not patentably distinct from each other because the copending claims and the instant claims are related as

species-genus. That is, the steps of the instant claims, including analysis of paired reads and unpaired reads, are included in the steps of the copending claims.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

CONCLUSION

7. Claims 31, 34-42, 44-49, 52-56, and 61-68 are free of the prior art, but are rejected for another reason. No claims are allowable.

8. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

9. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KENNETH R HORLICK whose telephone number is (571)272-0784. The examiner can normally be reached on Mon. - Thurs. 8:30 - 6:30.


Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

08/04/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

<i>Search Notes</i> 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
inventor name search	02/27/2020	KH
updated parent searches in USPAT and PGPUB	02/27/2020	KH
reviewed parent applications and references therein	02/27/2020	KH
updated in USPAT and PGPUB	08/04/2020	KH

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	
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Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (02-18)

Approved for use through 11/30/2020. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

U.S.PATENTS						Remove
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	10287631	B2	2019-05-14	Salk et al.	Entire Document
	2	10370713	B2	2019-08-06	Salk et al.	Entire Document
	3	10385393	B2	2019-08-20	Salk et al.	Entire Document
	4	10388403	B2	2019-08-20	Rava et al.	Entire Document
	5	10604804	B2	2020-03-31	Salk et al.	Entire Document
If you wish to add additional U.S. Patent citation information please click the Add button.						Add

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Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20100264331	A1	2010-10-21	Sacko et al.	Entire Document

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

2	20190271040	A1	2019-09-05	Salk et al.	Entire Document
3	20190292597	A1	2019-09-26	Salk et al.	Entire Document
4	20190338358	A1	2019-11-07	Salk et al.	Entire Document
5	20190352714	A1	2019-11-21	Salk et al.	Entire Document

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FOREIGN PATENT DOCUMENTS

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	1							

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NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵
	1	CLARK, T.A. et al. "Analytical Validation of a Hybrid Capture Based Next-Generation Sequencing Clinical Assay for Genomic Profiling of Cell-Free Circulating Tumor DNA," J. Mol. Diagnostics (2018) 20(5):686-702	
	2	KAMPS-HUGHES, N. et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data" PLOS One (2018)	

**INFORMATION DISCLOSURE
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Application Number	16714579
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First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

3	LENNON, N.J. et al. "Technological considerations for genome-guided diagnosis and management of cancer" Gen Med (2016) 8:112
4	MISHRA, S. et al. "Different Facets of Copy Number Changes: Permanent, Transient, and Adaptive" Mol Cell Biol (2016) 36(7):1050-1063
5	MOENCH, S. "Genomic Profiling Using Guardant 360 Cell-Free DNA-Based Assay vs Tumor-Based Genotyping Assays in Advanced NSCLC, CANCER THERAPY ADVISOR (Feb. 28, 2019), https://www.cancertherapyadvisor.com/home/news/conferencecoverage/american-association-for-cancer-research-aacr/aacr-2019/genomic-profiling-using-guardant-360-cell-free-dna-based-assay-vs-tumor-based-genotyping-assays-in-advanced-nsclc/ (last accessed Nov. 30, 2019)
6	NEWMAN, A. et al. "Integrated digital error suppression for improved detection of circulating tumor DNA" Nature Biotech (2016) 34(5):547-555
7	ODEGAARD, J.I. et al. "Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies" Clin Canc Res (2018) 24(15):3539-3549
8	YOU, SHI et al. "Liquid Biopsy to Identify Actionable Genomic Alterations" Am Soc Clin Onc (2018) 978
9	PAWELETZ, C.P. et al. "Bias-corrected targeted next-generation sequencing for rapid, multiplexed detection of actionable alterations in cell-free DNA from advanced lung cancer patients" Clin Canc Res (2016) 22(4):915-922
10	PHALLEN, J. et al. "Direct detection of early-stage cancers using circulating tumor DNA" Sci Trans Med (2017) Vol. 9, Issue 403, eaan2415DOI: 10.1126/scitranslmed.aan2415
11	SATHIRAPONGSASUTI, J.F. et al. "Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV" Bioinformatics (2011) 27(19):2648-2654
12	SHIROGUCHI, et al. Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes. Proc Natl Acad Sci U S A. 2012 Supplemental Information (8 pages)
13	TRAPNELL, C. et al. "How to map billions of short reads onto genomes" Nature Biotech (2009) 27(5):455-457

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
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Application Number	16714579	
Filing Date	2019-12-13	
First Named Inventor	AmirAli TALASAZ	
Art Unit	1636	
Examiner Name		
Attorney Docket Number	42534-708.305	

14	VAN LOO, P. et al. "Allele-specific copy number analysis of tumors" PNAS (2010) 107(39):16910-16915
15	WANG, T.T. et al. "High efficiency error suppression for accurate detection of low-frequency variants" NAR (2019) 47 (15):

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	08/04/2020
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579		
Filing Date	2019-12-13		
First Named Inventor	AmirAli TALASAZ		
Art Unit	1636		
Examiner Name			
Attorney Docket Number	42534-708.305		

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

- The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.
 A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-06-03
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli TALASAZ et al.	Confirmation No.: 3111
Serial Number: 16/714,579	Customer No.: 115823
Filing Date: December 13, 2019	Group Art Unit: 1637
Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: Kenneth R. HORLICK

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE TO FINAL OFFICE ACTION

Sir:

This communication is in response to the Final Office Action mailed on August 10, 2020. The shortened statutory period for reply expires November 10, 2020, therefore, this response is timely filed. Applicant respectfully requests consideration of the above-referenced application in view of the following amendments and remarks:

Amendments to the Specification appear on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 10 of this paper.

Electronic Acknowledgement Receipt

EFS ID:	40302137
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	17-AUG-2020
Filing Date:	13-DEC-2019
Time Stamp:	14:31:16
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2020-08-17_GH0004US- CON4_FOAResponse.pdf	157582 <small>63ba09c55232ba1faacc7e20d189be5ffadc 7781</small>	yes	11

00508

Multipart Description/PDF files in .zip description			
Document Description		Start	End
Applicant Arguments/Remarks Made in an Amendment		10	11
Claims		3	9
Specification		2	2
Response After Final Action		1	1

Warnings:

Information:

Total Files Size (in bytes):	157582
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

REMARKS

Claims 31, 34-42, 44-49, 52-56, and 61-68 are pending. Applicant has amended claims 31, 35, 37-42, 44-45, 47-49, 53, 55-56, 61-64, and 66-68 for clarity and antecedent basis. These amendments are fully supported by the application as filed throughout the specification, for example, at least at paragraphs [00153]-[00159]. No new matter is added by these amendments.

Rejection- Nonstatutory Double Patenting

Claims 31, 34-42, 44-49, 52-56, and 61-68 were rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claim 2 of U.S. Patent No. 9,920,366. Claims 31, 34-42, 44-49, 52-56, and 61-68 were provisionally rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 31, 33-41, 47-49, 51, 57-59, and 61-71 of copending Application No. 16/601,168, claims 54-73 of copending Application No. 15/892,178, and claims 61-90 of copending Application No. 16/672,267.

Without conceding in the basis of rejection and solely to advance prosecution, Applicant hereby submits terminal disclaimers over the aforementioned patent and applications. Accordingly, Applicant respectfully requests that this rejection be withdrawn.

It shall be understood herein that any instance in which Applicant has addressed certain comments set forth by the Office shall not be construed as a concession to other comments or arguments advanced by the Office. Any circumstance in which Applicant has amended or cancelled a claim also does not mean that Applicant concedes to the arguments or positions advanced by the Office with respect to that claim or other claims pending herein.

CONCLUSION

Applicant believes that the present application is now in condition for examination and respectfully requests that the Examiner expedite the prosecution of this application to allowance. The Commissioner is authorized to charge any underpayment, or credit any overpayment, to Deposit Account No. 60-2231 (Attorney Docket No. GH0004US-CON4).

USSN: 16/714,579

August 17, 2020

Page 11 of 11

Respectfully submitted,

GUARDANT HEALTH, INC.

Date: August 17, 2020

By: /Timothy A. Hott/

Timothy A. Hott

Registration No.: 67740

GUARDANT HEALTH, INC.

505 Penobscot Drive

Redwood City, CA 94063

Customer No. 115823

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application. The foregoing amendments are without prejudice and do not constitute an admission regarding the patentability of the amended subject matter and should not so be construed. Applicant reserves the right to pursue the subject matter of the canceled claims in this or any other appropriate patent application.

Listing of Claims:

1. - 30. (Cancelled).

31. (Currently amended): A method for analyzing sequencing reads of double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a sample of a subject, comprising:

(a) tagging a plurality of double-stranded cfDNA molecules from a population of double-stranded cfDNA molecules ~~obtained~~ from the sample ~~[[to]]~~ with a set of library adaptors comprising a plurality of molecular barcodes to generate tagged parent polynucleotides,

wherein the tagging comprises ligating a plurality of library adaptors from the set of library adaptors to the plurality of double-stranded cfDNA molecules from the population using ~~at least~~ more than a 10X molar excess of library adaptors as compared to the double-stranded cfDNA molecules ~~[[in]]~~ of the population;

wherein the tagging ~~yields~~ produces at least 20% of the double-stranded cfDNA molecules ~~[[from]]~~ of the populations having library adaptors ligated to both ends of a molecule of the double-stranded cfDNA molecules;

(b) amplifying a plurality of the tagged parent polynucleotides to produce progeny polynucleotides;

(c) sequencing ~~at least a subset~~ a plurality of the progeny polynucleotides to produce a set of sequencing reads; and

(d) determining, based at least on sequence information from the molecular barcodes, individual double-stranded cfDNA molecules from among the tagged parent polynucleotides for which either (1) both a Watson strand and a Crick strand of the individual double-stranded cfDNA molecule are detected or (2) only one of a Watson

strand or a Crick strand of the individual double-stranded cfDNA molecule is detected from a plurality of sequencing reads from the set of sequencing reads.

32. (Cancelled).

33. (Cancelled).

34. (Previously presented): The method of claim 31, wherein the sample is selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, and tears.

35. (Currently amended): The method of claim 31, wherein the plurality of library adaptors ~~[[are]]~~ is ligated to the plurality of double-stranded cfDNA molecules by blunt-end ligation or sticky-end ligation.

36. (Previously presented): The method of claim 31, wherein the ligating comprises using more than a 100X molar excess of library adaptors as compared to the double-stranded cfDNA molecules.

37. (Currently amended): The method of claim 31, wherein the tagging produces at least 40% of the double-stranded cfDNA molecules ~~from the sample are attached to~~ of the population having library adaptors ~~[[at]]~~ ligated to both ends of a molecule of the double-stranded cfDNA molecules.

38. (Currently amended): The method of claim 31, wherein the plurality of molecular barcodes ~~[[in]]~~ of the plurality of library adaptors ~~[[have]]~~ has between 2 ~~[[to]]~~ and 100,000 different molecular barcode sequences.

39. (Currently amended): The method of claim 31, wherein the plurality of molecular barcodes ~~[[in]]~~ of the plurality of library adaptors ~~[[have]]~~ has between 5 ~~[[to]]~~ and 100 different molecular barcode sequences.

40. (Currently amended): The method of claim 39, wherein ~~[[the]]~~ molecular barcodes of the plurality of molecular barcodes have a length of between 5 ~~[[to]]~~ and 20 nucleotides.

41. (Currently amended): The method of claim 31, wherein a plurality of the plurality of molecular barcodes have different sequences from one another and have an edit distance of at least 1 nucleotide base between one another.

42. (Currently amended): The method of claim 31, wherein ~~[[the]]~~ library adaptors of the plurality of library adaptors are Y-shaped adaptors.

43. (Cancelled).
44. (Currently amended): The method of claim 31, further comprising selectively enriching a plurality of the amplified progeny polynucleotides for ~~a plurality of~~ genomic regions of interest.
45. (Currently amended): The method of claim 44, wherein the ~~plurality of~~ genomic regions of interest comprises exon sequences.
46. (Previously presented): The method of claim 44, wherein the genomic regions of interest comprise sequences from one or more genes selected from the group consisting of:
ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
47. (Currently amended): The method of claim 44, wherein selectively enriching the ~~subset plurality of amplified~~ the progeny polynucleotides for ~~[[a]] the plurality of~~ genomic regions of interest comprises using a set of probes that hybridize to at least a portion of the ~~plurality of~~ genomic regions of interest.
48. (Currently amended): The method of claim 44, further comprising amplifying a ~~subset plurality of the~~ enriched ~~amplified~~ progeny polynucleotides.
49. (Currently amended): A method for analyzing double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a sample of a subject, comprising:
(a) ~~exposing tagging a population~~ plurality of double-stranded cfDNA molecules from a population of double-stranded cfDNA molecules ~~obtained~~ from the sample ~~[[to]]~~ with a set of library adaptors comprising a plurality of molecular barcodes to generate tagged parent polynucleotides,
wherein ~~exposing the tagging~~ comprises ligating a plurality of library adaptors from the set of library adaptors to ~~[[some]]~~ the plurality of ~~[[the]]~~ double-stranded cfDNA molecules ~~[[form]]~~ from the sample population using at least more than a 10X

molar excess of library adaptors as compared to the double-stranded cfDNA molecules ~~[[in]]~~ of the population;

wherein ~~exposing yields~~ the tagging produces at least 20% of the double-stranded cfDNA molecules ~~[[from]]~~ of the population having library adaptors ligated to both ends of a molecule of the double-stranded cfDNA molecules;

- (b) ~~subjecting~~ amplifying a plurality of the tagged parent polynucleotides to ~~amplification reactions under conditions that yield amplified~~ progeny polynucleotides;
- (c) determining nucleotide sequences of a plurality of ~~amplified~~ the progeny polynucleotides; and
- (d) analyzing a plurality of the nucleotide sequences with a ~~programed~~ programmed computer processor, the analyzing comprising:

mapping a plurality of the nucleotide sequences to a reference sequence to ~~generate~~ produce mapped sequences,

grouping a plurality of the mapped sequences into families based on a combination of sequence information from the molecular barcodes and start and stop positions of the mapped sequences, wherein a family of the families is representative of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides, and

identifying a plurality of the families as having nucleotide sequences representing either (1) both a Watson strand and a Crick strand of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides or (2) only one of a Watson strand or a Crick strand of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides.

50. (Cancelled).

51. (Cancelled).

52. (Previously presented): The method of claim 49, wherein the sample is selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool, and tears.

53. (Currently amended): The method of claim 49, wherein the plurality of library adaptors [[are]] is ligated to the plurality of double-stranded cfDNA molecules by blunt-end ligation or sticky-end ligation.
54. (Previously presented): The method of claim 49, wherein the ligating comprises using more than a 100X molar excess of library adaptors as compared to the double-stranded cfDNA molecules.
55. (Currently amended): The method of claim 49, wherein the tagging produces at least 40% of the double-stranded cfDNA molecules ~~from the sample are attached to~~ of the population having library adaptors [[at]] ligated to both ends of a molecule of the double-stranded cfDNA molecules.
56. (Currently amended): The method of claim 49, further comprising selectively enriching a plurality of the ~~amplified~~ progeny polynucleotides for genomic regions of interest prior to determining the nucleotide sequences ~~sequencing~~, wherein the genomic regions of interest comprise sequences from one or more genes selected from the group consisting of:
ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
57. - 60. (Cancelled).
61. (Currently amended): The method of claim 49, wherein double-stranded cfDNA molecules of the plurality of double-stranded cfDNA molecules are non-uniquely tagged, wherein the double-stranded cfDNA molecules that map to a mappable base position of a reference sequence are tagged with a number of different molecular barcodes ranging from at least 2 [[and]] to fewer than a number of the double-stranded cfDNA molecules that map to the mappable base position.
62. (Currently amended): The method of claim 49, wherein nucleotide sequences ~~generated~~ determined from a Watson strand of an individual double-stranded cfDNA molecule from

among the tagged parent polynucleotides are distinguished from nucleotide sequences generated from a Crick strand of ~~[[an]]~~ the individual double-stranded cfDNA molecule from among the tagged parent polynucleotides based ~~at least~~ on sequence information from the molecular barcodes and/or sequence information from endogenous nucleotide sequences of the progeny polynucleotides.

63. (Currently amended): The method of claim 31, wherein the tagging comprises non-uniquely tagging the plurality of double-stranded cfDNA molecules, wherein double-stranded cfDNA molecules of the plurality of double-stranded cfDNA molecules that map to a mappable base position of a reference sequence are tagged with a number of different molecular barcodes ranging from at least 2 ~~[[and]]~~ to fewer than a number of the double-stranded cfDNA molecules that map to the mappable base position.
64. (Currently amended): The method of claim 31, wherein sequencing reads ~~generated~~ produced from a Watson strand of a double-stranded cfDNA molecule from among the tagged parent polynucleotides are distinguished from sequencing reads generated from a Crick strand of ~~[[a]]~~ the double-stranded cfDNA molecule from among the tagged parent polynucleotides based ~~at least~~ on sequence information from the molecular barcodes and/or sequence information from endogenous nucleotide sequences of the progeny polynucleotides.
65. (Previously presented): The method of claim 31, further comprising, prior to (d), mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence to produce mapped sequencing reads ~~prior to (d).~~
66. (Currently amended): The method of claim 65, further comprising grouping a plurality of the mapped sequencing reads ~~mapping to the reference sequence~~ into families based on a combination of sequence information from the molecular barcodes and start and stop positions of the mapped sequencing reads ~~that map to the reference sequence~~, wherein a family of the families is representative of an individual double-stranded cfDNA molecule from among the tagged parent polynucleotides.
67. (Currently amended): The method of claim 31, further comprising:
- (e) for a plurality of genetic loci of a reference sequence, estimating a number of ~~total~~ tagged parent polynucleotides that map to the genetic locus ~~one or more genetic loci~~ of ~~[[a]]~~ the reference sequence based at least on quantitative measures of the

individual double-stranded cfDNA molecules determined in (d) that map to the genetic locus ~~one or more genetic loci~~.

68. (Currently amended): The method of claim 67, wherein estimating the number of ~~total~~ tagged parent polynucleotides is further based on inferring, from at least the quantitative measures of the individual double-stranded cfDNA molecules determined in (d), a quantitative measure of individual double-stranded cfDNA molecules that map to the genetic locus ~~each of the one or more genetic loci~~ for which neither a Watson strand ~~[[or]]~~ nor a Crick strand ~~[[was]]~~ is detected.

AMENDMENTS TO THE SPECIFICATION

Please amend paragraph [0001] as follows:

[0001] This application is a continuation of U.S. Application No. 16/672,267, filed November 1, 2019, which is a continuation of U.S. Application No. 16/601,168, filed October 14, 2019, which is a continuation of U.S. Application No. 15/892,178, filed February 8, 2018, which is a continuation of U.S. Application No. 14/861,989, filed September 22, 2015 (now U.S. Patent 9,920,366, issued March 20, 2018), which is a continuation application of International Application No. PCT/US2014/072383, filed December 24, 2014, which application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/921,456, filed December 28, 2013, and U.S. Provisional Application No. 61/948,509, filed March 5, 2014, each of which is entirely incorporated herein by reference.

Doc Code: DIST.E.FILE Document Description: Electronic Terminal Disclaimer - Filed	PTO/SB/25 PTO/SB/26 U.S. Patent and Trademark Office Department of Commerce
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Electronic Petition Request	TERMINAL DISCLAIMER TO OBIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION AND TERMINAL DISCLAIMER TO OBIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT
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Application Number	16714579
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Filing Date	13-Dec-2019
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First Named Inventor	AmirAli TALASAZ
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Attorney Docket Number	42534-708.305
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Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
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<input checked="" type="checkbox"/> Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action <input checked="" type="checkbox"/> This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.
--

Owner	Percent Interest
GUARDANT HEALTH, INC.	100 %

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

16601168 filed on 10/14/2019
15892178 filed on 02/08/2018
16672267 filed on 01/07/2020

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

The owner(s) with percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

9920366

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:

- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

- Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.
- I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.

Applicants claims the following fee status:

- Small Entity
- Micro Entity
- Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

- An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application
Registration Number 67740
- A sole inventor
- A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application
- A joint inventor; all of whom are signing this request

Signature

/Timothy A. Hott/

00521

Name	Timothy A. Hott
------	-----------------

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

Electronic Patent Application Fee Transmittal

Application Number:	16714579			
Filing Date:	13-Dec-2019			
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS			
First Named Inventor/Applicant Name:	AmirAli TALASAZ			
Filer:	Timothy A Hott/Michelle Chan			
Attorney Docket Number:	42534-708.305			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				160

Doc Code: DISQ.E.FILE

Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 16714579

Filing Date: 13-Dec-2019

Applicant/Patent under Reexamination: TALASAZ

Electronic Terminal Disclaimer filed on August 17, 2020

APPROVED

This patent is subject to a terminal disclaimer

DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

Electronic Acknowledgement Receipt

EFS ID:	40302205
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	17-AUG-2020
Filing Date:	13-DEC-2019
Time Stamp:	14:35:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$160
RAM confirmation Number	E20208GE35006043
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Terminal Disclaimer-Filed (Electronic)	eTerminal-Disclaimer.pdf	37860	no	3
			772c53d343a55b9faf40c76906313714f2da2fda		

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	30334	no	2
			07ea12c2c425d03d188895a58ce132299e8a235e		

Warnings:

Information:

Total Files Size (in bytes):	68194
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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/714,579	Filing Date 12/13/2019	<input type="checkbox"/> To be Mailed
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ENTITY: LARGE SMALL MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE (\$)	FEE (\$)
<input type="checkbox"/> BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
<input type="checkbox"/> SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
<input type="checkbox"/> EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 = *		x \$ 100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 = *		x \$ 460 =	
<input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)	RATE (\$)	ADDITIONAL FEE (\$)
AMENDMENT	08/17/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		
	Total (37 CFR 1.16(i))	* 29	Minus	** 29	= 0	x \$ 100 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0	x \$ 460 =	0
<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))							
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
						TOTAL ADD'L FEE	0
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		
	Total (37 CFR 1.16(i))	*	Minus	**	=	x \$ 0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x \$ 0 =	
<input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))							
<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
						TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.						LIE	
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".						/MARSHA R RICHARDS/	
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".							
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.							

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



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NOTICE OF ALLOWANCE AND FEE(S) DUE

115823 7590 08/27/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

Table with 2 columns: EXAMINER (HORLICK, KENNETH R), ART UNIT (1637), PAPER NUMBER

DATE MAILED: 08/27/2020

Table with 5 columns: APPLICATION NO. (16/714,579), FILING DATE (12/13/2019), FIRST NAMED INVENTOR (AmirAli TALASAZ), ATTORNEY DOCKET NO. (42534-708.305), CONFIRMATION NO. (3111)

TITLE OF INVENTION: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

Table with 7 columns: APPLN. TYPE (nonprovisional), ENTITY STATUS (UNDISCOUNTED), ISSUE FEE DUE (\$1000), PUBLICATION FEE DUE (\$0.00), PREV. PAID ISSUE FEE (\$0.00), TOTAL FEE(S) DUE (\$1000), DATE DUE (11/27/2020)

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies. If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above. If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)". For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: **(571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the **ISSUE FEE** and **PUBLICATION FEE** (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

115823 7590 08/27/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/714,579	12/13/2019	AmirAli TALASAZ	42534-708.305	3111

TITLE OF INVENTION: **METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS**

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	11/27/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
HORLICK, KENNETH R	1637	435-006120

<p>1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).</p> <p><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.</p> <p><input type="checkbox"/> "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. Use of a Customer Number is required.</p>	<p>2. For printing on the patent front page, list</p> <p>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively, _____ 1</p> <p>(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. _____ 2</p> <p>_____ 3</p>
---	---

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE _____ (B) RESIDENCE: (CITY and STATE OR COUNTRY) _____

Please check the appropriate assignee category or categories (will not be printed on the patent) : Individual Corporation or other private group entity Government

4a. Fees submitted: Issue Fee Publication Fee (if required) Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

Electronic Payment via EFS-Web Enclosed check Non-electronic payment by credit card (Attach form PTO-2038)

The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

Applicant certifying micro entity status. See 37 CFR 1.29

Applicant asserting small entity status. See 37 CFR 1.27

Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____



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Alexandria, Virginia 22313-1450
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Table with columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO., EXAMINER, ART UNIT, PAPER NUMBER. Includes application details for AmirAli TALASAZ and examiner HORLICK, KENNETH R.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be ~~disclosed~~, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability

Application No. 16/714,579	Applicant(s) TALASAZ et al.	
Examiner KENNETH R HORLICK	Art Unit 1637	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

- 1. This communication is responsive to the response filed 08/17/20.
 A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
- 2. An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
- 3. The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.
- 4. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
 - a) All b) Some *c) None of the:
 - 1. Certified copies of the priority documents have been received.
 - 2. Certified copies of the priority documents have been received in Application No. _____.
 - 3. Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

- 5. CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
- 6. DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- 1. Notice of References Cited (PTO-892)
- 2. Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date _____.
- 3. Examiner's Comment Regarding Requirement for Deposit
of Biological Material _____.
- 4. Interview Summary (PTO-413),
Paper No./Mail Date _____.
- 5. Examiner's Amendment/Comment
- 6. Examiner's Statement of Reasons for Allowance
- 7. Other _____.

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Continuation of 3. The allowed claim(s) is/are: 31,34-42,44-49,52-56 and 61-68

EXAMINER'S COMMENTS


1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. The terminal disclaimer filed on 08/17/20 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of US '366 or of any patent issuing from US applications '168, '178, or 267 has been reviewed and is accepted. The terminal disclaimer has been recorded.

3. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KENNETH R HORLICK whose telephone number is (571)272-0784. The examiner can normally be reached on Mon. - Thurs. 8:30 - 6:30.
Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.
Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

08/24/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Search Notes 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner


US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
inventor name search	02/27/2020	KH
updated parent searches in USPAT and PGPUB	02/27/2020	KH
reviewed parent applications and references therein	02/27/2020	KH
updated in USPAT and PGPUB	08/04/2020	KH
updated in USPAT and PGPUB	08/24/2020	KH

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
NONE	NONE	08/24/2020	KH

/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	
--	--

<i>Index of Claims</i> 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637


✓	Rejected
=	Allowed

-	Cancelled
÷	Restricted

N	Non-Elected
I	Interference

A	Appeal
O	Objected


CLAIMS										
<input checked="" type="checkbox"/> Claims renumbered in the same order as presented by applicant <input type="checkbox"/> CPA <input checked="" type="checkbox"/> T.D. <input type="checkbox"/> R.1.47										
CLAIM		DATE								
Final	Original	03/03/2020	08/10/2020	08/24/2020						
1	31	✓	✓	=						
	32	✓	-							
	33	✓	-							
2	34	✓	✓	=						
3	35	✓	✓	=						
4	36	✓	✓	=						
5	37	✓	✓	=						
6	38	✓	✓	=						
7	39	✓	✓	=						
8	40	✓	✓	=						
9	41	✓	✓	=						
10	42	✓	✓	=						
	43	✓	-							
11	44	✓	✓	=						
12	45	✓	✓	=						
13	46	✓	✓	=						
14	47	✓	✓	=						
15	48	✓	✓	=						
16	49	✓	✓	=						
	50	✓	-							
	51	✓	-							
17	52	✓	✓	=						
18	53	✓	✓	=						
19	54	✓	✓	=						
20	55	✓	✓	=						
21	56	✓	✓	=						
	57	✓	-							
	58	✓	-							
	59	✓	-							
	60	✓	-							
22	61		✓	=						
23	62		✓	=						
24	63		✓	=						
25	64		✓	=						
26	65		✓	=						
27	66		✓	=						
28	67		✓	=						
29	68		✓	=						

Issue Classification 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637

CPC						
Symbol					Type	Version
C12Q	/	1	/	6869	F	2013-01-01
C12Q	/	1	/	6886	I	2013-01-01
G16B	/	15	/	00	A	2019-02-01
C12Q	/	2600	/	158	A	2013-01-01
C12Q	/	2535	/	122	A	2013-01-01

CPC Combination Sets				
Symbol	Type	Set	Ranking	Version
/	/			

NONE	Total Claims Allowed:	
(Assistant Examiner)	(Date)	29
/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	24 August 2020	O.G. Print Claim(s)
(Primary Examiner)	(Date)	1
		O.G. Print Figure
		NONE

Issue Classification 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637


INTERNATIONAL CLASSIFICATION			
CLAIMED			
C12P	/	19	/ 34

NON-CLAIMED			
/	/	/	/

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS
435	6.12

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					
435	91.2					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	29	
/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	24 August 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

Issue Classification 	Application/Control No. 16/714,579	Applicant(s)/Patent Under Reexamination TALASAZ et al.
	Examiner KENNETH R HORLICK	Art Unit 1637

Claims renumbered in the same order as presented by applicant
 CPA
 T.D.
 R.1.47

CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	31	8	40	16	49		58	28	67						
	32	9	41		50		59	29	68						
	33	10	42		51		60								
2	34		43	17	52	22	61								
3	35	11	44	18	53	23	62								
4	36	12	45	19	54	24	63								
5	37	13	46	20	55	25	64								
6	38	14	47	21	56	26	65								
7	39	15	48		57	27	66								

NONE	Total Claims Allowed:	
(Assistant Examiner)	(Date)	29
/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	24 August 2020	O.G. Print Claim(s)
(Primary Examiner)	(Date)	1
		O.G. Print Figure
		NONE



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BIB DATA SHEET

CONFIRMATION NO. 3111

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
16/714,579	12/13/2019	435	1637	42534-708.305
	RULE			

APPLICANTS

GUARDANT HEALTH, INC., Redwood City, CA;

INVENTORS

AmirAli TALASAZ, Atherton, CA;
 Stefanie Ann Ward MORTIMER, Morgan Hill, CA;

**** CONTINUING DATA *******

This application is a CON of 16/672,267 01/07/2020
 which is a CON of 16/601,168 10/14/2019
 which is a CON of 15/892,178 02/08/2018
 which is a CON of 14/861,989 09/22/2015 PAT 9920366
 which is a CON of PCT/US2014/072383 12/24/2014
 which claims benefit of 61/948,509 03/05/2014
 and claims benefit of 61/921,456 12/28/2013

**** FOREIGN APPLICATIONS *******

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ****

Foreign Priority claimed <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		CA	11	30	2
Verified and /KENNETH R HORLICK/	Initials				
Acknowledged _____	Examiner's Signature				

ADDRESS

Wilson Sonsini Goodrich & Rosati / Guardant Health
 650 Page Mill Road
 Palo Alto, CA 94304
 UNITED STATES

TITLE

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

FILING FEE RECEIVED 2720	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	<input type="checkbox"/> All Fees
		<input type="checkbox"/> 1.16 Fees (Filing)
		<input type="checkbox"/> 1.17 Fees (Processing Ext. of time)
		<input type="checkbox"/> 1.18 Fees (Issue)
		<input type="checkbox"/> Other _____
		<input type="checkbox"/> Credit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli TALASAZ et al.	Confirmation No.: 3111
Serial Number: 16/714,579	Customer No.: 115823
Filing Date: December 13, 2019	Group Art Unit: 1637
Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: Kenneth R. HORLICK

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

OK TO ENTER: /K.R.H/

/K.R.H/ (08/24/2020) **AMENDMENT AND RESPONSE TO FINAL OFFICE ACTION**

Sir:

This communication is in response to the Final Office Action mailed on August 10, 2020. The shortened statutory period for reply expires November 10, 2020, therefore, this response is timely filed. Applicant respectfully requests consideration of the above-referenced application in view of the following amendments and remarks:

Amendments to the Specification appear on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 10 of this paper.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

U.S.PATENTS							Remove
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	
	1						

If you wish to add additional U.S. Patent citation information please click the Add button. Add

U.S.PATENT APPLICATION PUBLICATIONS							Remove
Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	
	1						

If you wish to add additional U.S. Published Application citation information please click the Add button. Add

FOREIGN PATENT DOCUMENTS								Remove
Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ² i	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T ⁵
	1							

If you wish to add additional Foreign Patent Document citation information please click the Add button Add

NON-PATENT LITERATURE DOCUMENTS				Remove
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.		T ⁵

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

1	EPO Preliminary Opinion, dated August 25, 2020, in EP2893040
2	LO, DENNIS Interview with Professor Dennis Lo, Qiagen News (2002)
3	Opponents Reply dated August 20, 2020 to Proprietor's Observation in EP2893040.
4	Opposition Form and Statement to EP3470533 filed August 6, 2020 by Foundation Medicine Inc.
5	US Provisional Appl. No. 61/625,623 filed 04/17/2012.

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-08-27
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ² i	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T ⁵
	1							

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T ⁵

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

1	Guardant Health, Inc. Response to Notices of Opposition in EP2893040 filed May 29, 2020.
2	PR2019-00634, Final Written Decision of U.S. Patent 9,840,743, dated August 18, 2020
3	PR2019-00652, Final Written Decision of U.S. Patent 9,834,822, dated August 18, 2020

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-08-27
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16714579
Filing Date:	13-Dec-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.305

Filed as Large Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	240	240
Total in USD (\$)				240

Electronic Acknowledgement Receipt

EFS ID:	40405951
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	27-AUG-2020
Filing Date:	13-DEC-2019
Time Stamp:	18:50:54
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$240
RAM confirmation Number	E20208QI51086096
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	2020-08-27_GH0004US- CON4_IDSTrans.pdf	145347 8faeb5f6b3a541ca5dc238b8cff9060230b7 2093	no	4
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	2020-08-27_GH0004US- CON4_SB08.pdf	1053393 27638a52eaaad7864492323aeda001e6789f 4078e	no	4
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
3	Information Disclosure Statement (IDS) Form (SB08)	2020-08-27_GH0004US- CON4_SB08A.pdf	1053256 00d644ef96366cae8a0c58fc0406675241ae 8fd2	no	4
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
4	Fee Worksheet (SB06)	fee-info.pdf	30478 7dfa9bb0cf7db4ab98d848caf72b787b48 439a5	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			2282474		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	AmirAli TALASAZ et al.	Confirmation No.:	3111
Serial No.:	16/714,579	Art Unit:	1637
Filing Date:	December 13, 2019	Examiner:	Kenneth R. HORLICK
Title:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:

(1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

(2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491

in an international application;

-- OR --

- (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

- (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

- B. *37 CFR § 1.97(c)*. Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b)*, above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

- a statement as specified in § 1.97 (e) provided concurrently herewith;

-- OR --

- a fee of \$240.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

- C. *37 CFR § 1.97 (d)*. Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

- i. a statement as specified in § 1.97 (e);

-- AND --

- ii. a fee of \$240.00 as set forth in § 1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

- D. *37 CFR § 1.97 (e)*. Statement.

- A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (c)*;

-- AND/OR --

- A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (d)*;

-- AND/OR --

- A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under *37 C.F.R. § 1.97(e) (1)* as provided for under MPEP 609.04(b) V.

- E. *Statement Under 37 C.F.R. § 1.704(d)*. Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure

statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. 37 CFR §1.98 (a) (2). The content of the Information Disclosure Statement is as follows:

Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. 37 CFR §1.98(a)(3). The Information Disclosure Statement includes non-English patents and/or references.

Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. 37 CFR §1.98(d). Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: 15/892,178

Information Disclosure Statement(s) filed on: 8/19/20; 8/26/20

AND

The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

USSN: 16/714,579

August 27, 2020

Page 4 of 4

- I. *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of \$240.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 60-2231(Docket No. GH0004US-CON4).

Respectfully submitted,

Dated: August 27, 2020

By: /Timothy A. Hott/
Timothy A. Hott, Reg. No. 67740

Customer No. 115823
GUARDANT HEALTH, INC.
505 Penobscot Drive
Redwood City, CA 94063

Document Description: Issue Fee Payment (PTO-85B)

Issue Fee Transmittal Form

Application Number	Filing Date	First Named Inventor	Atty. Docket No.	Confirmation No.
16714579	13-Dec-2019	AmirAli TALASAZ	42534-708.305	3111

TITLE OF INVENTION :

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

Entity Status	Application Type	Art Unit	Class - Subclass	EXAMINER
Regular Undiscounted	Utility under 35 USC 111(a)	1637	006120	KENNETH HORLICK
Issue Fee Due	Publication Due	Total Fee(s) Due	Date Due	Prev. Paid Fee
\$1000	\$0	\$1000	27-Nov-2020	\$0

1.Change of Correspondence Address and/or Indication Of Fee Address (37 CFR 1.33 & 1.363)

Current Correspondence Address:	Current Indicated Fee Address :
115823 Guardant Health / WSGR 650 Page Mill Road Palo Alto CA 94304 UNITED STATES 650-493-9300 Patents@guardanthealth.com	
<input type="checkbox"/> Change of correspondence address requested, system generated AIA/122-EFS form attached	<input type="checkbox"/> Fee Address indication requested, system generated SB/47-EFS form attached

2.Entity Status**Change in Entity Status**

Applicant certifying micro entity status; system generated Micro Entity certification form attached. See 37 CFR 1.29.

Note: Absent a valid certification of micro entity status, issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
 If this box is checked, you will be prompted to choose a micro entity status on the gross income basis (37 CFR 1.29(a)) or the institution of higher education basis (37 CFR 1.29(d)), and make the applicable certification online.

 Applicant asserting small entity status. See 37 CFR 1.27.

Note: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

 Applicant changing to regular undiscounted fee status.

Note: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

Document Description: Issue Fee Payment (PTO-85B)

3.The Following Fee(s) Are Submitted:

Issue Fee

I authorize USPTO to apply my previously paid issue fee to the current fees due

Publication Fee

The Director is hereby authorized to apply my previously paid issue fee to the current fee due and to charge deficient fees to Deposit Account Number _____

Advance Order - # of copies _____

If **in addition** to the payment of the issue fee amount submitted with this form, there are any discrepancies in any amount(s) due, the Director is authorized to charge any deficiency, or credit any overpayment, to Deposit Account Number 602231.
The **issue fee must be submitted** with this form. **If payment of the issue fee does not accompany this form, checking this box and providing a deposit account number will NOT be effective to satisfy full payment of the fee(s) due.**

4.Firm and/or Attorney Names To Be Printed

NOTE: If no name is listed, no name will be printed

For printing on the patent front page, list to be displayed as entered

1. Timothy A. Hott

2.

3.

5.Assignee Name(s) and Residence Data To Be Printed

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

Name	City	State	Country	Category
GUARDANT HEALTH, INC.	Redwood City	CALIFORNIA	united states	corporation

6.Signature

I certify, in accordance with 37 CFR 1.4(d)(4) that I am an attorney or agent registered to practice before the Patent and Trademark Office who has filed and has been granted power of attorney in this application. I also certify that this Fee(s) Transmittal form is being transmitted to the USPTO via EFS-WEB on the date indicated below.

Signature	/Timothy A. Hott/	Date	10-19-2020
Name	Timothy A Hott	Registration Number	67740

Electronic Patent Application Fee Transmittal

Application Number:	16714579			
Filing Date:	13-Dec-2019			
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS			
First Named Inventor/Applicant Name:	AmirAli TALASAZ			
Filer:	Timothy A Hott/Michelle Chan			
Attorney Docket Number:	42534-708.305			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
UTILITY APPL ISSUE FEE	1501	1	1000	1000
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1000

Electronic Acknowledgement Receipt

EFS ID:	40885889
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	19-OCT-2020
Filing Date:	13-DEC-2019
Time Stamp:	20:57:30
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$1000
RAM confirmation Number	E20200IK57295119
Deposit Account	602231
Authorized User	Michelle Chan

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

00564

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Web85b.pdf	46091	no	2
			e65a225c2b6a9a847406dead1dc499c4ad3cc188		

Warnings:

Information:

2	Fee Worksheet (SB06)	fee-info.pdf	31942	no	2
			53beff323ef617f78a221ea20bce53722f014e56		

Warnings:

Information:

Total Files Size (in bytes):	78033
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/714,579	12/13/2019	AmirAli TALASAZ	42534-708.305	3111
115823	7590	10/20/2020	EXAMINER	
Guardant Health / WSGR 650 Page Mill Road Palo Alto, CA 94304			HORLICK, KENNETH R	
			ART UNIT	PAPER NUMBER
			1637	
			NOTIFICATION DATE	DELIVERY MODE
			10/20/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents@guardanthealth.com
patentdocket@wsg.com

CORRECTED
Notice of Allowability

Application No.
16/714,579

Applicant(s)
TALASAZ et al.

Examiner
KENNETH R HORLICK

Art Unit
1637

AIA (FITF) Status
Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. This communication is responsive to the IDS submissions of 08/27/20.
 A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2. An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.
4. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- Certified copies:**
- a) All b) Some *c) None of the:
- Certified copies of the priority documents have been received.
 - Certified copies of the priority documents have been received in Application No. _____.
 - Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
- * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
6. DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- Notice of References Cited (PTO-892)
- Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date 8/27/20; 8/27/20; 6/3/20 (corrected).
- Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
- Interview Summary (PTO-413),
Paper No./Mail Date _____.
- Examiner's Amendment/Comment
- Examiner's Statement of Reasons for Allowance
- Other _____.

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Continuation of 3. The allowed claim(s) is/are: 31,34-42,44-49,52-56 and 61-68

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (02-18)

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

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	2	10370713	B2	2019-08-06	Salk et al.	Entire Document	
	3	10385393	B2	2019-08-20	Salk et al.	Entire Document	
	4	10388403	B2	2019-08-20	Rava et al.	Entire Document	
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	1	20100264331	A1	2010-10-21	Sacko et al.	Entire Document	

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2	20190271040	A1	2019-09-05	Salk et al.	Entire Document
3	20190292597	A1	2019-09-26	Salk et al.	Entire Document
4	20190338358	A1	2019-11-07	Salk et al.	Entire Document
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NON-PATENT LITERATURE DOCUMENTS

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	1	CLARK, T.A. et al. "Analytical Validation of a Hybrid Capture Based Next-Generation Sequencing Clinical Assay for Genomic Profiling of Cell-Free Circulating Tumor DNA," J. Mol. Diagnostics (2018) 20(5):686-702	
	2	KAMPS-HUGHES, N. et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data" PLOS One (2018)	

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3	LENNON, N.J. et al. "Technological considerations for genome-guided diagnosis and management of cancer" Gen Med (2016) 8:112
4	MISHRA, S. et al. "Different Facets of Copy Number Changes: Permanent, Transient, and Adaptive" Mol Cell Biol (2016) 36(7):1050-1063
5	MOENCH, S. "Genomic Profiling Using Guardant 360 Cell-Free DNA-Based Assay vs Tumor-Based Genotyping Assays in Advanced NSCLC, CANCER THERAPY ADVISOR (Feb. 28, 2019), https://www.cancertherapyadvisor.com/home/news/conferencecoverage/american-association-for-cancer-research-aacr/aacr-2019/genomic-profiling-using-guardant-360-cell-free-dna-based-assay-vs-tumor-based-genotyping-assays-in-advanced-nsclc/ (last accessed Nov. 30, 2019)
6	NEWMAN, A. et al. "Integrated digital error suppression for improved detection of circulating tumor DNA" Nature Biotech (2016) 34(5):547-555
7	ODEGAARD, J.I. et al. "Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies" Clin Canc Res (2018) 24(15):3539-3549
8	YOU, SHI et al. "Liquid Biopsy to Identify Actionable Genomic Alterations" Am Soc Clin Onc (2018) 978
9	PAWELETZ, C.P. et al. "Bias-corrected targeted next-generation sequencing for rapid, multiplexed detection of actionable alterations in cell-free DNA from advanced lung cancer patients" Clin Canc Res (2016) 22(4):915-922
10	PHALLEN, J. et al. "Direct detection of early-stage cancers using circulating tumor DNA" Sci Trans Med (2017) Vol. 9, Issue 403, eaan2415DOI: 10.1126/scitranslmed.aan2415
11	SATHIRAPONGSASUTI, J.F. et al. "Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV" Bioinformatics (2011) 27(19):2648-2654
12	SHIROGUCHI, et al. Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes. Proc Natl Acad Sci U S A. 2012 Supplemental Information (8 pages)
13	TRAPNELL, C. et al. "How to map billions of short reads onto genomes" Nature Biotech (2009) 27(5):455-457

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14	VAN LOO, P. et al. "Allele-specific copy number analysis of tumors" PNAS (2010) 107(39):16910-16915
15	WANG, T.T. et al. "High efficiency error suppression for accurate detection of low-frequency variants" NAR (2019) 47(15):e87

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Examiner Signature	/KENNETH R HORLICK/	Date Considered	10/15/2020
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Art Unit	1636		
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Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

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See attached certification statement.

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 A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-06-03
Name/Print	Timothy A. Hott	Registration Number	67740

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3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
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Doc description: Information Disclosure Statement (IDS) Filed

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	First Named Inventor	AmirAli TALASAZ
	Art Unit	1636
	Examiner Name	
	Attorney Docket Number	42534-708.305

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Attorney Docket Number	42534-708.305	

/K.R.H/	1	Guardant Health, Inc. Response to Notices of Opposition in EP2893040 filed May 29, 2020.
/K.R.H/	2	PR2019-00634, Final Written Decision of U.S. Patent 9,840,743, dated August 18, 2020
/K.R.H/	3	PR2019-00652, Final Written Decision of U.S. Patent 9,834,822, dated August 18, 2020

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Name/Print	Timothy A. Hott	Registration Number	67740

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	Attorney Docket Number	42534-708.305

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.					T ⁵

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1636
Examiner Name	
Attorney Docket Number	42534-708.305

/K.R.H/	1	EPO Preliminary Opinion, dated August 25, 2020, in EP2893040
/K.R.H/	2	LO, DENNIS Interview with Professor Dennis Lo, Qiagen News (2002)
/K.R.H/	3	Opponents Reply dated August 20, 2020 to Proprietor's Observation in EP2893040.
/K.R.H/	4	Opposition Form and Statement to EP3470533 filed August 6, 2020 by Foundation Medicine Inc.
/K.R.H/	5	US Provisional Appl. No. 61/625,623 filed 04/17/2012.

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	10/15/2020
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579		
Filing Date	2019-12-13		
First Named Inventor	AmirAli TALASAZ		
Art Unit	1636		
Examiner Name			
Attorney Docket Number	42534-708.305		

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

- That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

- That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

- The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-08-27
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.



APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/714,579	11/24/2020	10844433	42534-708.305	3111

115823 7590 11/04/2020
Guardant Health / WSGR
650 Page Mill Road
Palo Alto, CA 94304

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

GUARDANT HEALTH, INC., Redwood City, CA;
AmirAli TALASAZ, Atherton, CA;
Stefanie Ann Ward MORTIMER, Morgan Hill, CA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

CERTIFICATION AND REQUEST FOR CONSIDERATION OF AN INFORMATION DISCLOSURE STATEMENT FILED AFTER PAYMENT OF THE ISSUE FEE UNDER THE QPIDS PROGRAM

Non-Provisional Application Number: 16/714,579	Filing Date: 2019-12-13
First Named Inventor: AmirAli TALASAZ	Title of Invention: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

THE UNDERSIGNED HEREBY CERTIFIES AND REQUESTS THE FOLLOWING FOR THE ABOVE-IDENTIFIED APPLICATION.

1. Consideration is requested of the information disclosure statement (IDS) submitted herewith, which is being filed after payment of the issue fee.
2. Check the box next to the appropriate selection:
 Each item of information contained in the IDS was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the IDS. See 37 CFR 1.97(e)(1).
OR
 No item of information contained in the IDS was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the IDS was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the IDS. See 37 CFR 1.97(e)(2).
OR
 See attached certification statement in compliance with 37 CFR 1.97(e).
3. Please charge the IDS fee set forth in 37 CFR 1.17(p) to Deposit Account No. 602231.
4. A Petition to Withdraw from Issue After Payment of the Issue Fee (37 CFR 1.313(c)(2)), including the petition fee set forth in 37 CFR 1.17(h), is submitted herewith as a **Web-based ePetition**. **WARNING:** Do not submit the petition as a follow-on paper via EFS-Web. Submit the petition as a Web-based ePetition by signing on to EFS-Web as a registered user, selecting the radio button next to "Existing application/patent," and then selecting the radio button next to "ePetition (for automatic processing and immediate grant, if all petitions requirements are met)." Failure to use the Web-based ePetition interface will result in automatic entry of the RCE.
5. A request for continued examination (RCE) under 37 CFR 1.114 and the RCE fee under 37 CFR 1.17(e) are submitted herewith.
6. The RCE will be treated as a "conditional" RCE. In the event the examiner determines that any item of information contained in the IDS necessitates the reopening of prosecution in the application, the undersigned understands that (i) the RCE will be processed and treated as an RCE under 37 CFR 1.114 and therefore (ii) the IDS fee under 37 CFR 1.17(p) will be returned in accordance with 37 CFR 1.97(b)(4). In the event that no item of information in the IDS necessitates reopening prosecution, the undersigned understands that the RCE will not be processed and the RCE fee under 37 CFR 1.17(e) will be returned.
7. This certification and request is being filed as a **Web-based ePetition** and is not accompanied by an amendment to the application. Inclusion of an amendment will result in automatic entry of the RCE.

Signature / Timothy A. Hott/	Date 2020-11-09
Name (Print/Typed) Timothy A. Hott	Practitioner Registration Number (If applicable) 67740

Note: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below.*

*Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

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1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	AmirAli TALASAZ et al.	Confirmation No.:	3111
Serial No.:	16/714,579	Art Unit:	1637
Filing Date:	December 13, 2019	Examiner:	Kenneth R. HORLICK
Title:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT
UNDER 37 CFR § 1.97

Commissioner for Patents:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP § 609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in § 1.56.

A. 37 CFR § 1.97 (b). This Information Disclosure Statement should be considered by the Office because:

(1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under § 1.53 (d);

-- OR --

(2) It is being filed within 3 months of entry of the national stage as set forth in § 1.491

in an international application;

-- OR --

- (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

- (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under § 1.114.

- B. *37 CFR § 1.97(c)*. Although this Information Disclosure Statement is being filed after the period specified in *37 CFR § 1.97(b)*, above, it is filed before the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, this Information Disclosure Statement should be considered because it is accompanied by one of:

- a statement as specified in §1.97 (e) provided concurrently herewith;

-- OR --

- a fee of \$260.00 as set forth in § 1.17 (p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

- C. *37 CFR § 1.97 (d)*. Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under § 1.113, (2) a notice of allowance under § 1.311, or (3) an action that otherwise closes prosecution in the application, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

- i. a statement as specified in § 1.97 (e);

-- AND --

- ii. a fee of \$260.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

- D. *37 CFR §1.97 (e)*. Statement.

- A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (c)*;

-- AND/OR --

- A statement is provided herewith to satisfy the requirement under *37 CFR §§ 1.97 (d)*;

-- AND/OR --

- A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under *37 C.F.R. § 1.97(e) (1)* as provided for under MPEP 609.04(b) V.

- E. *Statement Under 37 C.F.R. §1.704(d)*. Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office or is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure

statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. 37 CFR §1.98 (a) (2). The content of the Information Disclosure Statement is as follows:

Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are not enclosed.

-- AND/OR --

Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98 (a) (2) (iii).

G. 37 CFR §1.98(a)(3). The Information Disclosure Statement includes non-English patents and/or references.

Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

Pursuant to 37 CFR §1.98(a) (3) (ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. 37 CFR §1.98(d). Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: 15/892,178

Information Disclosure Statement(s) filed on: 11/9/2020

AND

The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

USSN: 16/714,579

November 9, 2020

Page 4 of 4

- I. *Fee Authorization*. The Commissioner is hereby authorized to charge the above-referenced fees of \$260.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 60-2231(Docket No. GH0004US-CON4).

Respectfully submitted,

Dated: November 9, 2020

By: /Timothy A. Hott/
Timothy A. Hott, Reg. No. 67740

Customer No. 115823
GUARDANT HEALTH, INC.
505 Penobscot Drive
Redwood City, CA 94063

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.305

U.S.PATENTS							Remove
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	
	1						

If you wish to add additional U.S. Patent citation information please click the Add button.

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U.S.PATENT APPLICATION PUBLICATIONS							Remove
Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	
	1	20130096011	A1	2013-04-18	Rava et al.	Entire Document	

If you wish to add additional U.S. Published Application citation information please click the Add button.

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FOREIGN PATENT DOCUMENTS								Remove
Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ² i	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T ⁵
	1	2012148477	WO	A1	2012-11-01	FODOR et al.	Entire Document	

If you wish to add additional Foreign Patent Document citation information please click the Add button

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NON-PATENT LITERATURE DOCUMENTS							Remove
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.					T ⁵

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.305

1	JIANG et al. "Basics in Bioinformatics Lecture Notes of the Graduate Summer School on Bioinformatics of China" Springer Heidelberg New York Dordrecht London (2013)
2	Opposition Form and Statement to EP3378952 filed November 4, 2020 by Dirk Buhler
3	Opposition Form and Statement to EP3378952 filed November 5, 2020 by Foundation Medicine Inc.
4	Opposition Form and Statement to EP3378952 filed November 5, 2020 by Grunecker

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	<input type="text"/>	Date Considered	<input type="text"/>
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.305

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-11-09
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal

Application Number:	16714579			
Filing Date:	13-Dec-2019			
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS			
First Named Inventor/Applicant Name:	AmirAli TALASAZ			
Filer:	Timothy A Hott/Michelle Chan			
Attorney Docket Number:	42534-708.305			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
PETITION FEE- 37 CFR 1.17(H) (GROUP III)	1464	1	140	140
RCE- 1ST REQUEST	1801	1	1360	1360
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)			1500	



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Decision Date : November 9, 2020

In re Application of :

AmirAli TALASAZ

DECISION ON PETITION

UNDER CFR 1.313(c)(2)

Application No : 16714579

Filed : 13-Dec-2019

Attorney Docket No : 42534-708.305

This is an electronic decision on the petition under 37 CFR 1.313(c)(2), filed November 9, 2020, to withdraw the above-identified application from issue after payment of the issue fee.

The petition is **GRANTED**.

The above-identified application is withdrawn from issue for consideration of a submission under 37 CFR 1.114 (request for continued examination). See 37 CFR 1.313(c)(2).

Petitioner is advised that the issue fee paid in this application cannot be refunded. If, however, this application is again allowed, petitioner may request that it be applied towards the issue fee required by the new Notice of Allowance.

Telephone inquiries concerning this decision should be directed to the Patent Electronic Business Center (EBC) at 866-217-9197.

This application file is being referred to Technology Center AU 1637 for processing of the request for continuing examination under 37 CFR 1.114 .

Office of Petitions

Electronic Acknowledgement Receipt

EFS ID:	41076903
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.305
Receipt Date:	09-NOV-2020
Filing Date:	13-DEC-2019
Time Stamp:	20:19:31
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$ 1500
RAM confirmation Number	E2020A9K19275774
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Petition automatically granted by EFS	petition-request.pdf	31616	no	2
			5058c9c7d07615e1e0e6ad7756f220625d8792a5		

Warnings:

Information:

2	Request for Continued Examination (RCE)	2020-11-09_GH0004US-CON4_RCE.pdf	1364279	no	3
			6575a2f92144fd05d41c98f6671380c83614e5dc		

Warnings:

Information:

3	Quick Path Information Disclosure Statement	2020-11-09_GH0004US-CON4_QPIDS.pdf	196117	no	2
			498d0def7daa2a463a9486a76cab14cc76b070d		

Warnings:

Information:

4	Transmittal Letter	2020-11-09_GH0004US-CON4_IDSTrans.pdf	145465	no	4
			197bb6005edbcf5dde908a9630d05509af8e7142		

Warnings:

Information:

5	Information Disclosure Statement (IDS) Form (SB08)	2020-11-09_GH0004US_CON4-SB08.pdf	1053471	no	4
			397bbb296954f6db2037f338065f75c9336680ca		

Warnings:

Information:

6	Fee Worksheet (SB06)	fee-info.pdf	31983	no	2
			e0c8e721cc5e7fe573663ac6249791cb7f7fa748		

Warnings:

Information:

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Petition Request	PETITION TO WITHDRAW AN APPLICATION FROM ISSUE AFTER PAYMENT OF THE ISSUE FEE UNDER 37 CFR 1.313(c)
Application Number	16714579
Filing Date	13-Dec-2019
First Named Inventor	AmirAli TALASAZ
Art Unit	1637
Examiner Name	KENNETH HORLICK
Attorney Docket Number	42534-708.305
Title	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

An application may be withdrawn from issue for further action upon petition by the applicant. To request that the Office withdraw an application from issue, applicant must file a petition under this section including the fee set forth in § 1.17(h) and a showing of good and sufficient reasons why withdrawal of the application from issue is necessary.

APPLICANT HEREBY PETITIONS TO WITHDRAW THIS APPLICATION FROM ISSUE UNDER 37 CFR 1.313(c).

A grantable petition requires the following items:

- (1) Petition fee; and
- (2) One of the following reasons:
 - (a) Unpatentability of one or more claims, which must be accompanied by an unequivocal statement that one or more claims are unpatentable, an amendment to such claim or claims, and an explanation as to how the amendment causes such claim or claims to be patentable;
 - (b) Consideration of a request for continued examination in compliance with § 1.114 (for a utility or plant application only); or
 - (c) Express abandonment of the application. Such express abandonment may be in favor of a continuing application, but not a CPA under 37 CFR 1.53(d).

Petition Fee

<input type="radio"/> Small Entity
<input type="radio"/> Micro Entity
<input checked="" type="radio"/> Regular Undiscounted

Reason for withdrawal from issue

- One or more claims are unpatentable
- Consideration of a request for continued examination (RCE) (List of Required Documents and Fees)
- Applicant hereby expressly abandons the instant application (any attorney/agent signing for this reason must have power of attorney pursuant to 37 CFR 1.32(b)).

RCE request, submission, and fee.

- I certify, in accordance with 37 CFR 1.4(d)(4) that :
- The RCE request ,submission, and fee have already been filed in the above-identified application on
 - Are attached.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

- An attorney or agent registered to practice before the Patent and Trademark Office who has been given power of attorney in this application.
- An attorney or agent registered to practice before the Patent and Trademark Office, acting in a representative capacity.
- A sole inventor
- A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application
- A joint inventor; all of whom are signing this e-petition

Signature	/Timothy A. Hott/
Name	Timothy A. Hott
Registration Number	67740



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/714,579	12/13/2019	AmirAli TALASAZ	42534-708.305	3111
115823	7590	11/27/2020	EXAMINER	
Guardant Health / WSGR 650 Page Mill Road Palo Alto, CA 94304			HORLICK, KENNETH R	
			ART UNIT	PAPER NUMBER
			1637	
			NOTIFICATION DATE	DELIVERY MODE
			11/27/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents@guardanthealth.com
patentdocket@wsgr.com

CORRECTED
Notice of Allowability

Application No.
16/714,579

Applicant(s)
TALASAZ et al.

Examiner
KENNETH R HORLICK

Art Unit
1637

AIA (FITF) Status
Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. This communication is responsive to the submission of 11/09/20.
 A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2. An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.
4. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- Certified copies:**
- a) All b) Some *c) None of the:
- Certified copies of the priority documents have been received.
 - Certified copies of the priority documents have been received in Application No. _____.
 - Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
- * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
- Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
6. DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- Notice of References Cited (PTO-892)
- Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date 11/9/20.
- Examiner's Comment Regarding Requirement for Deposit
of Biological Material _____.
- Interview Summary (PTO-413),
Paper No./Mail Date. _____.
- Examiner's Amendment/Comment
- Examiner's Statement of Reasons for Allowance
- Other _____.

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Continuation of 3. The allowed claim(s) is/are: 31,34-42,44-49,52-56 and 61-68

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16714579
	Filing Date	2019-12-13
	First Named Inventor	AmirAli TALASAZ
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.305

U.S.PATENTS							Remove
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	
	1						

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS							Remove
Examiner Initial*	Cite No	Publication Number	Kind Code ¹	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	
/K.R.H./	1	20130096011	A1	2013-04-18	Rava et al.	Entire Document	

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS								Remove
Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ² i	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T ⁵
/K.R.H./	1	2012148477	WO	A1	2012-11-01	FODOR et al.	Entire Document	

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS							Remove
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.					T ⁵

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.305

/K.R.H/	1	JIANG et al. "Basics in Bioinformatics Lecture Notes of the Graduate Summer School on Bioinformatics of China" Springer Heidelberg New York Dordrecht London (2013)
/K.R.H/	2	Opposition Form and Statement to EP3378952 filed November 4, 2020 by Dirk Buhler
/K.R.H/	3	Opposition Form and Statement to EP3378952 filed November 5, 2020 by Foundation Medicine Inc.
/K.R.H/	4	Opposition Form and Statement to EP3378952 filed November 5, 2020 by Grunecker

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	11/23/2020
--------------------	---------------------	-----------------	------------

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.

**INFORMATION DISCLOSURE
STATEMENT BY APPLICANT**
(Not for submission under 37 CFR 1.99)

Application Number	16714579
Filing Date	2019-12-13
First Named Inventor	AmirAli TALASAZ
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.305

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-11-09
Name/Print	Timothy A. Hott	Registration Number	67740

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.



United States Patent and Trademark Office

Office of the Chief Financial Officer

Document Code:WFEE

User :C46472

Refund Accounting Date:12/01/2020

Effective Date	Sale Item Reference Number	Refund Total
11/09/2020	16714579	\$1,360.00

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method	Account Number
I2020B1723215005	1801	RCE- 1ST REQUEST	\$1,360.00	DA	602231



United States Patent and Trademark Office

Office of the Chief Financial Officer

Document Code:WFEE

User :C46472

Sale Adjustment Accounting Date:12/01/2020

Effective Date	Sale Accounting Date	Sale Item Reference Number
11/09/2020	12/01/2020	16714579

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method
I2020B1723215005	1801	RCE- 1ST REQUEST	\$1,360.00	DA



APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/714,579	01/12/2021	10889858	42534-708.305	3111

115823 7590 12/22/2020
Guardant Health / WSGR
650 Page Mill Road
Palo Alto, CA 94304

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

GUARDANT HEALTH, INC., Redwood City, CA;
AmirAli TALASAZ, Atherton, CA;
Stefanie Ann Ward MORTIMER, Morgan Hill, CA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli Talasaz, et at.

Application No.: 16/714,579

Filing Date: December 13, 2019

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Group Art Unit: 1637

Examiner: Kenneth R. HORLICK

Confirmation No.: 3111

Customer No.: 115823

FILED ELECTRONICALLY ON: September 2, 2021

**PETITION UNDER 37 C.F.R. § 1.78 TO ACCEPT AN
UNINTENTIONALLY DELAYED PRIORITY CLAIM UNDER 35 U.S.C. § 120**

Mail Stop Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is a petition under 37 C.F.R. § 1.78(e) to accept an unintentionally delayed priority claim.

The full and proper priority in this application 16/714,579 should read:

This application is a continuation of U.S. Application No. 16/601,168, filed October 14, 2019, which is a continuation of U.S. Application No. 15/892,178, filed February 8, 2018, which is a continuation of U.S. Application No. 14/861,989, filed September 22, 2015 (now U.S. Patent 9,920,366, issued March 20, 2018), which is a continuation application of International Application No. PCT/US2014/072383, filed December 24, 2014, which application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/921,456, filed December 28, 2013, and U.S. Provisional Application No. 61/948,509, filed March 5, 2014, each of which is entirely incorporated herein by reference.

The entire delay between the date the benefit claim was due under 37 CFR 1.78(d)(3) and the date this corrected benefit claim is being filed was unintentional.

Accordingly, it is hereby respectfully petitioned under 37 C.F.R. § 1.78 that the Office accept an unintentionally delayed claim for priority in connection with the above referenced application. In support of this petition, Applicant submits:

- i. a corrected Application Data Sheet showing the requested change, along with an amendment to the specification correcting the cross-reference priority paragraph in the specification;
- ii. the petition fee set forth in 37 C.F.R. § 1.17(m) and any additional fees that may be required; and
- iii. a statement that the entire period of delay between the date the priority claim was due and the date this priority claim is filed was unintentional.

The Commissioner is authorized to charge all required fees to Deposit Account No. **23-2415** (Docket No. 42534-708.305).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Date: September 2, 2021

By: /Dawson J. Wong/
Dawson J. Wong, Ph.D., J.D.
Reg. No. 76,533

650 Page Mill Road
Palo Alto, CA 94304-1050
Direct Dial: (202) 973-8940
Customer No. 115823

AMENDMENTS TO THE SPECIFICATION

Please amend paragraph [0001] as follows:

[0001] This application is ~~a continuation of U.S. Application No. 16/672,267, filed January 7, 2020,~~ which is a continuation of U.S. Application No. 16/601,168, filed October 14, 2019, which is a continuation of U.S. Application No. 15/892,178, filed February 8, 2018, which is a continuation of U.S. Application No. 14/861,989, filed September 22, 2015 (now U.S. Patent 9,920,366, issued March 20, 2018), which is a continuation application of International Application No. PCT/US2014/072383, filed December 24, 2014, which application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/921,456, filed December 28, 2013, and U.S. Provisional Application No. 61/948,509, filed March 5, 2014, each of which is entirely incorporated herein by reference.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli Talasaz, et at.

Application No.: 16/714,579

Filing Date: December 13, 2019

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Group Art Unit: 1637

Examiner: Kenneth R. HORLICK

Confirmation No.: 3111

Customer No.: 115823

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

REQUEST FOR CORRECTION OF INVENTORSHIP
IN A PATENT UNDER 37 CFR §1.324

Dear Commissioner:

Applicant hereby requests that the inventorship of the above-referenced patent be amended pursuant to 37 C.F.R. §1.324 to name only the actual inventors.

The Office is requested to amend the inventorship to **ADD** the following omitted inventor: Helmy Eltoukhy

37 C.F.R. 1.324 Correction of inventorship for Patent. The inventive entity was set forth in error in an executed oath or declaration under 37 C.F.R. § 1.63 in a nonprovisional application. The oath or declaration incorrectly omitted an inventor. This error arose without deceptive intent on the part of the person who through error was incorrectly named.

In support of this Request, Applicant provides:

- (1) A statement from each person being added as an inventor and/or from each person being deleted as an inventor that the error in inventorship occurred without deceptive intention on his or her part;
 - (2) A statement from current named inventors agreeing to add inventors;
 - (3) The processing fee set forth in 37 C.F.R. § 1.20(b); and
 - (4) If an assignment has been executed by any of the original named inventors, the written consent of the assignee.
- Fee Authorization.** The Commissioner is hereby authorized to charge the above-referenced fees of **\$160.00** and charge any additional fees or credit any overpayment

associated with this communication to Deposit Account No. 23-2415 (Docket No. 42534-708.305).

Respectfully submitted,

Dated: September 2, 2021

By: /Dawson J. Wong/
Dawson J. Wong, Ph.D., J.D.
Registration No. 76,533

WILSON SONSINI GOODRICH & ROSATI
650 Page Mill Road
Palo Alto, California 94304
Telephone: (202) 973-8940
Customer No. 115823

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: AmirAli Talasaz, et al.

Serial Number: 16/714,579

Filing Date: December 13, 2019

Issued Patent No.: 10,889,858

Issued: January 12, 2021

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

CONFIRMATION NO: 3111

Customer Number: 115823

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

STATEMENT FROM EACH PERSON BEING ADDED
PURSUANT TO 37 CFR §1.324

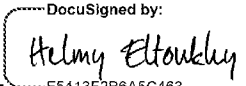
I, Helmy Eltoukhy, declare as follows:

Upon review of the above-referenced U.S. Patent No. 10,889,858 (hereinafter referred to as "Patent"), it is my belief that I am an inventor of the claimed subject matter described in the patent and I agree that I should be added to the Patent as co-inventor.

The error in inventorship occurred without deceptive intent on my part.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 8/31/2021

By: /  /
F5413F2B6A5C463...
Helmy Eltoukhy

Attorney Docket No. GH0004US-CON4
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: AmirAli Talasaz, et al.
Serial Number: 16/714,579
Filing Date: December 13, 2019
Issued Patent No.: 10,889,858
Issued: January 12, 2021
Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

CONFIRMATION NO: 3111
Customer Number: 115823

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

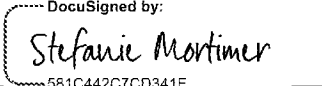
**STATEMENT FROM CURRENT NAMED INVENTOR AGREEING TO ADD AN
INVENTOR PURSUANT TO 37 C.F.R. § 1.324**

I, Stefanie Ann Ward Mortimer, declare as follows:

Upon review of the above-referenced U.S. Patent No. 10,889,858 (hereinafter referred to as "Patent"), it is my belief that Helmy Eltoukhy is a co-inventor of the claimed subject matter described in the Patent, and I agree that Helmy Eltoukhy should be added to the Patent as co-inventor.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the or any patent issued thereon.

Dated: 8/31/2021

By: /  /
Stefanie Ann Ward Mortimer

Attorney Docket No. GH0004US-CON4
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: AmirAli Talasaz, et al.
Serial Number: 16/714,579
Filing Date: December 13, 2019
Issued Patent No.: 10,889,858
Issued: January 12, 2021
Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

CONFIRMATION NO: 3111
Customer Number: 115823

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

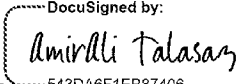
**STATEMENT FROM CURRENT NAMED INVENTOR AGREEING TO ADD AN
INVENTOR PURSUANT TO 37 C.F.R. § 1.324**

I, AmirAli Talasaz, declare as follows:

Upon review of the above-referenced U.S. Patent No. 10,889,858 (hereinafter referred to as "Patent"), it is my belief that Helmy Eltoukhy is a co-inventor of the claimed subject matter described in the Patent, and I agree that Helmy Eltoukhy should be added to the Patent as co-inventor.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the or any patent issued thereon.

Dated: 8/31/2021

By: /  /
AmirAli Talasaz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: AmirAli TALASAZ, et al.

Serial Number: 16/714,579

Filing Date: December 13, 2019

Issued Patent No.: 10,889,858

Issued: January 12, 2021

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

CONFIRMATION NO: 3111

Customer Number: 115823

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

CONSENT OF ASSIGNEE TO AMENDMENT OF INVENTORSHIP
PURSUANT TO 37 CFR §1.324

Guardant Health, Inc. states that it is:

- The assignee of the entire right, title and interest, or
- An assignee of less than the entire right, title and interest. The extent of ownership is 50%,

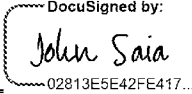
in the patent application identified above by virtue of either:

- An assignment from the inventor(s) of the patent application identified above. The assignments were recorded in the U.S. Patent and Trademark Office at Reel 052452, Frame 0149 and Reel 057327, Frame 0961.
- Copies of assignments or other documents in the chain of title are attached.

The undersigned is authorized to act on behalf of the Assignee.

The Assignee hereby assents to the Amendment of Inventorship requested in Request for Correction of Inventorship in a Patent, in the above-referenced issued patent, filed herewith.

Dated: 8/31/2021

DocuSigned by:

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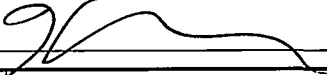
 Signature

John Saia
Printed Name

SVP and General Counsel
Title

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
<p>As the below named inventor, I hereby declare that:</p> <p>This declaration is directed to: <input type="checkbox"/> The attached application, or <input checked="" type="checkbox"/> United States application or PCT international application number <u>14/861,989</u> filed on <u>September 22, 2015</u>.</p> <p>The above-identified application was made or authorized to be made by me.</p> <p>I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.</p> <p>I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.</p> <p style="text-align: center;">WARNING:</p> <p>Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.</p>	
<p>LEGAL NAME OF INVENTOR</p> <p>Inventor: <u>Helmy Eltokhy</u> Date (Optional): <u>11/10/15</u></p> <p>Signature: </p>	
<p>Note: An application data sheet (PTO/AIA/14 or equivalent), including naming the entire inventive entity, must accompany this form. Use an additional PTO/SB/AIA01 form for each additional inventor.</p>	

This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Inventor(s): AmirAli Talasaz, et at.

Application No.: 16/714,579

Filing Date: December 13, 2019

Issued Patent No.: 10,889,858

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Group Art Unit: 1637

Examiner: Kenneth R. HORLICK

Confirmation No.: 3111

Customer No.: 115823

FILED ELECTRONICALLY ON: September 2, 2021

**REQUEST FOR CERTIFICATE OF CORRECTION OF THE ISSUED PATENT
FOR APPLICANT MISTAKE (37 C.F.R. §1.323)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
ATTN: Certificate of Correction Branch

Sir:

It is noted that there are errors in the above noted Patent's Priority Claim and a named inventor. The mistakes are of a minor nature or character, and correction as detailed herein does not involve such change in the patent as would constitute new matter or would require re-examination of the patent. A certificate of correction is therefore requested.

Attached hereto is Form PTO/SB/44 which is suitable for printing.

Applicant respectfully requests correction to the language in the issued Patent in order to correct an error in the named inventors. This error was made without deceptive intent. The changes requested are:

On the Title page, item (72), Inventors: add inventor --Helmy Eltoukhy,
Atherton, CA (US)--

In reviewing the issued Patent, Applicant noted the priority claim contained an error. The exact column and line number or page and item where the error is shown incorrectly in the above-noted Patent are:

On the Title page, Item (63), Related U.S. Application Data:

Delete “Continuation of application No. 16/672,267, filed on Jan. 7, 2020, which is a continuation of application No. 16/601,168, filed on Oct. 14, 2019, which is a continuation of application No. 15/892,178, filed Feb. 8, 2018, which is a continuation of application No. 14/861,989, filed on Sep. 22, 2015, now Pat. No. 9,920,366, which is a continuation of application No. PCT/US2014/072383, filed on Dec. 24, 2014.”

Replace with --Continuation of application No. 16/601,168, filed on Oct. 14, 2019, which is a continuation of application No. 15/892,178, filed Feb. 8, 2018, which is a continuation of application No. 14/861,989, filed on Sep. 22, 2015, now Pat. No. 9,920,366, which is a continuation of application No. PCT/US2014/072383, filed on Dec. 24, 2014.--

In support of this request, Applicant would like to draw the Office’s attention to the Request for Corrected Inventorship under 37 C.F.R. §1.48, Petition under 37 C.F.R. §1.78 and supplemental Application Data Sheet submitted on September 2, 2021. Applicant respectfully requests the Office review the documents submitted and issue a Certificate of Correction.

FEE AUTHORIZATION

Please charge the processing fee under 37 CFR 1.20(a) to Deposit Account No. **23-2415** (Docket No. 42534-708.305).

The Commissioner is authorized to charge any additional fees which may be required, including petition fees, or credit any overpayment to Deposit Account No. 23-2415 (Docket No. 42534-708.305).

Respectfully submitted,

Dated: September 2, 2021

By: /Dawson J. Wong/

Dawson J. Wong, Ph.D., J.D.
Reg. No. 76,533

WILSON SONSINI GOODRICH & ROSATI
650 Page Mill Road
Palo Alto, California 94304
Direct Dial: (202) 973-8940
Customer No. 115823

**UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION**Page 1 of 1

PATENT NO. : 10,889,858
APPLICATION NO.: 16/714,579
ISSUE DATE : 2021-01-12
INVENTOR(S) : AmirAli TALASAZ;, et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title page, item (72), Inventors: add inventor --Helmy Eltoukhy, Atherton, CA (US)--

On the Title page, item (63), Related U.S. Application Data:

Delete "Continuation of application No. 16/672,267, filed on Jan. 7, 2020, which is a continuation of application No. 16/601,168, filed on Oct. 14, 2019, which is a continuation of application No. 15/892,178, filed Feb. 8, 2018, which is a continuation of application No. 14/861,989, filed on Sep. 22, 2015, now Pat. No. 9,920,366, which is a continuation of application No. PCT/US2014/072383, filed on Dec. 24, 2014."

Replace with --Continuation of application No. 16/601,168, filed on Oct. 14, 2019, which is a continuation of application No. 15/892,178, filed Feb. 8, 2018, which is a continuation of application No. 14/861,989, filed on Sep. 22, 2015, now Pat. No. 9,920,366, which is a continuation of application No. PCT/US2014/072383, filed on Dec. 24, 2014.--

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):

Wilson Sonsini Goodrich & Rosati
650 Page Mill Road
Palo Alto, California 94304

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

00625

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli Talasaz, et at.

Application No.: 16/714,579

Filing Date: December 13, 2019

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Group Art Unit: 1637

Examiner: Kenneth R. HORLICK

Confirmation No.: 3111

Customer No.: 115823

Mail Stop Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION UNDER 37 C.F.R. § 1.182

Dear Commissioner:

In accordance with 37 C.F.R. §1.182, this paper requests that the United States Patent and Trademark Office (“USPTO”) expedite review of the Petition to Accept an Unintentionally Delayed Priority Claim filed on September 2, 2021. In support of this petition, Applicant submits the petition fee set forth in 37 C.F.R. § 1.17(f) of \$420, and any additional fees that may be required.

The Commissioner is authorized to charge all required fees to Deposit Account No. 23-2415 (Docket No. 42534-708.305).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI
A Professional Corporation

Date: September 2, 2021

By: /Dawson J. Wong/
Dawson J. Wong, Ph.D., J.D.
Reg. No. 76,533

650 Page Mill Road
Palo Alto, CA 94304-1050
Direct Dial: (202) 973-8940
Customer No. 115823

Electronic Patent Application Fee Transmittal

Application Number:	16714579			
Filing Date:	13-Dec-2019			
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS			
First Named Inventor/Applicant Name:	AmirAli TALASAZ			
Filer:	Dawson J. Wong/Cecilia Hui			
Attorney Docket Number:	42534-708.305			
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
PETITION FEE- 37 CFR 1.17(F) (GROUP I)	1462	1	420	420
PET. DELAY SUB OR RESTORE PRIORITY-CLAIM	1454	1	2100	2100
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Post-Allowance-and-Post-Issuance:				
CERTIFICATE OF CORRECTION	1811	1	160	160
PROCESSING FEE CORRECTING INVENTORSHIP	1816	1	160	160
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2840

Electronic Acknowledgement Receipt

EFS ID:	43668637
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Dawson J. Wong/Cecilia Hui
Filer Authorized By:	Dawson J. Wong
Attorney Docket Number:	42534-708.305
Receipt Date:	02-SEP-2021
Filing Date:	13-DEC-2019
Time Stamp:	13:27:34
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$2840
RAM confirmation Number	E202192D28240831
Deposit Account	232415
Authorized User	Cecilia Hui

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.21 (Miscellaneous fees and charges)

00631

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	42534_708305_SupplADS.pdf	135144	no	9
			acf57827051b77139a86124bddc686a1dda a1f85		

Warnings:

Information:

This is not an USPTO supplied ADS fillable form

2	Petition for review by the Office of Petitions	42534_708305_178Petition.pdf	115855	no	3
			4f5c09f146fe38af78deb4279e31f9cf2e3c3 629		

Warnings:

Information:

3	Petition for review by the Office of Petitions	42534_708305_Request_CorrectInventor.pdf	731181	no	6
			d9a38af16ee3dcb698af23192683228ec75c 2229		

Warnings:

Information:

4	Oath or Declaration filed	42534_708305_EltoukhyDEC.pdf	66437	no	1
			0b3245f7ee27de76e113a3f8afe5a56299ea 35ce		

Warnings:

Information:

5	Request for Certificate of Correction	42534_708305_Request_CoC.pdf	218534	no	5
			2000f3c69bd963af9b8dce9fcaa0aba8dd8 2be5		

Warnings:

Information:

6	Petition for review by the PCT legal office	42534_708305_PetitionExpedite.pdf	111841	no	2
			746c306704a2b46f732f3a102d7fda806f9 b2c1		

Warnings:

Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	45102	no	2
			ccc0dd555290994c67da0d9cf4edf976e09de4c0		
Warnings:					
Information:					
Total Files Size (in bytes):				1424094	
<p>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</p> <p><u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p>					

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

<input type="checkbox"/>	Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)
--------------------------	---

Inventor Information:

Inventor 1					<input type="button" value="Remove"/>
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	AmirAli		TALASAZ		
Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service					
City	Atherton	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1	505 Penobscot Drive				
Address 2					
City	Redwood City	State/Province	CA		
Postal Code	94063	Country i	US		
Inventor 2					<input type="button" value="Remove"/>
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Stefanie	Ann Ward	MORTIMER		
Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service					
City	Morgan Hill	State/Province	CA	Country of Residence	US
Mailing Address of Inventor:					
Address 1	2000 Willow Springs Road				
Address 2					
City	Morgan Hill	State/Province	CA		
Postal Code	95037	Country i	US		
Inventor 3					<input type="button" value="Remove"/>
Legal Name					
Prefix	Given Name	Middle Name	Family Name	Suffix	
	Helmy		ELTOUKHY		
Residence Information (Select One) <input checked="" type="radio"/> US Residency <input type="radio"/> Non US Residency <input type="radio"/> Active US Military Service					

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Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305	
		Application Number		
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS			

City	<u>Atherton</u>	State/Province	<u>CA</u>	Country of Residence	<u>US</u>
------	-----------------	----------------	-----------	----------------------	-----------

Mailing Address of Inventor:

Address 1	<u>505 Penobscot Drive</u>				
Address 2					
City	<u>Redwood City</u>	State/Province	<u>CA</u>		
Postal Code	<u>94063</u>	Country	<u>US</u>		

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button.

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

An Address is being provided for the correspondence information of this application.

Customer Number	115823		
Email Address	<u>patents@guardanthealth.com</u>	<input type="button" value="Add Email"/>	<input type="button" value="Remove Email"/>
Email Address	<u>patentdocket@wsgr.com</u>	<input type="button" value="Add Email"/>	<input type="button" value="Remove Email"/>

Application Information:

Title of the Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		
Attorney Docket Number	<u>42534-708.305</u>	Small Entity Status Claimed	<input type="checkbox"/>
Application Type	<u>Nonprovisional</u>		
Subject Matter	<u>Utility</u>		
Total Number of Drawing Sheets (if any)	<u>11</u>	Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Publication Information:

<input type="checkbox"/> Request Early Publication (Fee required at time of Request 37 CFR 1.219)
<input type="checkbox"/> Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.			
Please Select One:	<input checked="" type="radio"/> Customer Number	<input type="radio"/> US Patent Practitioner	<input type="radio"/> Limited Recognition (37 CFR 11.9)
Customer Number	115823		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending			Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
	Continuation of	46672267	2019-11-01		
Prior Application Status	Pending			Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
46672267	Continuation of	16601168	2019-10-14		
Prior Application Status	Pending			Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
16601168	Continuation of	15892178	2018-02-08		
Prior Application Status	Patented			Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15892178	Continuation of	14861989	2015-09-22	9920366	2018-03-20

00636

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Prior Application Status	Pending	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
14861989	Continuation of	PCTUS2014072383	2014-12-24
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCTUS2014072383	Claims benefit of provisional	61948509	2014-03-05
Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCTUS2014072383	Claims benefit of provisional	61921456	2013-12-28
Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the Add button.			

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)
Additional Foreign Priority Data may be generated within this form by selecting the Add button.			

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Assignee Legal Representative under 35 U.S.C. 117 Joint Inventor

Person to whom the inventor is obligated to assign. Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here.

Organization Name GUARDANT HEALTH, INC.

Mailing Address Information For Applicant:

Address 1	505 Penobscot Drive		
Address 2			
City	Redwood City	State/Province	CA
Country	US	Postal Code	94063
Phone Number		Fax Number	
Email Address	patents@guardanthealth.com		

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	42534-708.305
		Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS		

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here.

Prefix	Given Name	Middle Name	Family Name	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1				
Address 2				
City		State/Province		
Country ⁱ	Postal Code			
Phone Number	Fax Number			
Email Address				

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Dawson Wong/		Date (YYYY-MM-DD)	2021-09-02	
First Name	Dawson	Last Name	Wong	Registration Number	76533

Additional Signature may be generated within this form by selecting the Add button.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.305
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.



UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

MAILED

OCT 18 2021

INTERNATIONAL PATENT LEGAL ADM

Guardant Health / WSGR
650 Page Mill Road
Palo Alto CA 94304

In re Application of
GUARDANT HEALTH, INC.
Application No.: 16/714,579
Filing Date: December 13, 2019
Attorney Docket No.: 42534-708.305

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DECISION ON PETITION
UNDER 37 CFR 1.78(e)

This is a decision on the petition under 37 CFR 1.78(e), filed September 2, 2021, to accept an unintentionally delayed claim under 35 U.S.C. 120 for the benefit of priority to one or more prior-filed nonprovisional or international applications.

Petition to Expedite

This is a decision on the petition under 37 CFR 1.182, filed September 2, 2021, to expedite the consideration of the above-identified petition filed on September 2, 2021. The petition fee has been paid.

The petition under 37 CFR 1.182 is **GRANTED**.

Petition Under 37 CFR 1.78(e)

Under 37 CFR 1.78(e), a petition to accept an unintentionally delayed claim under 35 U.S.C. 120 for the benefit of a prior-filed application must be accompanied by:

- (i) the reference required by 35 U.S.C. 120 and 37 CFR 1.78(d)(2) to the prior-filed application, unless previously submitted;
- (ii) the petition fee set forth in 37 CFR 1.17(m); and

- (iii) a statement that the entire delay between the date the claim was due under 37 CFR 1.78(d)(3) and the date the claim was filed was unintentional. The Director may require additional information where there is a question whether the delay was unintentional.

Item (i) has not been met. The petition requests that the benefit claim that the instant application is a continuation of application number 16/672,267 be deleted. The instant application has issued as a patent. A priority claim may not be deleted with a Certificate of Correction. Such a priority claim may have been relied upon in the decision to allow the application. I.e., the deletion of a priority claim is not a mistake of minor character. See, e.g., MPEP § 1481.

With regard to item (ii), the petition fee set forth in 37 CFR 1.17(m) has been submitted.

With regard to item (iii), the statement of unintentional delay contained in the petition differs slightly from the language contained in 37 CFR 1.78(e)(3) and is hereby construed as a statement that the entire delay between the date the claim was due under 1.78(d)(3) and the date the claim was filed was unintentional. If this interpretation is incorrect, applicant is required to immediately notify the Office. As construed, the provided statement of unintentional delay is acceptable.

For the reasons above, the petition under 37 CFR 1.78(e) is **DISMISSED** without prejudice.

Any further correspondence with respect to this matter may be filed electronically via EFS-Web selecting the document description "Petition for review and processing by the PCT Legal Office" or by mail addressed to Mail Stop PCT, Commissioner for Patents, International Patent Legal Administration, P.O. Box 1450, Alexandria, Virginia 22313-1450, with the contents of the letter marked to the attention of the International Patent Legal Administration.

/Daniel Stemmer/

Daniel Stemmer
PCT Legal Examiner
International Patent Legal Administration
571-272-3301

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): AmirAli Talasaz, et at.

Application No.: 16/714,579

Patent: 10,889,858

Confirmation No.: 3889

Filing Date: December 13, 2019

Customer No.: 021971

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Group Art Unit: 1637

Examiner: Kenneth R. HORLICK

Confirmation No.: 3111

Customer No.: 115823

FILED ELECTRONICALLY ON: November 23, 2021

**RENEWED PETITION UNDER 37 C.F.R. § 1.78 TO ACCEPT AN
UNINTENTIONALLY DELAYED PRIORITY CLAIM UNDER 35 U.S.C. § 120**

Mail Stop Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is a renewed petition under 37 C.F.R. § 1.78(e) to accept an unintentionally delayed priority claim, in response to the decision on petition under 37 CFR 1.78(e) mailed October 18, 2021.

The full and proper priority in the present case should read this application 16/714,579 should read (strike out used to show changes):

This application is a Continuation of application No. ~~16/672,267, filed on Jan. 7, 2020, which is a continuation~~ of U.S. Application No. 16/601,168, filed October 14, 2019, which is a

continuation of U.S. Application No. 15/892,178, filed February 8, 2018, which is a continuation of U.S. Application No. 14/861,989, filed September 22, 2015 (now U.S. Patent 9,920,366, issued March 20, 2018), which is a continuation application of International Application No. PCT/US2014/072383, filed December 24, 2014, which application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/921,456, filed December 28, 2013, and U.S. Provisional Application No. 61/948,509, filed March 5, 2014, each of which is entirely incorporated herein by reference.

An explanation as to why the error requiring deletion of the priority claim is clear from the record, why the priority claim being deleted could not have been relied on as a source for priority, and why deletion of this priority claim has no effect on the ability of the present application to claims priority to the remaining applications in the priority chain is provided in the accompanying request for certificate of correction.

Accordingly, it is hereby respectfully petitioned under 37 C.F.R. § 1.78 that the Office accept an unintentionally delayed claim for priority in connection with the above referenced application. In support of this petition, Applicant has submitted or submits:

- i. A corrected Application Data Sheet showing the requested change (filed September 2, 2021);
- ii. The petition fee set forth in 37 C.F.R. § 1.17(m) and any additional fees that may be required (paid September 2, 2021); and
- iii. A statement that the entire period of delay between the date the claim was due under 1.78(d)(3) and the date the priority claim was filed was unintentional (provided September 2, 2021).
- iv. A request for a certificate of correction reflecting the correct priority claim as well as unrelated change of inventorship is filed herewith.

The Commissioner is authorized to charge all required fees to Deposit Account No. 16-605 (Docket No. 065777-492387).

Date: November 23, 2021

Customer No. 115823

Respectfully submitted,

/ Joe Liebeschuetz /

Joe Liebeschuetz
Reg. 37,505

**UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION**Page 1 of 1

PATENT NO. : 10,889,858

APPLICATION NO.: 16/714,579

ISSUE DATE : 2021-01-12

INVENTOR(S) : AmirAli TALASAZ et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title page, item (72) Inventors: add inventor --Helmy Eltoukhy, Atherton CA (US)--

On the Title page, item (63), Related U.S. Application Data:

Delete "of application No. 16/672,267, filed on Jan. 7, 2020, which is a continuation"

At col. 1, lines 6-7, please delete "a continuation of U.S. application Ser. No. 16/672,267, filed Nov. 1, 2019, which is"

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Inventor(s): AmirAli Talasaz, et at.

Application No.: 16/714,579

Filing Date: December 13, 2019

Issued Patent No.: 10,889,858

Title: METHODS AND SYSTEMS FOR
DETECTING GENETIC VARIANTS

Group Art Unit: 1637

Examiner: Kenneth R. HORLICK

Confirmation No.: 3111

Customer No.: 115823

FILED ELECTRONICALLY ON: November 22, 2021

**REQUEST FOR CERTIFICATE OF CORRECTION OF THE ISSUED PATENT
FOR (37 C.F.R. §1.322)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
ATTN: Certificate of Correction Branch

Sir:

There are errors in the above-noted Patent's Priority Claim and in omission of a named inventor. The mistakes are of a minor nature or character, and correction as detailed herein does not involve such change in the patent as would constitute new matter or would require re-examination of the patent. A certificate of correction is therefore requested.

Attached hereto is Form PTO/SB/44 which is suitable for printing.

Applicant respectfully requests correction to the language in the issued Patent in order to correct an error in the named inventors. This error was made without deceptive intent. The changes requested are:

On the Title page, item (72), Inventors: add inventor --Helmy Eltoukhy,
Atherton, CA (US)--

A related petition to change inventorship was filed September 2, 2021 but does not appear to have been acted on.

Correction of the priority claim is also requested. It is immediately apparent on the face of the issued Patent that the priority claim contains an error. The exact column and line number or page and item where the error occurs in the above-noted Patent are: on the Title page, Item (63), Related U.S. Application Data, which should be corrected as follows:

Continuation of application No. 16/601,168, filed on Oct. 14, 2019, which is a continuation of application No. 15/892,178, filed Feb. 8, 2018, which is a continuation of application No. 14/861,989, filed on Sep. 22, 2015, now Pat. No. 9,920,366, which is a continuation of application No. PCT/US2014/072383, filed on Dec. 24, 2014.

A corresponding correction is requested in the Cross-Reference to related Applications at col. 1, lines 5-21.

Applicant respectfully submits that the need for this unintentionally delayed claim for priority and Certificate of Correction has arisen at least in part as a result of an error by the Office. At the time of filing the present case on December 13, 2019, Applicant made a proper benefit claim in the Application Data Sheet, based on a presumption that the Application No. 16/672,267, filed on November 1, 2019, would be accorded a Filing Date of November 1, 2019.

On December 19, 2019 (6 days after the present case was filed), Applicant received a Notice of Incomplete Application in Application No. 16/672,267, indicating a filing date of November 1, 2019 was not accorded. On January 10, 2020, a Filing Receipt was received in Application No. 16/672,267, indicating a Filing Date of January 7, 2020. As a result of that later granted Filing Date in Application No. 16/672,267, the benefit claim became defective because there was not co-pendency between Application No. 16/672,267 (with a Filing Date of January 7, 2020) and the present case (with a Filing Date of December 13, 2019).

However, such defect went unnoticed by the USPTO and the Applicant. In particular, the USPTO did not notify Applicant of any defective benefit claim in the present case, and in fact recorded that full benefit claim in PAIR (where it is still viewable). Moreover, in all of the Filing Receipts (of January 9, 2020 and January 22, 2020), no problem with the benefit claim was mentioned by the Office in any communication during prosecution, and U.S. Patent No.

10,889,858 was granted with the uncorrected benefit claim printed on the face of the patent.

Applicant requests grant of the petition for correction of the benefit claim under 1.78(e) and the corresponding Certificate of Correction to correct the error in the benefit claim. The requested correction is merely the removal of Application No. 16/672,267 from the priority chain. The present case then claims priority directly to Application No. 16/601,168 rather than through 16/672,267 as an intermediate (as noted on the certificate of correction). Application Nos. 16/601,168 and 16/672,267 have identical specifications. The removal of 16/672,267 represents correction of an error immediately apparent from the face of the present patent because the present patent cannot claim priority to an application with a later filing date than its own. Nor could the Examiner have relied on an application having a later filing date than that of the present patent as a source of priority. It is also clear from the record that removal of 16/672,267 has no effect on the propriety of claiming priority to the remaining applications in the priority chain, on which the Examiner may have relied, because the present application has co-pendency with 16/601,168, through which priority can be claimed to each of the earlier applications in the priority chain.

Because the facially inappropriate claim to priority to 16/672,267 could not have been relied on for an earlier effective filing date in prosecution and has no effect on the propriety of claiming priority to the remaining applications in the priority chain, its removal does not require any further examination.

Applicant respectfully submits that such mistake in the patent was incurred at least in part through the fault of the Office not notifying application of the priority claim defect, and is clearly disclosed by the records of the Office. Accordingly, Applicant respectfully requests the Director to issue a certificate of correction stating the fact and nature of such mistake to be recorded in the records of patents for the U.S. Patent No. 10,889,858, pursuant to 35 U.S.C. 254 and 37 CFR 1.322.

In the alternative, should the Office find that such mistake in the patent was incurred through Applicant's mistake (which Applicant does not concede), then Applicant respectfully submits that such mistake constitutes a mistake of minor character and does not involve a change which would constitute new matter or require reexamination and is clear from the record to be appropriate (as previously pointed out above), thereby satisfying the statutory requirements of 35 U.S.C. 255, 37 CFR 1.323 and MPEP 1481.03. Although removal of a

priority claim may sometimes require further examination that is not the case here because as related above, the removal corrects an error immediately apparent from the record, which could not have been relied on for an earlier effective filing date and the removal does not change the effective filing date of the application. By implication, the requested correction to the priority claim does not seek to materially affect the scope or meaning of the patent, at least because there would be no change to the earliest effective filing date nor the specification of the U.S. Patent No. 10,889,858; therefore, the requested correction does not impact the examination and allowance of the Application No. 16/714,579 nor the issuance of the corresponding U.S. Patent No. 10,889,858. Accordingly, Applicant respectfully requests the Director to issue a certificate of correction stating the fact and nature of such mistake to be recorded in the records of patents for the U.S. Patent No. 10,889,858, pursuant to 35 U.S.C. 255 and 37 CFR 1.323.

FEE AUTHORIZATION

The processing fee under 37 CFR 1.20(a) was paid September 2, 2021. However, if a further processing or other fees are due please charge to Deposit Account No. 16-605 (Docket No. 42534-708.305).

The Commissioner is authorized to charge any additional fees which may be required, including petition fees, or credit any overpayment to Deposit Account No. 16-605 (Docket No. 065777-492387).

Respectfully submitted,

Dated: November 23, 2021

By: / Joe Liebeschuetz /

Joe Liebeschuetz
Reg. No. 37,505

Customer No. 115823
Tel 650 838 2038

Electronic Acknowledgement Receipt

EFS ID:	44358809
Application Number:	16714579
International Application Number:	
Confirmation Number:	3111
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	AmirAli TALASAZ
Customer Number:	115823
Filer:	Joe Liebeschuetz
Filer Authorized By:	
Attorney Docket Number:	42534-708.305
Receipt Date:	23-NOV-2021
Filing Date:	13-DEC-2019
Time Stamp:	21:32:39
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Petition for review by the Office of Petitions	42534-708-305_renewedpet.pdf	110647 <small>a39d21b85cba0c8a01af874a7e5ad7d2e117782</small>	no	2

Warnings:

00653

Information:					
2	Request for Certificate of Correction	42534-708-305_coc.pdf	281302	no	2
			af19b2129102d279445f16f86c68a2492686376d		

Warnings:

Information:

3	Request for Certificate of Correction	412192001.pdf	119398	no	4
			634a0178dc21c8de7d4af0ccd490f64b5ca40429		

Warnings:

Information:

Total Files Size (in bytes):			511347
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.