PTO/AIA/15 (10-17)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	42534-708.303
First Named Inventor	Helmy ELTOUKHY
Title	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [✔] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [✔] **Specification** [*Total Pages* _____] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [✔] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 11]
6. **Inventor's Oath or Declaration** [*Total Pages* 2] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [✔] A copy from a prior application (37 CFR 1.63(d))
7. [✔] **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

ADDRESS TO:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee _____
11. [✔] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [✔] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [✔] **Other:** Certification and Request for Prioritized Examination

*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

[✘] The address associated with Customer Number: 115823 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Timothy A. Hott/	Date	2019-10-14
Name (Print/Type)	Timothy A. Hott	Registration No. (Attorney/Agent)	67740

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	42534-708.303
	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
	Helmy		ELTOUKHY	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
Atherton	CA	US

Mailing Address of Inventor:

Address 1	505 Penobscot Drive
Address 2	
City	Redwood City
State/Province	CA
Postal Code	94063
Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	AmirAli		TALASAZ	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
Atherton	CA	US

Mailing Address of Inventor:

Address 1	505 Penobscot Drive
Address 2	
City	Redwood City
State/Province	CA
Postal Code	94063
Country	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).

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	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	42534-708.303	**Small Entity Status Claimed**	☐
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:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	115823		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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		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	Expired			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)
14861989	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)
14861989	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

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).

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	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

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. Add

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.

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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.

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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	42534-708.303
	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 — Remove

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.

Clear

● 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. 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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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)	2019-10-14
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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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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

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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 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 CooperationTreaty.

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.

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. 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 adaptors. In some embodiments, at least 4 terminal bases are identical in all of the library adaptors.

[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 adaptors 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 phylogentic 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, n 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 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

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. <u>Methods for processing and/or analyzing a nucleic acid sample</u>**

[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 ($2x10^{11}$) 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/orsomatic 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 then 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, medulloeptithelioma, 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 ^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, 25and 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 a 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. <u>Sequencing</u>**

[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. <u>Determining consensus sequence reads</u>**

[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 N1, N2, and N3 as the numbers of double-strands, single-strands, and unseen fragments, respectively. Thus, N=N1+N2+N3 (N1 and N2 are known from the sequence readouts, and N and N3 are unknown). If the formula is solved for N (or N3), then N3 (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 N1 double-strands has the following equation: N1=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×p×(1-p). Thus, seeing all N2 single-strands has the following equation: N2=N×2×p×(1-p).

[00169] "p" is also unknown. To solve p, the ratio of N1 to N2 is used to solve for "p":

$$R = \frac{N1}{N2} = \frac{Np^2}{2Np(1-p)} = \frac{p^2}{2p(1-p)} = \frac{p}{2(1-p)}$$

Once "p" is found, N can be found. After N is found, can be found N3 = N − N1 − N2.

[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. <u>Grouping</u>**

[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. <u>CNV Detection</u>**

[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. <u>Sequence Variant Detection</u>**

[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 phylogentic 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 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.

[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. <u>Pooling Methods</u>**

[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. <u>Computer Control Systems</u>**

[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 processer 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 H_2O. 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÷2 PQ = N.
1000 / P(2) = 500÷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÷2500 = P.
0.8 = P.
1000/ P(2) = N.
1000÷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, turn-over, 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	**Chromo-some**	**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.

PTO/AIA/01 (06-12)
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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 14/861,989

filed 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.

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LEGAL NAME OF INVENTOR

Inventor: AmirAli Talasaz Date (Optional): 11/20/15

Signature:

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.

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LEGAL NAME OF INVENTOR

Inventor: Helmy Eltoukhy Date (Optional): 11/10/15

Signature: _____

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.

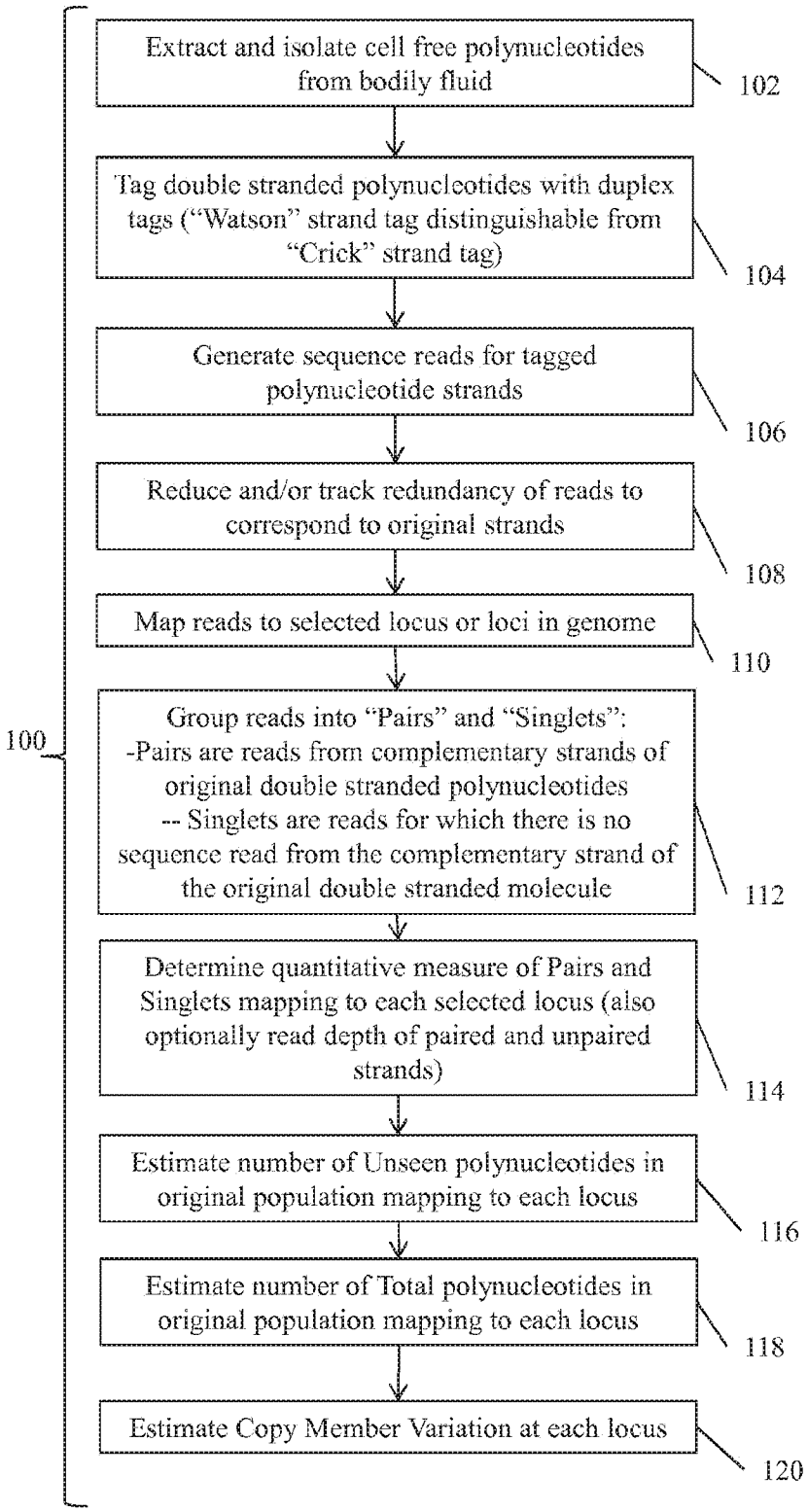

Extract and isolate cell free polynucleotides from bodily fluid
102
Tag double stranded polynucleotides with duplex tags ("Watson" strand tag distinguishable from "Crick" strand tag)
104
Generate sequence reads for tagged polynucleotide strands
106
Reduce and/or track redundancy of reads to correspond to original strands
108
Map reads to selected locus or loci in genome
110
Group reads into "Pairs" and "Singlets":
-Pairs are reads from complementary strands of original double stranded polynucleotides
-- Singlets are reads for which there is no sequence read from the complementary strand of the original double stranded molecule
112
Determine quantitative measure of Pairs and Singlets mapping to each selected locus (also optionally read depth of paired and unpaired strands)
114
Estimate number of Unseen polynucleotides in original population mapping to each locus
116
Estimate number of Total polynucleotides in original population mapping to each locus
118
Estimate Copy Member Variation at each locus
120
100

Fig. 1

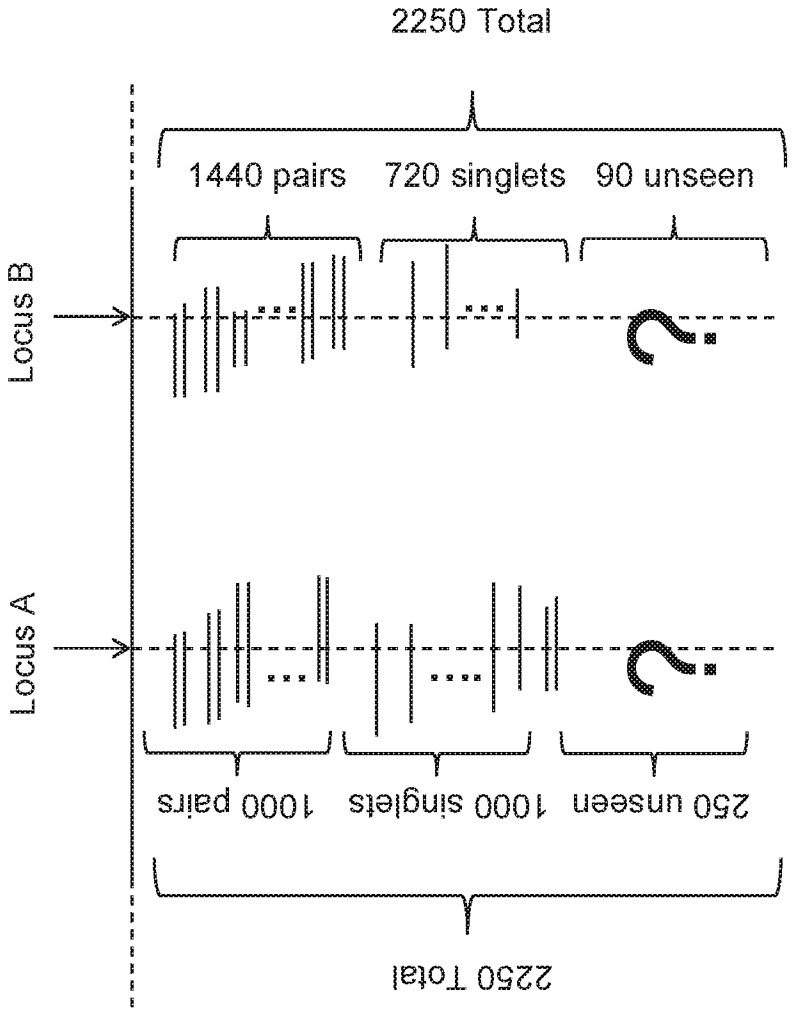

2250 Total
1440 pairs
720 singlets
90 unseen
Locus B
Locus A
1000 pairs
1000 singlets
250 unseen
2250 Total

Fig. 2

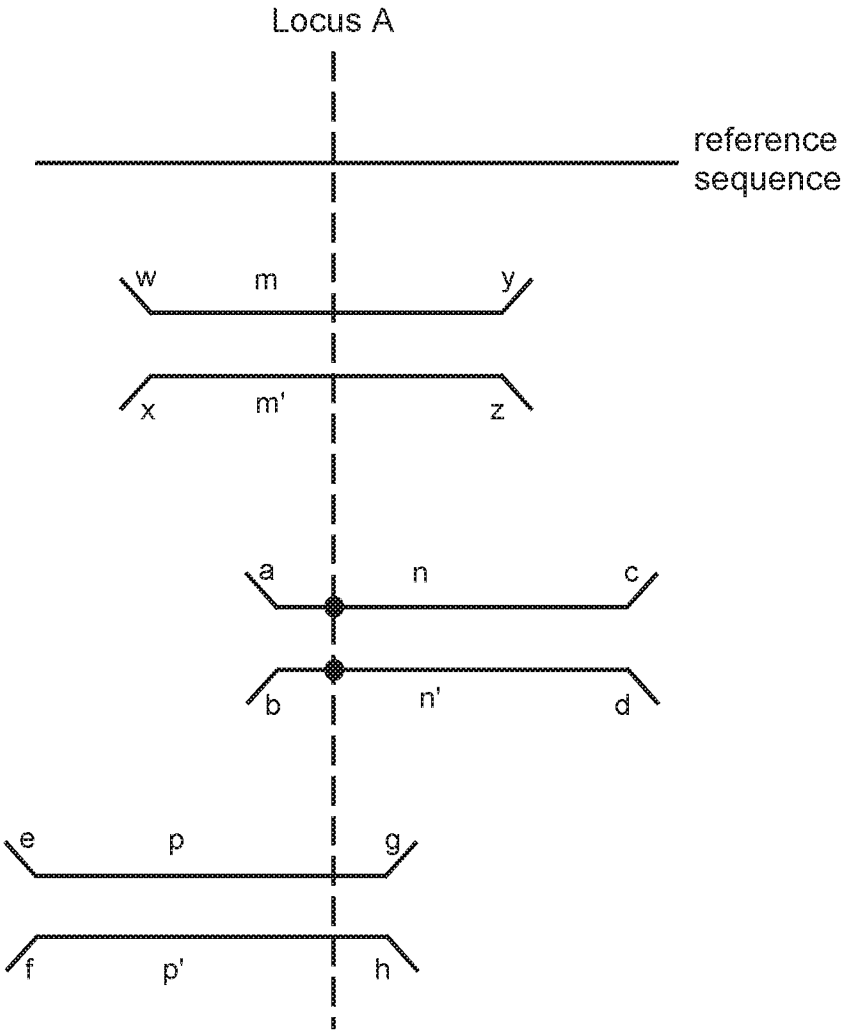


Fig. 3

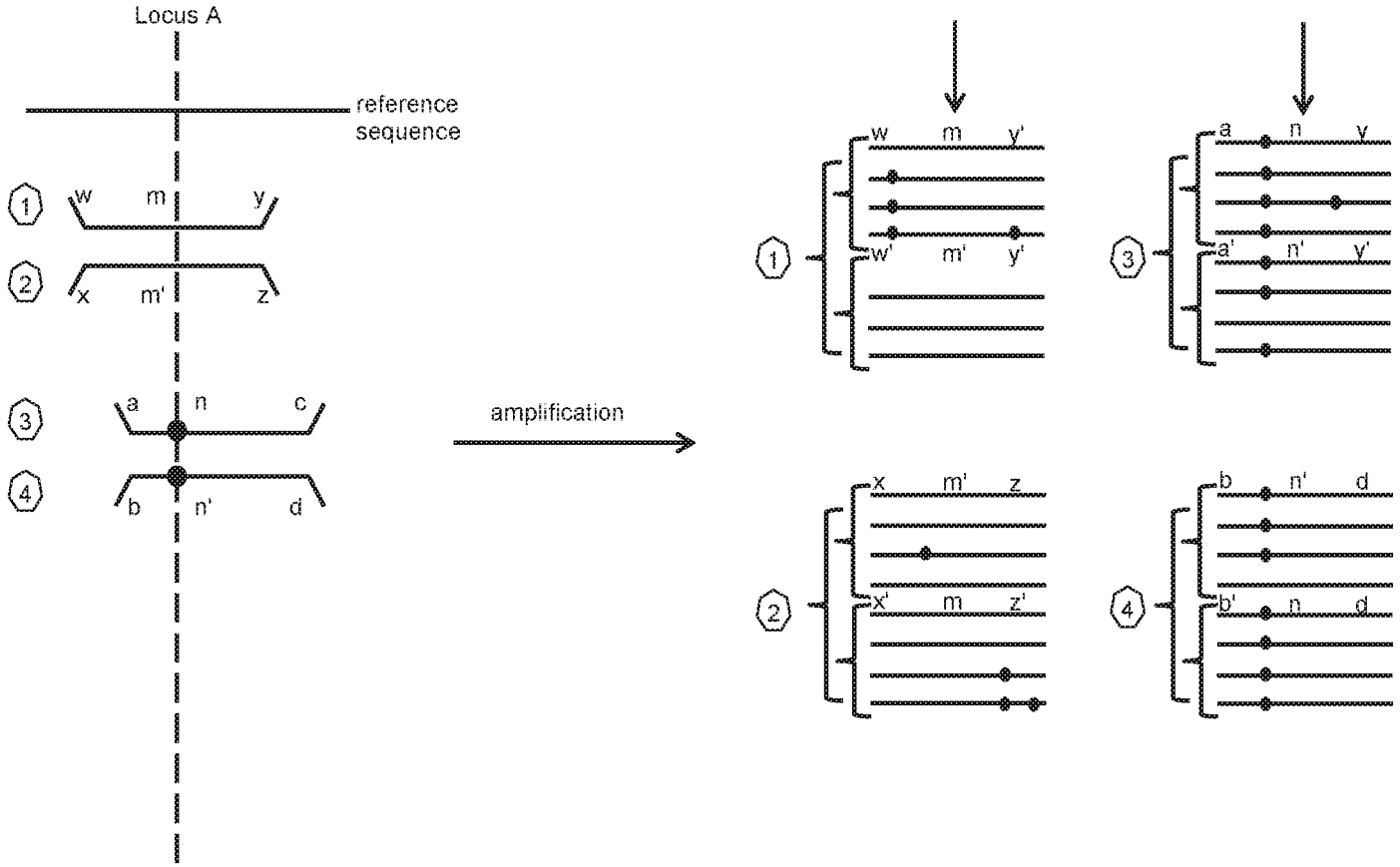

Locus A
reference sequence
1
w m y
2
x m' z
3
a n c
4
b n' d
amplification
w m y'
w' m' y'
a n y
a' n' y'
x m' z
x' m z'
b n' d
b' n d

Fig. 4A

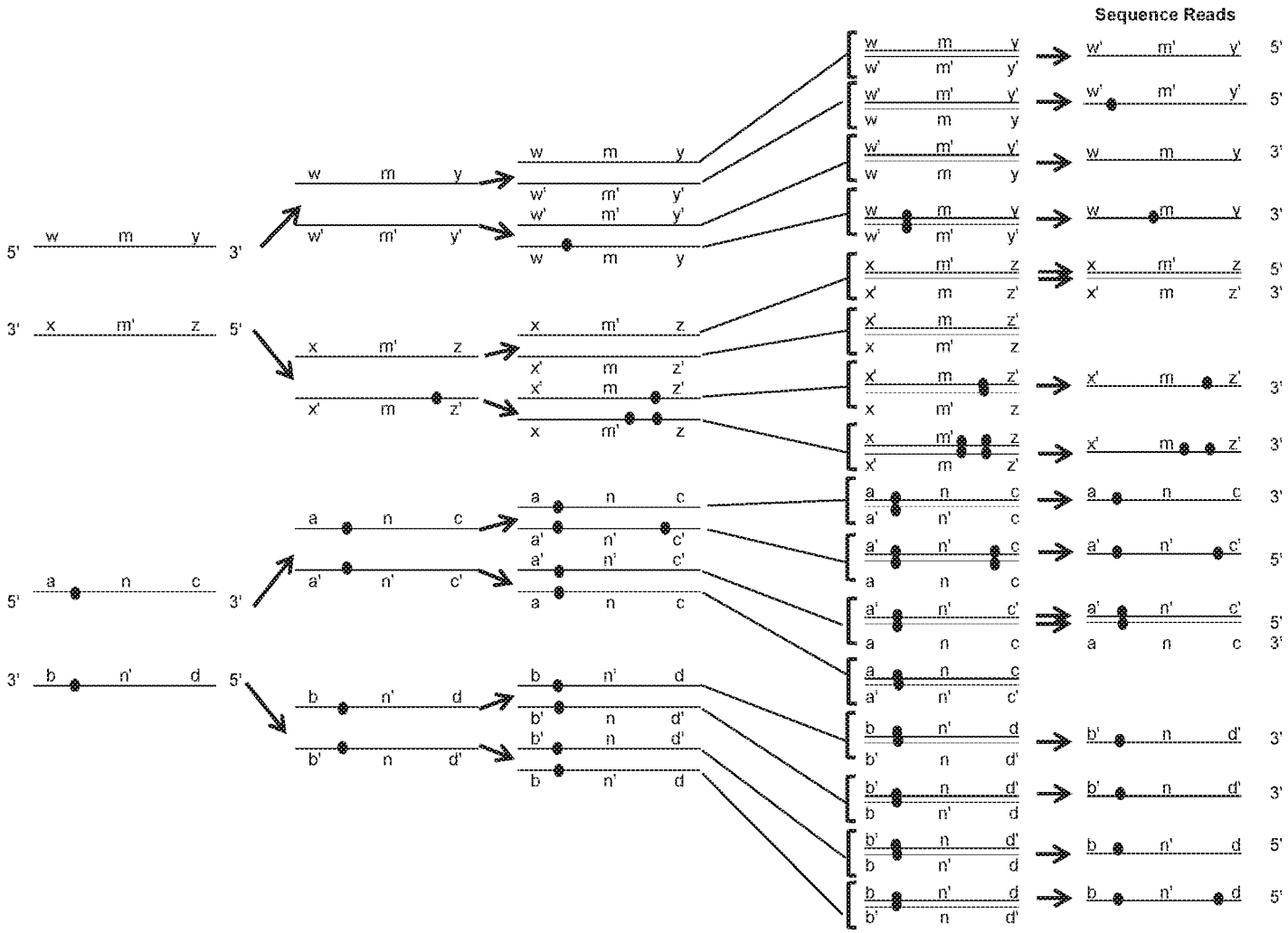


Fig. 4B

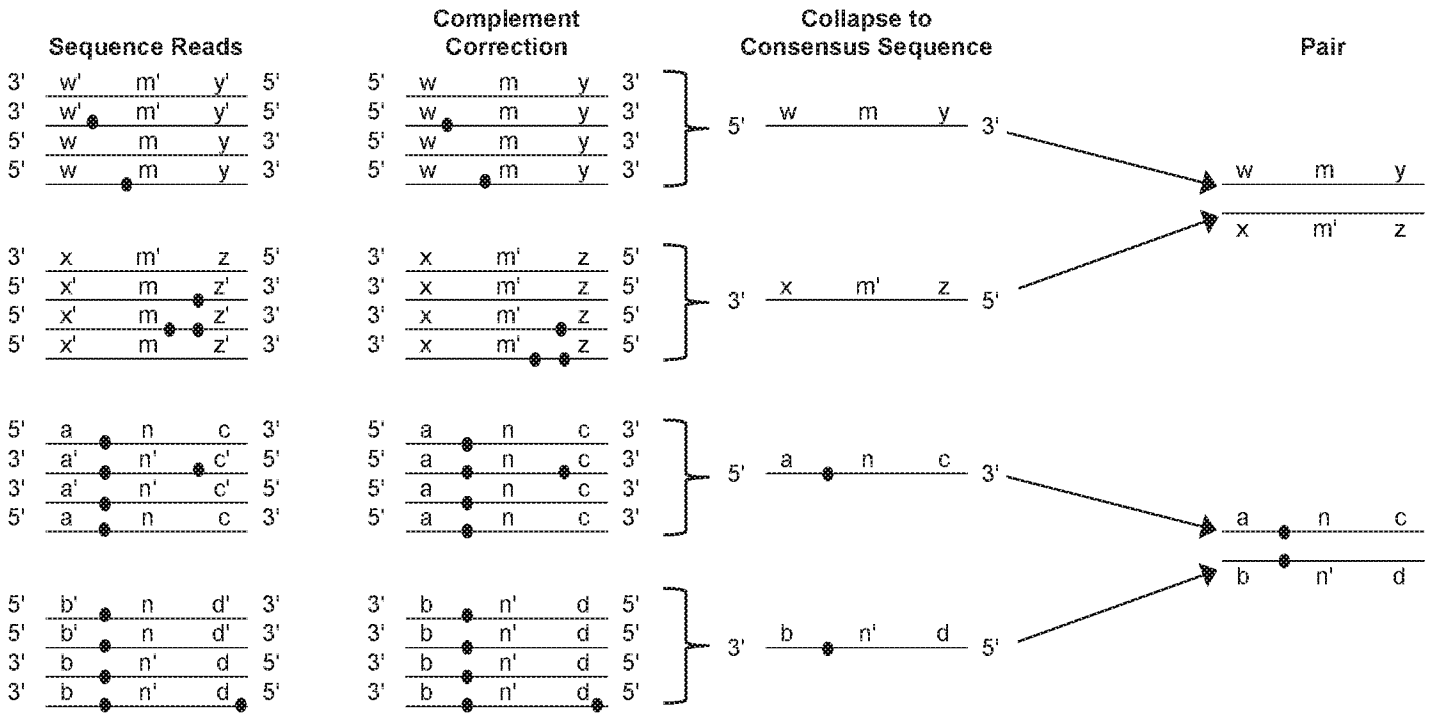

Sequence Reads
Complement Correction
Collapse to Consensus Sequence
Pair
3' w' m' y' 5'
3' w' m' y' 5'
5' w m y 3'
5' w m y 3'
5' w m y 3'
5' w m y 3'
5' w m y 3'
5' w m y 3'
3' x m' z 5'
5' x' m z' 3'
5' x' m z' 3'
5' x' m z' 3'
3' x m' z 5'
3' x m' z 5'
3' x m' z 5'
3' x m' z 5'
3' x m' z 5'
5' a n c 3'
3' a' n' c' 5'
3' a' n' c' 5'
5' a n c 3'
5' a n c 3'
5' a n c 3'
5' a n c 3'
5' a n c 3'
5' b' n d' 3'
5' b' n d' 3'
3' b n' d 5'
3' b n' d 5'
3' b n' d 5'
3' b n' d 5'
3' b n' d 5'
3' b n' d 5'

Fig. 4C

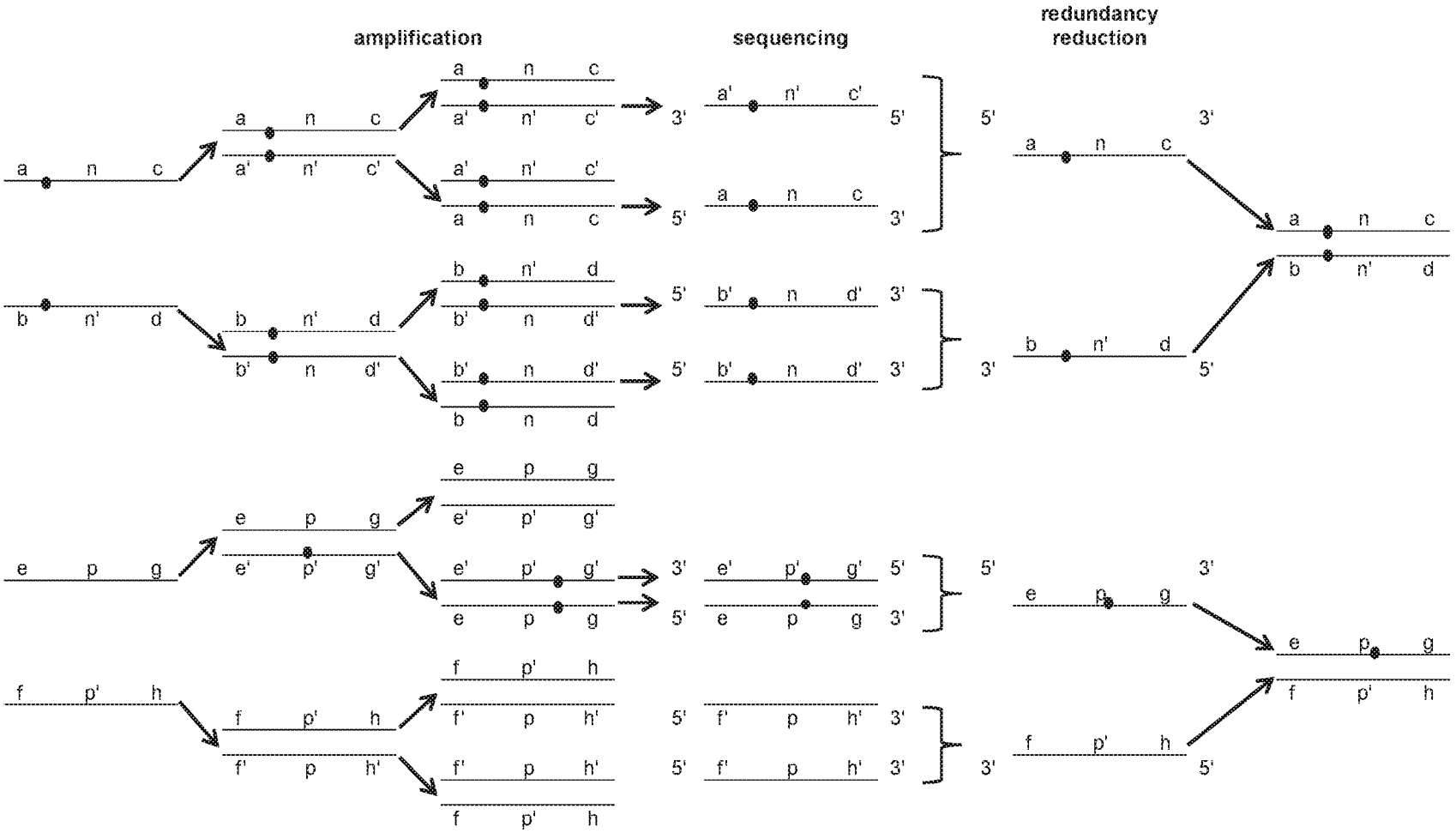

amplification
sequencing
redundancy reduction
a n c
a' n' c'
b n' d
b' n d'
e p g
e' p' g'
f p' h
f' p h'
3'
5'

Fig. 5

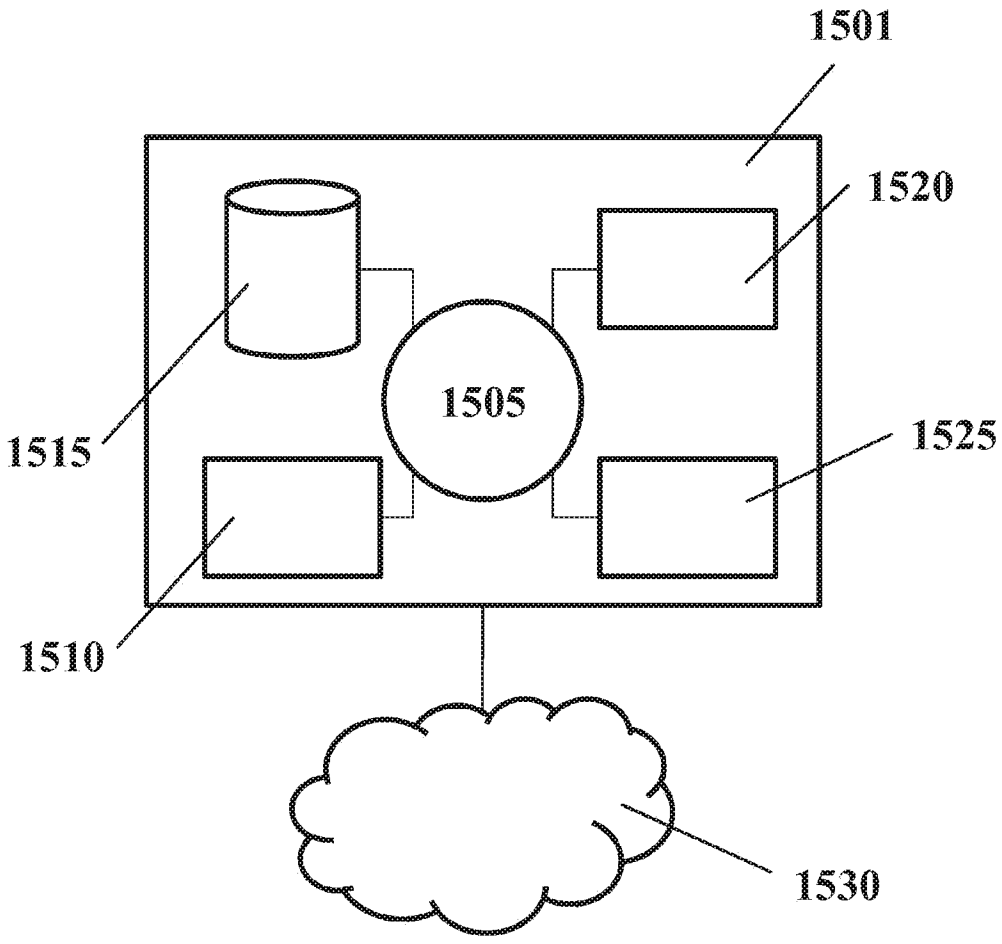


Fig. 6

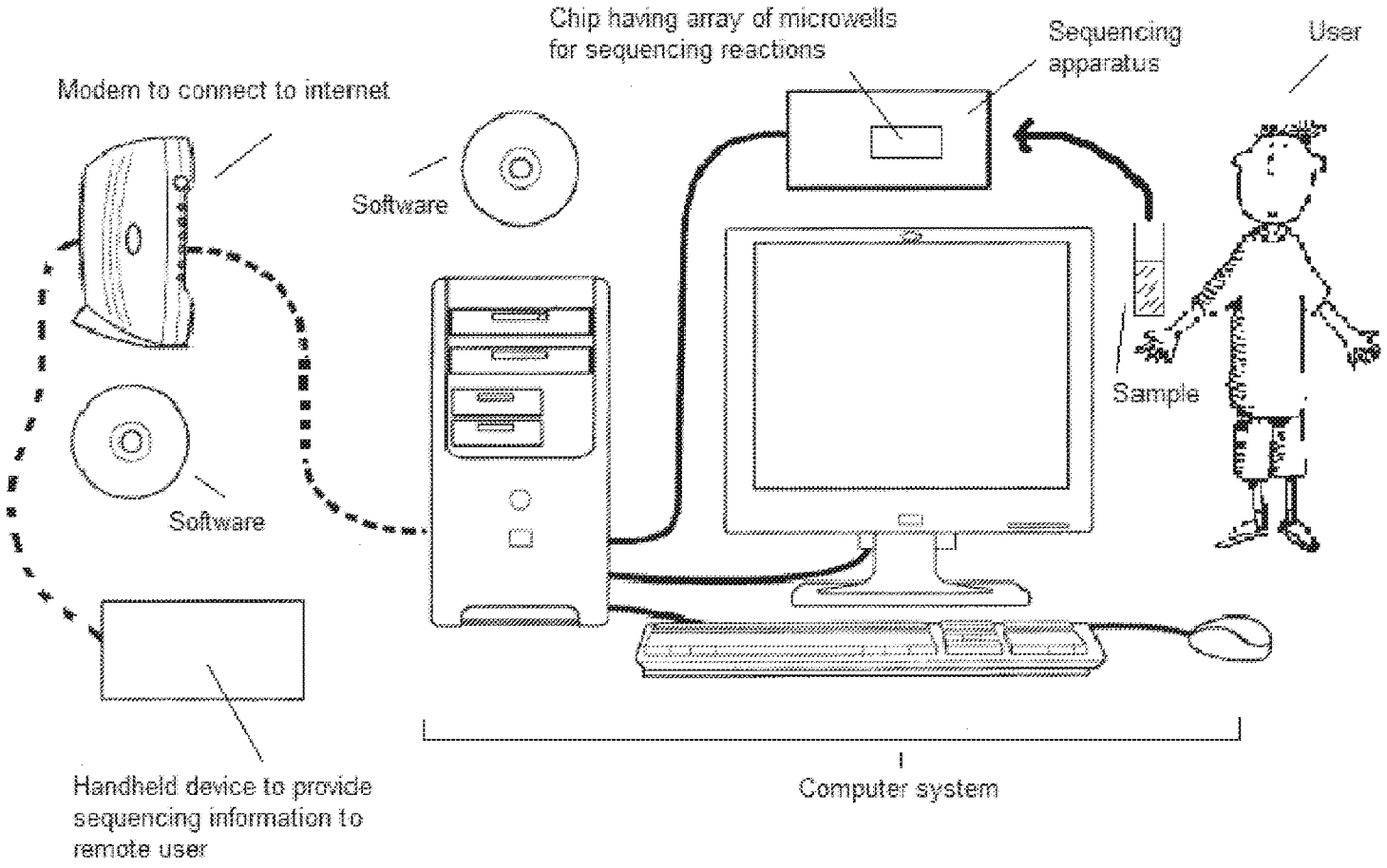

Chip having array of microwells for sequencing reactions
Sequencing apparatus
User
Modem to connect to internet
Software
Software
Sample
Handheld device to provide sequencing information to remote user
Computer system

Fig. 7

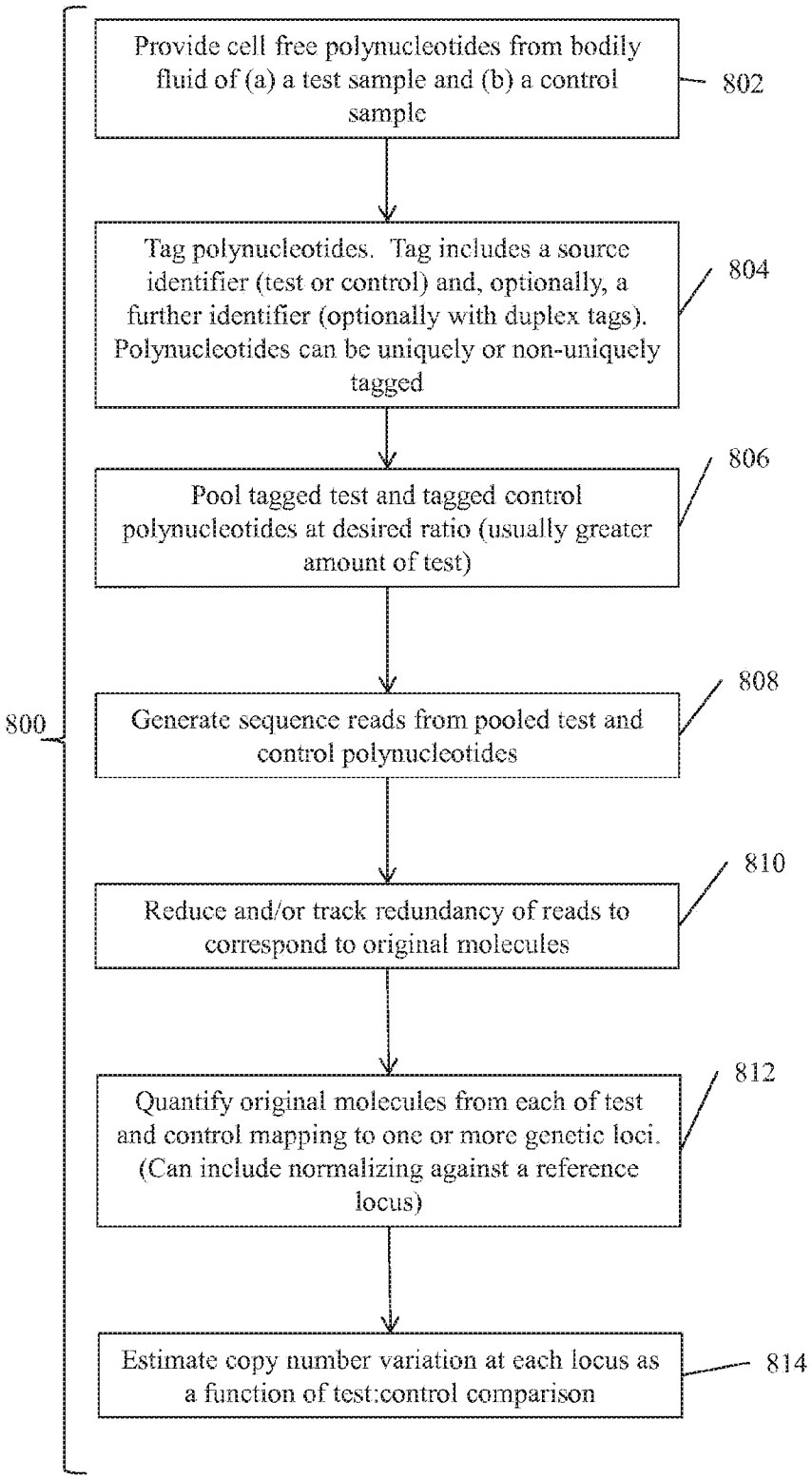

Provide cell free polynucleotides from bodily fluid of (a) a test sample and (b) a control sample
802
Tag polynucleotides. Tag includes a source identifier (test or control) and, optionally, a further identifier (optionally with duplex tags). Polynucleotides can be uniquely or non-uniquely tagged
804
Pool tagged test and tagged control polynucleotides at desired ratio (usually greater amount of test)
806
Generate sequence reads from pooled test and control polynucleotides
808
800
Reduce and/or track redundancy of reads to correspond to original molecules
810
Quantify original molecules from each of test and control mapping to one or more genetic loci. (Can include normalizing against a reference locus)
812
Estimate copy number variation at each locus as a function of test:control comparison
814

Fig. 8

Fig. 9A

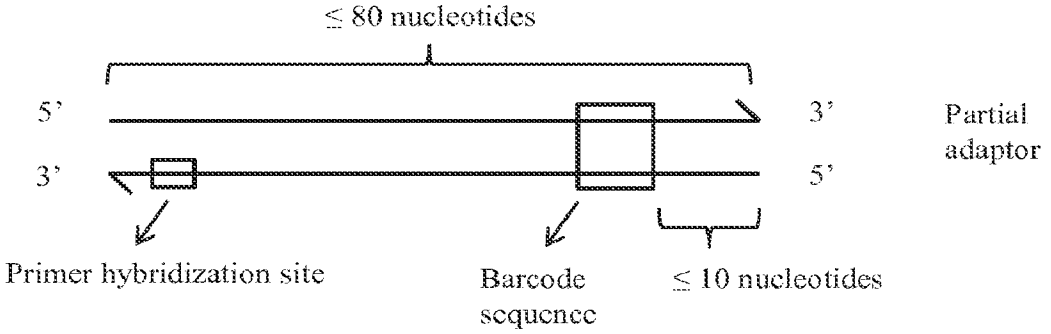


Fig. 9B

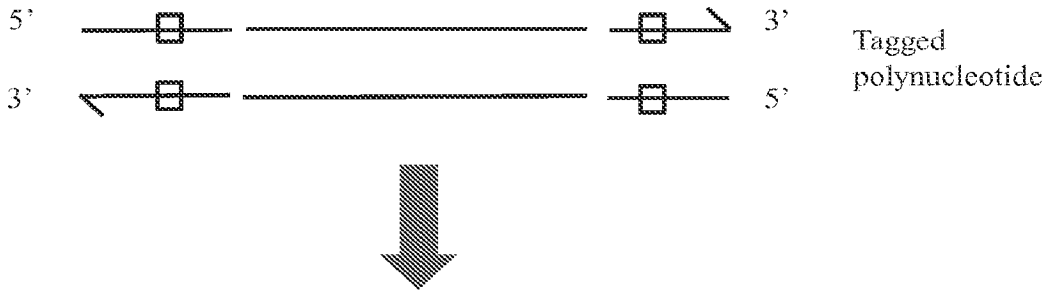
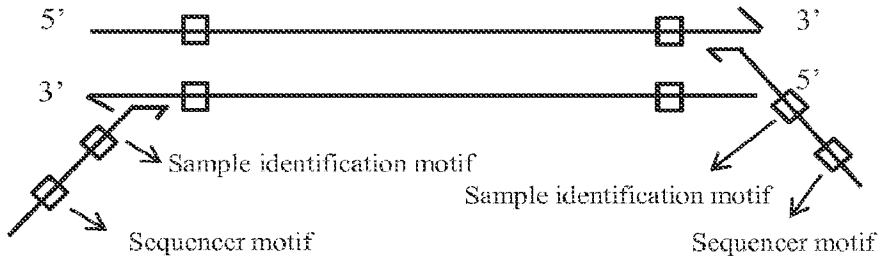


Fig. 9C



Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	GH0004US-C2_42534-708.303

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
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:	37449872
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	GH0004US-C2_42534-708.303
Receipt Date:	14-OCT-2019
Filing Date:	
Time Stamp:	16:59:15
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	E20190DG59460012
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 1.16 (National application filing, search, and examination fees)

37 CFR 1.17 (Patent application and reexamination processing fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	TrackOne Request	2019-10-14_GH0004US-CON2_T1Req.pdf	129506 1399b7032e66b7561e05f1d87f22dba6efe31c8d	no	2
Warnings:					
Information:					
2	Transmittal of New Application	2019-10-14_GH0004US-CON2_AppTrans.pdf	293620 71a26bc7e25b13583a9cc757401f90056163a781	no	2
Warnings:					
Information:					
3	Application Data Sheet	2019-10-14_GH0004US-CON2_ADS.pdf	1874412 2c3bd6382aea740a3ec96766ec8a5e1eb3614180	no	9
Warnings:					
Information:					
4		2019-10-14_GH0004US_CON2_SpecAsFiled.pdf	626337 da2b9711d73fc96a785d8379cb15c79ca0623312	yes	91

Multipart Description/PDF files in .zip description

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

Warnings:					
Information:					
5	Oath or Declaration filed	2019-10-14_GH0004US-CON2_ParentDec.pdf	198149 9b1fdd215bf3349ea7dfbe0758e33756228765fb	no	2

Warnings:					
Information:					
6	Power of Attorney	2019-10-14_GH0004US-CON2_ParentPoA.pdf	190194 ff69efd3f616ea4d4f0569e8551fc9e7bce85453	no	3
Warnings:					
Information:					
7	Drawings-only black and white line drawings	2019-10-14_GH0004US-CON2_Figs.pdf	2550181 97cf1eb2e8fae70ef9de5a96fb471e6f046d9975	no	11
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	41700 c106219af0254a577bb9af1b76b15f742a960d08	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	5904099		

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.

<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.
<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.
<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.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

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

First Named Inventor:	Helmy ELTOUKHY	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 October 14, 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 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.

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE

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

☐

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	/[signature]/	Date *	9/5/13
Name	Helmy Eltoukhy	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.

5753826_1.DOC

PTO/AIA/96 (08-12)
Approved for use through 01/31/2013. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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, 2015

Titled: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

Guardant 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.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

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☐ 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/	February 22, 2016
Signature	Date
Ali R. Alemozafar	68,180
Printed or Typed Name	Title or Registration Number

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303

CONFIRMATION NO. 1052

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

FORMALITIES LETTER

OC000000113063354

Date Mailed: 12/04/2019

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 1½ 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.

Items 1) and 2) provide general guidance related to requirements for sequence disclosures.

1) 37 CFR 1.821(c) requires that patent applications which contain disclosures of nucleotide and/or amino acid sequences that fall within the definitions of 37 CFR 1.821(a) must contain a "Sequence Listing", *as a separate part of the disclosure*, which presents the nucleotide and/or amino acid sequences and associated information using the symbols and format in accordance with the requirements of 37 CFR 1.821-1.825. This "Sequence Listing" part of the disclosure may be submitted:
   a) via EFS-Web (see Section I.1 of the Legal Framework for EFS-Web (https://www.uspto.gov/patents-application-process/filing-online/legal-framework-efs-web), hereinafter "Legal Framework") as an ASCII text file, together with an incorporation-by-reference of the material in the ASCII text file in a separate paragraph of the specification identifying:
      i) the name of the ASCII text file;
      ii) the date of creation; and
      iii) the size of the ASCII text file in bytes;
   b) on compact disc(s) in duplicate according to 37 CFR 1.52(e)(1)(ii) and (4), labeled according to 37 CFR 1.52(e)(6), together with a statement that the duplicate compact discs are identical and an

incorporation-by-reference of the material in the ASCII text file according to 37 CFR 1.52(e)(5) in a separate paragraph of the specification identifying:

i) the name of the ASCII text file;
ii) the date of creation; and
iii) the size of the ASCII text file in bytes;

c) via EFS-Web as a PDF (not recommended); or
d) on paper.

2) 37 CFR 1.821(e) requires that a *copy* of the "Sequence Listing" must also be submitted in computer readable form (CRF) in accordance with the requirements of 37 CFR 1.824.
   a) If a "Sequence Listing" ASCII text file submitted via EFS-Web on the application filing date complies with the requirements of 37 CFR 1.824(a)(2)-(6) and (b), and applicant has not filed a "Sequence Listing" as a PDF, the text file will serve as both the "Sequence Listing" required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e), and the statement of identity under the "Legal Framework" is not required.
   b) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed via EFS-Web as a PDF, then the "Legal Framework" requires submission of a statement that the "Sequence Listing" content of the PDF copy and the ASCII text file copy submitted via EFS-Web are identical.
   c) If the "Sequence Listing" required by 37 CFR 1.821(c) is filed on paper or compact disc, then 37 CFR 1.821(f) requires submission of a statement that the "Sequence Listing" content of the paper or compact disc copy and the CRF are identical.

Specific deficiencies and the required response to this notice are as follows:

- This application fails to comply with the requirements of 37 CFR 1.821-1.825 because it does not contain a "Sequence Listing" as a separate part of the disclosure or a CRF. Applicant must provide:
  - A "Sequence Listing" part of the disclosure, as described above in item 1); **together with**
  - **An amendment specifically directing its entry into the application;**
  - A statement that the "Sequence Listing" includes no new matter; and
  - A statement that indicates support for the amendment in the application, as filed, as required by 37 CFR 1.825 and the "Legal Framework".
- If the "Sequence Listing" part of the disclosure is submitted according to item 1) a) or b) above, Applicant must also provide:
  - A substitute specification in compliance with 37 CFR 1.121(b)(3) and 1.125 inserting the required incorporation-by-reference paragraph, consisting of:
    - A copy of the previously-submitted specification, with deletions shown with strikethrough or brackets and insertions shown with underlining (marked-up version);
    - A copy of the amended specification without markings (clean version); and
    - A statement that the substitute specification contains no new matter.
- If the "Sequence Listing" part of the disclosure is submitted according to item 1) b), c), or d) above, Applicant must also provide:
  - A CRF; and
  - A statement according to item 2) b) or c) above.
- If applicant desires the CRF in this application to be identical with the CRF of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF (Form PTO/SB/93 may be used for this purpose).

For questions regarding compliance to these requirements, please contact:

- **For Rules interpretation, call (571) 272-2510**
- **For CRF submission help, call (571) 272-2510**
- **For PatentIn software program support technical assistance, call (866) 217-9197.**
- **Send e-mail correspondence for PatentIn software program help to ebc@uspto.gov**
- **PatentIn software is available at http://www.uspto.gov/patents/resources/tools/checker/patentinrel.jsp .**

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.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

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**.

/tle/

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/601,168	10/14/2019	1636	2720	42534-708.303	30	3

CONFIRMATION NO. 1052

FILING RECEIPT

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

OC000000113063353

Date Mailed: 12/04/2019

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)

Helmy ELTOUKHY, Atherton, CA;
AmirAli Talasaz, 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 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
and 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: 12/03/2019

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/601,168**

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

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.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

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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 AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

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No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/601,168

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	30 minus 20 =	* 10			OR	x 100 =	1000
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 =	*				x 460 =	0.00
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).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2720

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): ELTOUKHY et al.	Confirmation No.: 1052
Serial Number: 16/601,168	Customer No.: 115823
Filing Date: October 14, 2019	Group Art Unit: 1636
Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: To be assigned

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
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 December 4, 2019. The shortened statutory period for reply expires February 4, 2020; therefore, this response is timely filed. Applicant respectfully requests consideration of the above-referenced application in view of the following 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 9 of this paper.

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

CROSS-REFERENCE

[0001] This application 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 adaptors. In some embodiments, at least 4 terminal bases are identical in all of the library adaptors.

[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 adaptors 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 phylogentic 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, n 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 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

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. <u>Methods for processing and/or analyzing a nucleic acid sample</u>**

[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 ($2x10^{11}$) 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/orsomatic 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 then 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, medulloeptithelioma, 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 ^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, 25and 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 a 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. <u>Sequencing</u>**

[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. <u>Determining consensus sequence reads</u>**

[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 N1, N2, and N3 as the numbers of double-strands, single-strands, and unseen fragments, respectively. Thus, N=N1+N2+N3 (N1 and N2 are known from the sequence readouts, and N and N3 are unknown). If the formula is solved for N (or N3), then N3 (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 N1 double-strands has the following equation: N1=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×p×(1-p). Thus, seeing all N2 single-strands has the following equation: N2=N×2×p×(1-p).

[00169] "p" is also unknown. To solve p, the ratio of N1 to N2 is used to solve for "p":

$$R = \frac{N1}{N2} = \frac{Np^2}{2Np(1-p)} = \frac{p^2}{2p(1-p)} = \frac{p}{2(1-p)}$$

Once "p" is found, N can be found. After N is found, can be found N3 = N − N1 − N2.

[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. <u>CNV Detection</u>**

[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. <u>Sequence Variant Detection</u>**

[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 phylogentic 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 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.

[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. <u>Pooling Methods</u>**

[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. <u>Computer Control Systems</u>**

[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 processer 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 H_2O. 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÷2 PQ = N.

1000 / P(2) = 500÷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÷2500 = P.

0.8 = P.

1000/ P(2) = N.

1000÷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, turn-over, 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	**Chromo-some**	**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.

PTO/AIA/01 (06-12)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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 16/601,168 filed 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 INVENTOR

Inventor: Stefanie Ann Ward MORTIMER Date (Optional): _____

Signature: _____

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	Helmy ELTOUKHY et al.	Confirmation No.:	1052
Serial No.:	16/601,168	Art Unit:	
Filing Date:	October 14, 2019	Examiner:	
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: 11/7/19 and 11/27/19

AND

☒ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

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 14, 2020

By: /Timothy A. Hott/
Timothy A. Hott, Reg. No. 67740

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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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.303

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
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	First Named Inventor	Helmy ELTOUKHY
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	Attorney Docket Number	42534-708.303

	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)	
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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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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).

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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)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
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	9	Guardant Health vs. FMI Suppl Invalidity References dated February 15, 2019 (C.A. No. 17-cv-1616-LPS-CJB)	
	10	Guardant Health vs. FMI Supplemental Invalidity 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)	
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Name/Print	Timothy A. Hott	Registration Number	67740

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Attorney Docket Number	42534-708.303

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	
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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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☒ A certification statement is not submitted herewith.

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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)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	
	Examiner Name	
	Attorney Docket Number	42534-708.303

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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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)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.303

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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	
	11	Notice of allowance dated 08/04/2017 for US Application No.15/467,570	

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	13	Notice of allowance dated 08/29/2017 for US Application No. 15/492,659	
	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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	23	Office action dated 06/03/2016 for US Application No. 14/861,989.	
	24	Office action dated 06/12/2017 for US Application No. 15/492,659.	
	25	Office action dated 07/18/2017 for US Application No. 14/861,989	
	26	Office Action dated 07/30/2019 for U.S. Application No. 16/283,635.	
	27	Office action dated 08/06/2019 for US Application No. 14/855,301.	
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	30	Office action dated 10/20/2017 for US Application No. 14/425,189.	
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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	☒
	39	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Foundation Medicine Inc.	☐
	40	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Grunecker Patent-und Rechtsanwalte PartG mbB	☐
	41	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Personal Genome Diagnostics, Inc.	☐
	42	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Strawman Limited	☐
	43	Opposition Form and Statement to EP3087204 filed November 14, 2018	☐
	44	Pacific Biosciences. Template Preparation and Sequencing Guide. Publication date: 2014-10-14. Pacific Biosciences website http://www.pacificbiosciences.com/support/pubmap/documentation.html.	☐

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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.	☐
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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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First Named Inventor	Helmy ELTOUKHY
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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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Name/Print	Timothy A. Hott	Registration Number	67740

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First Named Inventor	Helmy ELTOUKHY
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	37	ZHANG, et al. The impact of next-generation sequencing on genomics. J Genet Genomics. 2011 Mar 20;38(3):95-109. doi: 10.1016/j.jgg.2011.02.003. Epub 2011 Mar 15.	
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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.

Doc Code: R48.REQ
Document Description: Request under Rule 48 correcting inventorship

PTO/AIA/40 (04-15)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

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	16/601,168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
Practitioner Docket Number	42534-708.303

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)). $ _____

PTO/AIA/40 (04-15)
Approved for use through 11/30/2020. OMB 0651-0031
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

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
Registration number 64470

☐ attorney or agent acting under 37 CFR 1.34
Registration number _____

Signature /Timothy A. Hott/

Typed or printed name Timothy A. Hott

Date January 14, 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.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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
	Helmy		ELTOUKHY	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ 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 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	AmirAli		TALASAZ	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ 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 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Stefanie	Ann Ward	MORTIMER	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

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	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).

☐ **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	42534-708.303	Small Entity Status Claimed	☐
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

PTO/AIA/14 (02-18)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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	42534-708.303
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

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:	◉ Customer Number	○ US Patent Practitioner	○ 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	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	Expired		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)
~~14861989~~ PCTUS2014072383	Claims benefit of provisional	61948509	2014-03-05

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	Application Number	
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	

Prior Application Status	Expired	Remove	
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
~~14861989~~ 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	42534-708.303
	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.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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.

Clear

(●) 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.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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	/Timothy A. Hott/			Date (YYYY-MM-DD)	2020-01-14
First Name	Timothy	Last Name	Hott	Registration Number	67740

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

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	42534-708.303
	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.**

Electronic Patent Application Fee Transmittal

Application Number:	16601168
Filing Date:	14-Oct-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.303

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:	38296109
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	14-JAN-2020
Filing Date:	14-OCT-2019
Time Stamp:	20:21:55
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	E20201DK22159136
Deposit Account	
Authorized User	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		2020-01-14_GH0004US-CON2_CorrPaperResponse.pdf	138081 a10fa29d9737f2ccf9c192bb508fde6fcecafad9	yes	10

Multipart Description/PDF files in .zip description

Document Description	Start	End
Applicant Arguments/Remarks Made in an Amendment	9	10
Claims	3	8
Specification	2	2
Applicant Response to Pre-Exam Formalities Notice	1	1

Warnings:

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2	Specification	2020-01-14_GH0004US_CON2_SubSpecAsFiled.pdf	567460 11d885572e215c1f60869c57dc32122b39dd0920	no	86

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3	Oath or Declaration filed	2020-01-14_GH0004US-CON_Mortimer_Dec.pdf	2445236 0eab8fa5008def7dbe9e9b96fe4e3d692f9b93ce	no	1

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Information:

4	Transmittal Letter	2020-01-14_GH0004US-CON2_IDSTrans.pdf	110273 e9f17368f15d8a5ab425fdfc5f1c5ed1a971773f	no	4

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5	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_1_OF_8.pdf	1067952 b241d9ab98246b8863f42c33b030fd89d7148de2	no	39
Warnings:					
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6	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_2_OF_8.pdf	1057321 589dac81f80b6c503238379c5358f5d5733b032f	no	10
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7	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_3_OF_8.pdf	1055740 7f6d084a799f7e42cdcf268d77798716bfa809c2	no	8
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8	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_4_OF_8.pdf	1056438 a8fa48fb45e63eb7f0253fdea3736b82a168db42	no	8
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9	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_5_OF_8.pdf	1057810 e6a92730f552bc730c72d62649f76d0fab45c616	no	8
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11	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_7_OF_8.pdf	1057627 d63b1b5aac828f97aef5753468ef7a4ec424e24e	no	8

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12	Information Disclosure Statement (IDS) Form (SB08)	2020-01-14_GH0004US-CON2_SB08_8_OF_8.pdf	1056618 c8f0e086586484688ecb062d450e2a9c96383390	no	7

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13	Request under Rule 48 correcting inventorship	2020-01-14_GH0004US-CON2_ReqCorrInv.pdf	159463 ba7a43d7d84b7426c5be19eedd0dbdf309756f5c	no	3

Warnings:

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14	Application Data Sheet	2020-01-14_GH0004US_CON2_CorrADS.pdf	1402990 876940c2ac0bc89a3afe771a543ff25ec9bfd7f2	no	8

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15	Fee Worksheet (SB06)	fee-info.pdf	30498 e8121fa5e7f4ed57260fc7451decb0af5cb9e807	no	2

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REMARKS

Claims

Claims 1-30 were previously pending, but are hereby cancelled without prejudice or disclaimer. Claims 31-60 are newly added, support for which may be found throughout application USSN 16/601,168 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.

Sequence Listing

The Notice to File Corrected Application Papers requested that a sequence listing be submitted under 37 CFR 1.821(c). However, Applicant respectfully notes that the specification does not disclose nucleic acid and/or amino acid sequences that would merit inclusion of a sequencing listing. Thus, no sequencing listing is believed to be required.

CONCLUSION

This paper fully addresses the rejections raised in the Notice to File Corrected Application Papers mailed December 4, 2019. 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-CON2).

Respectfully submitted,
GUARDANT HEALTH, INC.

Date: January 14, 2020 By: /Timothy A. Hott/

Timothy A. Hott
Registration No.: 67740

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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 detecting a presence or absence of somatic genetic variants in double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a sample of a human subject, the method comprising:

(a) ligating adapters comprising molecular barcodes to a population of double-stranded cfDNA molecules obtained from the sample with at least a 10X molar excess of adapters relative to the double-stranded cfDNA molecules in the population to generate tagged parent polynucleotides,

wherein at least 20% of the double-stranded cfDNA molecules are tagged with adapters comprising molecular barcodes at 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) selectively enriching a plurality of the progeny polynucleotides for target regions associated with cancer, whereby enriched progeny polynucleotides are generated;

(d) sequencing at least a subset of the enriched progeny polynucleotides to produce a set of sequencing reads;

(e) mapping a plurality of the sequencing reads to one or more reference sequences;

(f) grouping a plurality of the sequencing reads mapped in (e) into families of sequencing reads based at least on (i) sequence information from the molecular barcodes and (ii) the beginning base position and ending base position of the sequencing reads that map to the one or more reference sequences; and

(g) detecting, from among a plurality of families of sequencing reads, the presence or absence of one or more somatic genetic variants.

32. (New): The method of claim 31, wherein the human subject has cancer or is suspected of having cancer.
33. (New): The method of claim 31, wherein the population of double-stranded cfDNA molecules comprises 1 nanogram (ng) to 100 ng of double-stranded cfDNA molecules.
34. (New): The method of claim 31, wherein the sample is blood, plasma, or serum.
35. (New): The method of claim 31, wherein ligating comprises blunt-end ligation or sticky-end ligation.
36. (New): The method of claim 31, wherein at least 40% of the double-stranded cfDNA molecules are tagged with adapters comprising molecular barcodes at both ends of the cfDNA molecules.
37. (New): The method of claim 31, wherein more than a 90X molar excess of adapters relative to the double-stranded cfDNA molecules in the population is used to generate the tagged parent polynucleotides.
38. (New): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes comprising molecular barcodes having 2 to 1,000 different molecular barcode sequences.
39. (New): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes comprising molecular barcodes having 5 to 100 different molecular barcode sequences that have a length of 5 to 20 nucleotides.
40. (New): The method of claim 31, wherein the target regions associated with cancer comprise genetic sequences of 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.

41. (New): The method of claim 31, further comprising amplifying a plurality of the enriched progeny polynucleotides prior to sequencing.
42. (New): The method of claim 31, wherein the detecting comprises generating a set of consensus sequences from among the sequencing reads of the families.
43. (New): The method of claim 42, further comprising determining the presence or absence of the one or more somatic genetic variants in the set of consensus sequences as compared with the one or more reference sequences.
44. (New): The method of claim 42, further comprising generating a base call for a family among the families at a locus of a plurality of loci of the one or more reference sequences.
45. (New): The method of claim 44, further comprising measuring a frequency of one or more bases called at the locus from among a plurality of the families.
46. (New): The method of claim 42, further comprising identifying consensus sequences from the set of consensus sequences as (i) paired consensus sequences generated from sequencing reads representing a Watson strand and a Crick strand of a tagged parent polynucleotide or (ii) unpaired consensus sequences generated from sequencing reads representing either a Watson strand or a Crick strand.
47. (New): The method of claim 46, further comprising:
    (i) calculating a first quantitative measure of paired consensus sequences that map to a locus of one or more reference sequences, and
    (ii) calculating a second quantitative measure of unpaired consensus sequences that map to a locus of the one or more reference sequences.
48. (New): The method of claim 47, further comprising:
    (iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to a locus of the one or more reference sequences for which neither complementary strand of the double-stranded cfDNA molecules is detected in said set of consensus sequences, wherein the third quantitative measure is calculated based at least in part on the first and second quantitative measures.
49. (New): The method of claim 48, further comprising:
    (iv) calculating a fourth quantitative measure of a total number of double-stranded cfDNA molecules in the population of double-stranded cfDNA molecules in the

sample, wherein the total number of double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules in the sample, and wherein the total number of double-stranded cfDNA molecules is determined based at least in part on the first, second, and third quantitative measures.

50. (New): The method of claim 49, further comprising:

(v) calculating a fifth quantitative measure of a copy number variation (CNV) for each locus in a set of one or more loci.

51. (New): The method of claim 50, comprising determining the first, second, third, fourth, and fifth quantitative measures with a programmed computer processor.

52. (New): The method of claim 31, wherein the one or more somatic genetic variants comprises a single nucleotide variation (SNV), an insertion or deletion (indel), a CNV, or a gene fusion.

53. (New): The method of claim 31, further comprising detecting cancer in the human subject when the presence of the one or more somatic genetic variants is detected in double-stranded cfDNA molecules from the sample.

54. (New): The method of claim 32, further comprising administering a therapy to the human subject to treat the cancer.

55. (New): The method of claim 32, wherein the cancer is selected from the group consisting of 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, medulloeptithelioma, 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 Wilms Tumor.

56. (New): The method of claim 53, further comprising detecting a tumor burden above a clinical level.

57. (New): A method for detecting a presence or absence of somatic genetic variants in cell-free deoxyribonucleic acid (cfDNA) molecules from a bodily fluid sample of a human subject, the method comprising:

(a) ligating adapters comprising molecular barcodes to a population of cfDNA molecules obtained from the bodily fluid sample with at least a 10X molar excess of adapters relative to the cfDNA molecules in the population to generate tagged parent polynucleotides,

wherein at least 20% of the cfDNA molecules are tagged with molecular barcodes at both ends of a molecule of the cfDNA molecules;

(b) amplifying a plurality of the tagged parent polynucleotides to produce progeny polynucleotides;

(c) sequencing at least a plurality of the progeny polynucleotides to produce a set of sequencing reads; and

(d) detecting, from among a plurality of sequencing reads in the set of sequencing reads that map to one or more reference sequences, the presence or absence of one or more somatic genetic variants using sequence information from the molecular barcodes in combination with mapping positions of the sequencing reads that map to the one or more reference sequences.

58. (New): The method of claim 57, further comprising selectively enriching a plurality of the progeny polynucleotides for target regions associated with cancer.
59. (New): The method of claim 57, wherein the detecting comprises grouping the plurality of sequencing reads in the set of sequencing reads that map to one or more reference sequences into families, wherein a family comprises sequencing reads of progeny polynucleotides amplified from the same tagged parent polynucleotide.
60. (New): The method of claim 59, wherein the detecting further comprises generating a set of consensus sequences from among the sequencing reads of the families and determining the presence or absence of the one or more somatic genetic variants in the set of consensus sequences as compared with the one or more reference sequences.

AMENDMENTS TO THE SPECIFICATION

Please replace the specification with the substitute specification submitted herewith.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/601,168	10/14/2019	1637	2720	42534-708.303	30	2

CONFIRMATION NO. 1052

UPDATED FILING RECEIPT

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

OC000000114039183

Date Mailed: 01/17/2020

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Inventor(s)

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

Domestic Priority data as claimed by applicant

This application 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
and 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.

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/601,168**
Projected Publication Date: 04/23/2020
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

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 16/601,168

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	30 minus 20 =	* 10			OR	x 100 =	1000
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x 460 =	0.00
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).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0.00
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2720

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	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	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
16/601,168	10/14/2019	1636	2720	42534-708.303	30	2

CONFIRMATION NO. 1052

UPDATED FILING RECEIPT

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

OC000000011403941O

Date Mailed: 01/17/2020

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)

Helmy ELTOUKHY, Atherton, CA;
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 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
and 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: 01/16/2020
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/601,168**
Projected Publication Date: 04/23/2020
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.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

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 AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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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 U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303

CONFIRMATION NO. 1052

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

37 CFR 1.48 ACKNOWLEDGEMENT LETTER

OC000000114039164

Date Mailed: 01/17/2020

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 01/14/2020.

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**.

/tle/

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

115823 7590 01/23/2020

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

EXAMINER	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
01/23/2020	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

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 16/601,168	**Applicant(s)** ELTOUKHY et al.	
	Examiner DIANE C GOODWYN	**Art Unit** OPET	**AIA (FITF) Status** Yes

1. THE REQUEST FILED 14 October 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.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

115823 7590 02/18/2020

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
02/18/2020	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

Office Action Summary	Application No. 16/601,168	Applicant(s) ELTOUKHY 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 --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ 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.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 31-60 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 31-60 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, 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.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 10/14/19 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ 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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date See Continuation Sheet.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Continuation of Attachment(s) 2) Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date: 1/14/20; 1/14/20; 1/14/20; 1/14/20; 1/14/20; 1/14/20; 1/14/20; 1/14/20.

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 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(l)(l) - 706.02(l)(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 31-60 are 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 steps of the instant claims are included within the steps of the patent claims.

4. Claims 31-60 are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 10 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 claim are related as genus-species. That is, the steps of the instant claims are included within the steps of the patented claim.

5. Claims 57-60 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claim 42 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 claim are related as genus-species. That is, the steps of the instant claims are included within the steps of the copending claim.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claim has not in fact been patented.

PRIOR ART REJECTION

6. 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.

7. 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 Sacko et al. (US 2010/0264331).

Independent claim 31 is drawn to a method comprising: ligating adapters comprising barcodes to double-stranded cell-free DNA molecules, using at least a 10X molar excess of adapters, wherein at least 20% of DNA molecules are tagged; amplifying tagged molecules; selectively enriching for tagged molecules with target regions associated with cancer; sequencing enriched tagged strands to generate sequence reads; mapping sequence reads; grouping sequence reads into families; and detecting one or

more somatic genetic variants. Independent claim 57 is drawn to a similar but broader method which lacks the above steps of enriching, mapping, and grouping.

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; grouping sequence reads into families; collapsing sequence reads into consensus sequence reads, and detecting mutations (somatic genetic variants). 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 adapters is used such that at least 20% of molecules are tagged, nor wherein the double-stranded DNA is cell-free DNA.

Sacko et al. discloses that cell-free DNA from blood of cancer patients is associated with microsatellite mutations and instabilities, which are one type of somatic genetic variant (see paragraph 0005).

One of ordinary skill in the art would have been motivated to modify the method of Schmitt et al. by using at least a 10X molar excess of adapters such that 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). The skilled artisan would have been motivated to modify the method of Schmitt et al. by using cell-free DNA because Sacko et al. disclosed that cell-free DNA may contain somatic genetic variants associated with cancers. 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

8. No claims are free of the prior art.

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.

02/12/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Notice of References Cited	Application/Control No. 16/601,168	Applicant(s)/Patent Under Reexamination ELTOUKHY et al.	
	Examiner KENNETH R HORLICK	Art Unit 1637	Page 1 of 1

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20100264331-A1	10-2010	Sacko; Mory	G01N21/6428	250/459.1
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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/601,168	ELTOUKHY et al.
	Examiner	Art Unit
	KENNETH R HORLICK	1637

CPC - Searched*		
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CPC Combination Sets - Searched*		
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Search Notes		
Search Notes	Date	Examiner
inventor name search	02/11/2020	KH
updated parent searches in USPAT and PGPUB	02/11/2020	KH
reviewed parent applications and references therein	02/11/2020	KH

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	

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	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.303

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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)	
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	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)	
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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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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

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See attached certification statement.

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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)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.303

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)	2020-01-14
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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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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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	02/11/2020

*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.

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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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 Invalidity References dated February 15, 2019 (C.A. No. 17-cv-1616-LPS-CJB)	
	10	Guardant Health vs. FMI Supplemental Invalidity Contentions dated March 29, 2019 (C.A. No. 17-cv-1616-LPS-CJB)	
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	19	Guardant Health vs. PGDx Invalidity Claim Chart H, dated May 13, 2019 (C.A. No. 17-cv-1623-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

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)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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CONFIRMATION NO. 1052

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
16/601,168	10/14/2019 RULE	435	1637	42534-708.303

APPLICANTS

Guardant Health, Inc., Redwood City, CA;

INVENTORS

Helmy ELTOUKHY, Atherton, CA;
AmirAli Talasaz, Atherton, CA;
Stefanie Ann Ward Mortimer, Morgan Hill, CA;

**** CONTINUING DATA ***************************

This application 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
and claims benefit of 61/948,509 03/05/2014
and claims benefit of 61/921,456 12/28/2013

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01/16/2020

Foreign Priority claimed	☐ Yes ☑ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☐ Yes ☑ No		CA	11	30	2
Verified and Acknowledged	/KENNETH R HORLICK/ Examiner's Signature	Initials				

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650 Page Mill Road
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UNITED STATES

TITLE

METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

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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)	2020-01-14
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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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 record s.

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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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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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	
Examiner Name	
Attorney Docket Number	42534-708.303

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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.
	2	NEWMAN, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014 May;20(5):548-54. doi: 10.1038/nm.3519. Epub 2014 Apr 6.
	3	NG, S.B., et al., "Targeted capture and massively parallel sequencing of 12 human exomes," Nature 2009, 461(7261), 272-276
	4	NIELSEN, R. et al. "Genotype and SNP calling from next-generation sequencing data" Nature Reviews Genetics (2011) 12(6):443-451
	5	NORD, et al. Accurate and exact CNV identification from targeted high-throughput sequence data. BMC Genomics. 2011 Apr 12;12:184.
	6	Notice of Allowance dated 01/31/2017 for U.S. Patent Application No. 14/712,754.
	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
	11	Notice of allowance dated 08/04/2017 for US Application No.15/467,570

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	12	Notice of allowance dated 08/22/2014 for US Application No. 12/969,581.	
	13	Notice of allowance dated 08/29/2017 for US Application No. 15/492,659	
	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.	
	21	Office action dated 05/31/2016 for US Application No. 14/712,754.	
	22	Office action dated 06/01/2017 for US Application No. 15/467,570	

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	23	Office action dated 06/03/2016 for US Application No. 14/861,989.	
	24	Office action dated 06/12/2017 for US Application No. 15/492,659.	
	25	Office action dated 07/18/2017 for US Application No. 14/861,989	
	26	Office Action dated 07/30/2019 for U.S. Application No. 16/283,635.	
	27	Office action dated 08/06/2019 for US Application No. 14/855,301.	
	28	Office action dated 10/03/2013 for US Application No. 12/969,581.	
	29	Office Action dated 10/12/2016 for U.S. Patent Application No. 14/712,754.	
	30	Office action dated 10/20/2017 for US Application No. 14/425,189.	
	31	Office Action dated 11/21/2016 for U.S. Patent Application No. 14/855,301.	
	32	Office action dated 12/04/2015 for US Application No. 14/712,754.	
	33	Office action dated 12/07/2017 for US Application No. 14/855,301	

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	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.	☐
	40	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Grunecker Patent-und Rechtsanwalte PartGmbB	☐
	41	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Personal Genome Diagnostics, Inc.	☐
	42	Opposition Form and Statement to EP2893040 filed October 2, 2019 by Strawman Limited	☐
	43	Opposition Form and Statement to EP3087204 filed November 14, 2018	☐
	44	Pacific Biosciences. Template Preparation and Sequencing Guide. Publication date: 2014-10-14. Pacific Biosciences website http://www.pacificbiosciences.com/support/pubmap/documentation.html.	☐

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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.	☐
	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.	☐
	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	☐
	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	☐
	49	PERAKIS, S. et al. "Advances in Circulating Tumor DNA Analysis" Adv Clin Chem (2017) pp 1-81	☐
	50	PIHLAK, et al. Rapid genome sequencing with short universal tiling probes. Nature Biotechnology, 26: 676-684 (2008).	☐

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EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	02/11/2020

*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.

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Examiner Name	
Attorney Docket Number	42534-708.303

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).

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Signature	/Timothy A. Hott/	Date (YYYY-MM-DD)	2020-01-14
Name/Print	Timothy A. Hott	Registration Number	67740

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Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

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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.	
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	7	5424413	A	1995-06-13	Hogan et al.	
	8	5445934		1995-08-01	Fodor et al.	

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	9	5604097	A	1997-02-18	Brenner	
	10	5635352	A	1997-06-03	Urdea et al.	
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	19	5763175	A	1998-06-09	Brenner	

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	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.	
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	29	5981179	A	1999-11-09	Lorinez et al.	
	30	6013445	A	2000-01-11	Albrecht et al.	

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	31	6020124	A	2000-02-01	Sorenson	
	32	6040138	A	2000-03-21	Lockhart et al.	
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	40	6197506	B1	2001-03-06	Fodor et al.	
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	42	6242186	B1	2001-06-05	Salonen	
	43	6268152	B1	2001-07-31	Fodor et al.	
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	53	6440667	B1	2002-08-27	Fodor et al.	
	54	6440706	B1	2002-08-27	Vogelstein et al.	
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16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303

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PUBLICATION NOTICE

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): ELTOUKHY et al. Serial Number: 16/601,168 Filing Date: October 14, 2019 Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Confirmation No.: 1052 Customer No.: 115823 Group Art Unit: 1637 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 Office Action mailed on February 18, 2020. The shortened statutory period for reply expires May 18, 2020; therefore, this response is timely filed. Applicant respectfully requests reconsideration of the above-referenced application in view of the following remarks:

Amendments to the Claims begin on page 2 of this paper.
Remarks begin on page 9 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 classifying consensus sequences generated from sequencing reads derived from ~~detecting a presence or absence of somatic genetic variants in~~ double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a sample of a human subject, the method comprising:

(a) non-uniquely tagging ~~ligating adapters comprising molecular barcodes to~~ a population of double-stranded cfDNA molecules obtained from the sample with at least a 10X molar excess, relative to the double-stranded cfDNA molecules in the population, of adapters comprising molecular barcodes ~~relative to the double-stranded cfDNA molecules in the population~~ to generate non-uniquely tagged parent polynucleotides,

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, and

wherein at least 20% of the double-stranded cfDNA molecules are ~~tagged~~ attached with the adapters comprising molecular barcodes at both ends of a molecule of the double-stranded cfDNA molecules;

(b) amplifying a plurality of the non-uniquely tagged parent polynucleotides to produce progeny polynucleotides;

(c) ~~selectively~~ enriching a plurality of the progeny polynucleotides for target regions of interest ~~associated with cancer~~, whereby enriched progeny polynucleotides are generated;

(d) sequencing at least a subset of the enriched progeny polynucleotides to produce a set of sequencing reads;

(e) mapping a plurality of the sequencing reads to the ~~one or more~~ reference sequence[[s]];

(f) grouping a plurality of the sequencing reads mapped in (e) into families of sequencing reads based at least on (i) sequence information from the molecular barcodes and (ii) the beginning base position and ending base position of the sequencing reads that map to the ~~one or more~~ reference sequence[[s]]; ~~and~~

(g) generating a consensus sequence of each family from among a plurality of families ~~detecting, from among a plurality of families of sequencing reads, the presence or absence of one or more somatic genetic variants~~; and

(h) classifying a plurality of consensus sequences from the set of consensus sequences as (1) paired consensus sequences generated from sequencing reads representing a Watson strand and a Crick strand of a non-uniquely tagged parent polynucleotide or (2) unpaired consensus sequences generated from sequencing reads representing either a Watson strand or a Crick strand of a non-uniquely tagged parent polynucleotide.

32. (Cancelled).

33. (Previously presented): The method of claim 31, wherein the population of double-stranded cfDNA molecules comprises 1 nanogram (ng) to 100 ng of double-stranded cfDNA molecules.

34. (Previously presented): The method of claim 31, wherein the sample is blood, plasma, or serum.

35. (Currently amended): The method of claim 31, wherein ~~ligating~~ the adapters comprising molecular barcodes are attached to the double-stranded cfDNA molecules by ~~comprises~~ blunt-end ligation or sticky-end ligation.

36. (Currently amended): The method of claim 31, wherein at least 40% of the double-stranded cfDNA molecules are non-uniquely tagged with adapters comprising molecular barcodes at both ends of the cfDNA molecules.
37. (Currently amended): The method of claim 31, wherein more than a 90X molar excess of adapters relative to the double-stranded cfDNA molecules in the population is used to generate the non-uniquely tagged parent polynucleotides.
38. (Currently amended): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes ~~comprising molecular barcodes~~ having 2 to 1,000 different molecular barcode sequences.
39. (Currently amended): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes ~~comprising molecular barcodes~~ having 5 to 100 different molecular barcode sequences that have a length of 5 to 20 nucleotides.
40. (Currently amended): The method of claim 31, wherein the target regions of interest ~~associated with cancer~~ comprise genetic sequences of 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.
41. (Previously presented): The method of claim 31, further comprising amplifying a plurality of the enriched progeny polynucleotides prior to sequencing.
42. (Cancelled).
43. (Cancelled).
44. (Cancelled).
45. (Cancelled).
46. (Cancelled).
47. (Currently amended): The method of claim 31 [[46]], further comprising:

(i) calculating a first quantitative measure of paired consensus sequences that map to a locus of the ~~one or more~~ reference sequence[[s]], and

(ii) calculating a second quantitative measure of unpaired consensus sequences that map to a locus of the ~~one or more~~ reference sequence[[s]].

48. (Currently amended): The method of claim 47, further comprising:

(iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to a locus of the ~~one or more~~ reference sequence[[s]] for which neither complementary strand of the double-stranded cfDNA molecules is detected in said set of consensus sequences, wherein the third quantitative measure is calculated based at least in part on the first and second quantitative measures.

49. (Previously presented): The method of claim 48, further comprising:

(iv) calculating a fourth quantitative measure of a total number of double-stranded cfDNA molecules in the population of double-stranded cfDNA molecules in the sample, wherein the total number of double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules in the sample, and wherein the total number of double-stranded cfDNA molecules is determined based at least in part on the first, second, and third quantitative measures.

50. (Cancelled).

51. (Currently amended): The method of claim 49 [[50]], comprising determining the first, second, third, and fourth[[,]] ~~and fifth~~ quantitative measures with a programmed computer processor.

52. (Cancelled).

53. (Cancelled).

54. (Cancelled).

55. (Cancelled).

56. (Cancelled).

57. (Currently amended): A method for classifying unique sequence reads generated from sequencing reads derived from double-stranded ~~detecting a presence or absence of somatic genetic variants in~~ cell-free deoxyribonucleic acid (cfDNA) molecules from a bodily fluid sample of a human subject, the method comprising:

(a) ~~ligating adapters comprising molecular barcodes to~~ tagging a population of double-stranded cfDNA molecules obtained from the bodily fluid sample with at least a 10X molar excess of adapters comprising molecular barcodes, relative to the double-stranded cfDNA molecules in the population, to generate tagged parent polynucleotides,

wherein at least 20% of the cfDNA molecules are ~~tagged~~ ligated with the adapters comprising the molecular barcodes at 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 plurality of the progeny polynucleotides to produce a set of sequencing reads; ~~and~~

(d) mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence ~~detecting, from among a plurality of sequencing reads in the set of sequencing reads that map to one or more reference sequences, the presence or absence of one or more somatic genetic variants using sequence information from the molecular barcodes in combination with mapping positions of the sequencing reads that map to the one or more reference sequences~~;

(e) determining unique sequence reads from the set of sequencing reads based at least on the molecular barcode sequences, wherein a unique sequence read is representative of a tagged parent polynucleotide from among the tagged parent polynucleotides; and

(f) classifying a plurality of the unique sequence reads as either (1) paired sequences generated from sequencing reads representing a Watson strand and a Crick strand of a tagged parent polynucleotide or (2) unpaired sequences generated from sequencing reads representing either a Watson strand or a Crick strand of a tagged parent polynucleotide.

58. (Currently amended): The method of claim 57, further comprising selectively enriching a plurality of the progeny polynucleotides for target regions of interest ~~associated with cancer~~.

59. (Currently amended): The method of claim 57, wherein determining unique sequence reads ~~the detecting~~ comprises grouping the plurality of sequencing reads in the set of sequencing

reads that map to the ~~one or more~~ reference sequence[[s]] into families, wherein a family comprises sequencing reads of progeny polynucleotides amplified from the same tagged parent polynucleotide.

60. (Cancelled).

61. (New): The method of claim 57, further comprising:

(i) calculating a first quantitative measure of paired sequences that map to a locus of the reference sequence, and

(ii) calculating a second quantitative measure of unpaired sequences that map to a locus of the reference sequence.

62. (New): The method of claim 61, further comprising:

(iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to a locus of the reference sequence for which neither complementary strand of the double-stranded cfDNA molecules is detected in said set of unique sequence reads, wherein the third quantitative measure is calculated based at least in part on the first and second quantitative measures.

63. (New): The method of claim 62, further comprising:

(iv) calculating a fourth quantitative measure of a total number of double-stranded cfDNA molecules in the population of double-stranded cfDNA molecules in the sample, wherein the total number of double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules in the sample, and wherein the total number of double-stranded cfDNA molecules is determined based at least in part on the first, second, and third quantitative measures.

64. (New): The method of claim 63, comprising determining the first, second, third, and fourth quantitative measures with a programmed computer processor.

65. (New): The method of claim 57, wherein the molecular barcodes are from a set of molecular barcodes having 2 to 10,000 different molecular barcode sequences.

66. (New): The method of claim 57, wherein the molecular barcode sequences are predetermined sequences.

67. (New): The method of claim 57, wherein at least 40% of the double-stranded cfDNA molecules are tagged with adapters comprising molecular barcodes at both ends of the cfDNA molecules.
68. (New): The method of claim 57, wherein more than a 90X molar excess of adapters relative to the double-stranded cfDNA molecules in the population is used to generate tagged parent polynucleotides.
69. (New): The method of claim 57, further comprising quantifying a number of unique sequence reads identified from the set of sequencing reads.
70. (New): The method of claim 57, wherein determining unique sequence reads is further based on (1) a start base position of a given sequencing read from among the set of sequencing reads at which the given sequencing read starts aligning to the reference sequence, and (2) a stop base position of the given sequencing read at which the given sequencing read stops aligning to the reference sequences.
71. (New): The method of claim 31, further comprising determining a quantity of consensus sequences generated from among a plurality of families.

REMARKS

Claims 31-60 were pending prior to entry of the above-referenced claim amendments. Claims 32, 42-46, 50, 52-56 and 60 are hereby cancelled without disclaimer or prejudice. Claims 31, 35-40, 47-48, 51, 57-59 are amended. Claims 61-71 are newly added. Support for these amendments may be found throughout the application as filed, for example, at least at paragraphs [0021], [00115], [00119], and [00179]-[00180] as filed. No new matter is believed to be added by these amendments. Therefore, claims 31, 33-41, 47-49, 51, 57-59 and 61-71 are pending and set for examination.

Nonstatutory Double Patenting Rejections

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 claim 10 of U.S. Patent No. 9,920,366. Claims 57-60 were provisionally rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claim 42 of copending Application No. 15/892,178.

Without conceding in the basis of the rejections, and solely to expediate prosecution of this application, Applicant submits herewith terminal disclaimers over the '366 patent and the '178 application. Accordingly, Applicant respectfully requests that the respective nonstatutory double patenting rejections be withdrawn.

Applicant respectfully requests the Examiner to reconsider the non-statutory double patenting rejection over the '992 patent in view of the aforementioned claim amendments. If only a non-statutory double patenting rejection remains following entry of this response, Applicant respectfully requests the Examiner to telephone the undersigned attorney of record such that a Terminal Disclaimer may be considered and timely filed.

Applicant submits that the nonstatutory double patenting rejections to claims 32, 42-46, 50, 52-56 and 60 are moot in view of the cancellation of these claims.

Rejection under 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 Sacko et al. (U.S. 2010/0264331).

Without conceding in the basis of the rejection, and to expediate prosecution, independent claims 31 and 57 have been amended to clarify the claimed subject matter. Applicant submits that the asserted combination of Schmitt et al. and Sacko et al. does not meet all of the elements of independent claims 31 and 57 as amended, much less the elements of dependent claims 33-41, 47-49, 51, and 58-59. Moreover, a person of ordinary skill in the art would recognize that there was no 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 1/14/20), "[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 57 and dependent claims 33-41, 47-49, 51, and 58-59 are not obvious over Schmitt et al. in view of Sacko et al. For purposes solely to expedite the prosecution of this application, claims 32, 42-46, 50, 52-56 and 60 have been 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 Sacko 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

This paper fully addresses the rejections raised in the Office Action mailed February 18, 2020. Applicant believes that the present application is now in condition for allowance 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-CON2).

Respectfully submitted,

GUARDANT HEALTH, INC.

Date: May 6, 2020 By: /Timothy A. Hott/

Timothy A. Hott
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s):	Helmy ELTOUKHY et al.	Confirmation No.:	1052
Serial No.:	16/601,168	Art Unit:	1637
Filing Date:	October 14, 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: _____

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.

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-CON2).

Respectfully submitted,

Dated: May 6, 2020

By: /Timothy A. Hott/
Timothy A. Hott, Reg. No. 67740

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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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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	1	20130288244	A1	2013-10-31	Deciu et al.	Entire Document

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

	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	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
	3	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
	4	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)

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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	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

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)	2020-05-06
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 record s.

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:	16601168
Filing Date:	14-Oct-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.303

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:	39370523
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	06-MAY-2020
Filing Date:	14-OCT-2019
Time Stamp:	16:53:55
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	E202056G54146253
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		2020-05-06_GH0004US-CON2_OA1Response.pdf	156039 0f21d66065ef8d695040cb874ffb360cd17a0104	yes	11

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Claims	2	8
Applicant Arguments/Remarks Made in an Amendment	9	11

Warnings:

Information:

2	Transmittal Letter	2020-05-06_GH0004US-CON2_IDSTrans.pdf	144727 442482308d55dcd1667de93ab881e51726f98565	no	4

Warnings:

Information:

3	Information Disclosure Statement (IDS) Form (SB08)	2020-05-06_GH0004US-CON2_SB08.pdf	1053748 a661923140368 1cb46a647730d5e011c13273fdc	no	4

Warnings:

Information:

4	Non Patent Literature	CLARK_JMolDiag_2018_686-702pdf.pdf	3158287 a0919e0ddb3ec5f7ca0f3bb3ae016a43ad3b2eae	no	17

Warnings:

Information:

5	Non Patent Literature	PAWELETZ_ClinCancRes_2017_915-922.pdf	464079 c369c3784c7b07ce8e0a55ee7d5c9373f45dbcfa	no	16

Warnings:					
Information:					
6	Non Patent Literature	Phallen_SciTranslMed_2017.pdf	2398204 aaf91fd1b3e2177ce9a1fc5427081c5a67d4cce6	no	13
Warnings:					
Information:					
7	Non Patent Literature	SHIROGUCHI_PNAS_2012_SupplInfo.pdf	667103 3563ff1f6c63079ada69be1a2ffaca1ba5cb9bef	no	8
Warnings:					
Information:					
8	Fee Worksheet (SB06)	fee-info.pdf	30486 d29d223bef7b1e88aa840e9a5a5719145fefbc78	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	8072673		

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.

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

Electronic Petition Request	**TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION AND TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT**
Application Number	16601168
Filing Date	14-Oct-2019
First Named Inventor	Helmy ELTOUKHY
Attorney Docket Number	42534-708.303
Title of Invention	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ 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)

15892178 filed on 02/08/2018

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)

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/
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:	16601168
Filing Date:	14-Oct-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.303

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.: 16601168

Filing Date: 14-Oct-2019

Applicant/Patent under Reexamination: ELTOUKHY

Electronic Terminal Disclaimer filed on May 6, 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:	39370575
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	06-MAY-2020
Filing Date:	14-OCT-2019
Time Stamp:	16:56:57
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	E202056G56546310
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	Terminal Disclaimer-Filed (Electronic)	eTerminal-Disclaimer.pdf	36007 4e5a2616accfb3a3a42c825244f75e4ca9cb40c3	no	3
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30346 0a48fac525f53be6dd7ce8a87864af145cc99975	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	66353		

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.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/601,168	Filing Date 10/14/2019	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)			
FOR	NUMBER FILED	NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ 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 =	
☐ 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))					
* 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)			
AMENDMENT	05/06/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 27	Minus	** 30	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $460 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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".	/YOLANDA CHADWICK/
*** 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.

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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

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	20190271040	A1	2019-09-05	Salk et al.	Entire Document

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

	2	20190292597	A1	2019-09-26	Salk et al.	Entire Document
	3	20190338358	A1	2019-11-07	Salk et al.	Entire Document
	4	20190352714	A1	2019-11-21	Salk et al.	Entire Document

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]
	1	KAMPS-HUGHES, N. et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data"PLOS One (2018)	
	2	LENNON, N.J. et al. "Technological considerations for genome-guided diagnosis and management of cancer" Gen Med (2016) 8:112	
	3	MISHRA, S. et al. "Different Facets of Copy Number Changes: Permanent, Transient, and Adaptive" Mol Cell Biol (2016) 36(7):1050-1063	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

	4	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)
	5	NEWMAN, A. et al. "Integrated digital error suppression for improved detection of circulating tumor DNA" Nature Biotech (2016) 34(5):547-555
	6	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
	7	OU, SHI et al. "Liquid Biopsy to Identify Actionable Genomic Alterations" Am Soc Clin Onc (2018) 978
	8	SATHIRAPONGSASUTI, J.F. et al. "Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV" BioInformatics (2011) 27(19):2648-2654
	9	TRAPNELL, C. et al. "How to map billions of short reads onto genomes" Nature Biotech (2009) 27(5):455-457
	10	VAN LOO, P. et al. "Allele-specific copy number analysis of tumors" PNAS (2010) 107(39):16910-16915
	11	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 Add

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	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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 record s.

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:	16601168
Filing Date:	14-Oct-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.303

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:	39612876
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	03-JUN-2020
Filing Date:	14-OCT-2019
Time Stamp:	13:13:33
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	E202063D14012132
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	2020-06-03_GH0004US-CON2_IDSTrans.pdf	144106 94b734c6856ab25821fd064c31062e3c2cf158c7	no	4
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	2020-06-03_GH0004US-CON2_SB08.pdf	1054692 14f753a97c891014f32b8426796b173a70526c0a	no	5
Warnings:					
Information:					
3	Non Patent Literature	KAMPS-HUGHES_PLOSOne_2018.pdf	5707894 ea4d5cf7239d69ce8d4cdfae92b25404809533e1	no	18
Warnings:					
Information:					
4	Non Patent Literature	LENNON_GenMed_2016_112.pdf	689512 67e86d14248058462070 40bcbbef63ab7e600b00	no	10
Warnings:					
Information:					
5	Non Patent Literature	MISHRA_MCB_2016_1050-1063.pdf	1216913 5f63e365b9b13c1c13c502d1372bc57258b43782	no	14
Warnings:					
Information:					
6	Non Patent Literature	MOENCH_CancTherAdv_2019.pdf	640654 cbedcb9c9350ebc56dce20b0e103f70bf26863c3	no	3
Warnings:					
Information:					

7	Non Patent Literature	NEWMAN_NatMed_2014_547-555.pdf	1377423 69ec2aa88500b798bf011f81a5bfe5b8de41be93	no	9
Warnings:					
Information:					
8	Non Patent Literature	ODEGAARD_ClinCancRes_2018_3539-3549.pdf	1062206 cb51a9f4833c005db46554e71fa08573f616a364	no	11
Warnings:					
Information:					
9	Non Patent Literature	OU_ACSO_2018_978-997.pdf	664167 dde74d5088588343da8b04ede4fcf58466ae171e	no	20
Warnings:					
Information:					
10	Non Patent Literature	Sathirapongsasuti_BioInformatics_2011.pdf	484289 78ff01d10aa50d956f5f7a931c727355df943f9e	no	7
Warnings:					
Information:					
11	Non Patent Literature	TRAPNELL_NatBiotech_2009_455-457.pdf	183342 8d9e4daf2d810f2b71651c6febd734337a82b52a	no	3
Warnings:					
Information:					
12	Non Patent Literature	VAN_LOO_PNAS_2010_16910-16915.pdf	2450725 77581bb7574de0bfab9474778d4b67be852c428a	no	6
Warnings:					
Information:					
13	Non Patent Literature	WANG_NAR_2019.pdf	735593 e5227ff4b8dcb4d907196ad89e55c94d3b43e356	no	11
Warnings:					
Information:					

14	Fee Worksheet (SB06)	fee-info.pdf	30486 74444a5fcb1718e4704abe729a2b39c269f0ac72	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	16442002

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):	Helmy ELTOUKHY et al.	Confirmation No.:	1052
Serial No.:	16/601,168	Art Unit:	1637
Filing Date:	October 14, 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: _____

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.

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-CON2).

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
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

115823 7590 07/13/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

EXAMINER
HORLICK, KENNETH R

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/13/2020

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

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	10/13/2020

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)

115823 7590 07/13/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

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.

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.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

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	10/13/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
HORLICK, KENNETH R	1637	435-006120

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "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.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(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.

1 _____
2 _____
3 _____

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. _____

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.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

115823 7590 07/13/2020

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

EXAMINER	
HORLICK, KENNETH R	
ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/13/2020

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.
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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. 16/601,168	Applicant(s) ELTOUKHY 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 05/06/20.
   ☐ 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 5/6/20; 6/3/20.
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: 31,33-41,47-49,51,57-59 and 61-71

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 05/06/20 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of **U.S. patent '366, or any patent issuing from U.S. application '178,** 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.

07/07/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Index of Claims	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/601,168	ELTOUKHY et al.
	Examiner	Art Unit
	KENNETH R HORLICK	1637

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

CLAIMS

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	02/18/2020	07/07/2020							
1	31	✓	=							
	32	✓	-							
2	33	✓	=							
3	34	✓	=							
4	35	✓	=							
5	36	✓	=							
6	37	✓	=							
7	38	✓	=							
8	39	✓	=							
9	40	✓	=							
10	41	✓	=							
	42	✓	-							
	43	✓	-							
	44	✓	-							
	45	✓	-							
	46	✓	-							
11	47	✓	=							
12	48	✓	=							
13	49	✓	=							
	50	✓	-							
14	51	✓	=							
	52	✓	-							
	53	✓	-							
	54	✓	-							
	55	✓	-							
	56	✓	-							
15	57	✓	=							
16	58	✓	=							
17	59	✓	=							
	60	✓	-							
18	61		=							
19	62		=							
20	63		=							
21	64		=							
22	65		=							
23	66		=							
24	67		=							
25	68		=							
26	69		=							
27	70		=							
28	71		=							

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/601,168	ELTOUKHY et al.
	Examiner	**Art Unit**
	KENNETH R HORLICK	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)	28	
/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	07 July 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20200707

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/601,168	ELTOUKHY et al.
	Examiner	**Art Unit**
	KENNETH R HORLICK	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 (Assistant Examiner) (Date)	**Total Claims Allowed:** 28	
/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637 (Primary Examiner) 07 July 2020 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure NONE

U.S. Patent and Trademark Office Part of Paper No.: 20200707

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☑ 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	9	40	13	49	16	58	24	67						
	32	10	41		50	17	59	25	68						
2	33		42	14	51		60	26	69						
3	34		43		52	18	61	27	70						
4	35		44		53	19	62	28	71						
5	36		45		54	20	63								
6	37		46		55	21	64								
7	38	11	47		56	22	65								
8	39	12	48	15	57	23	66								

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	28	
/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	07 July 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	NONE

U.S. Patent and Trademark Office — Part of Paper No.: 20200707

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	16/601,168	ELTOUKHY et al.
	Examiner	Art Unit
	KENNETH R HORLICK	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	02/11/2020	KH
updated parent searches in USPAT and PGPUB	02/11/2020	KH
reviewed parent applications and references therein	02/11/2020	KH
updated in USPAT and PGPUB	07/07/2020	KH

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
NONE	NONE	07/07/2020	KH

/KENNETH R HORLICK/ Primary Examiner, Art Unit 1637	

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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
/K.R.H/	1	20130288244	A1	2013-10-31	Deciu et al.	Entire Document

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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							

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]

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/K.R.H/	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
/K.R.H/	2	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
/K.R.H/	3	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
/K.R.H/	4	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)

If you wish to add additional non-patent literature document citation information please click the Add button [Add]

EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	07/06/2020

*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.

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Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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)	2020-05-06
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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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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Attorney Docket Number	42534-708.303

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
/K.R.H/	1	10287631	B2	2019-05-14	Salk et al.	Entire Document
/K.R.H/	2	10370713	B2	2019-08-06	Salk et al.	Entire Document
/K.R.H/	3	10385393	B2	2019-08-20	Salk et al.	Entire Document
/K.R.H/	4	10388403	B2	2019-08-20	Rava et al.	Entire Document
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/K.R.H/	1	20190271040	A1	2019-09-05	Salk et al.	Entire Document

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

/K.R.H/	2	20190292597	A1	2019-09-26	Salk et al.	Entire Document
/K.R.H/	3	20190338358	A1	2019-11-07	Salk et al.	Entire Document
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/K.R.H/	1	KAMPS-HUGHES, N. et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data"PLOS One (2018)	
/K.R.H/	2	LENNON, N.J. et al. "Technological considerations for genome-guided diagnosis and management of cancer" Gen Med (2016) 8:112	
/K.R.H/	3	MISHRA, S. et al. "Different Facets of Copy Number Changes: Permanent, Transient, and Adaptive" Mol Cell Biol (2016) 36(7):1050-1063	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

/K.R.H/	4	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)
/K.R.H/	5	NEWMAN, A. et al. "Integrated digital error suppression for improved detection of circulating tumor DNA" Nature Biotech (2016) 34(5):547-555
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/K.R.H/	7	OU, SHI et al. "Liquid Biopsy to Identify Actionable Genomic Alterations" Am Soc Clin Onc (2018) 978
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EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	07/06/2020

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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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CONFIRMATION NO. 1052

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
16/601,168	10/14/2019 RULE	435	1637	42534-708.303

APPLICANTS

Guardant Health, Inc., Redwood City, CA;

INVENTORS

Helmy ELTOUKHY, Atherton, CA;
AmirAli Talasaz, Atherton, CA;
Stefanie Ann Ward Mortimer, Morgan Hill, CA;

**** CONTINUING DATA ***************************

This application 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
and claims benefit of 61/948,509 03/05/2014
and claims benefit of 61/921,456 12/28/2013

**** FOREIGN APPLICATIONS ***************************

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01/16/2020

Foreign Priority claimed	☐ Yes ☑ No	☐ Met after Allowance	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
35 USC 119(a-d) conditions met	☐ Yes ☑ No		CA	11	30	2
Verified and Acknowledged	/KENNETH R HORLICK/ Examiner's Signature	Initials				

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

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Helmy ELTOUKHY et al. Serial Number: 16/601,168 Filing Date: October 14, 2019 Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Confirmation No.: 1052 Customer No.: 115823 Group Art Unit: 1637 Examiner: Kenneth R. HORLICK

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AMENDMENT UNDER 37 C.F.R. §1.312

Dear Commissioner:

This communication is submitted under 37 C.F.R. §1.312 after mailing of the Notice of Allowance on July 13, 2020.

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

Remarks and **Conclusion** begin on page 9 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 classifying consensus sequences generated from sequencing reads derived from double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a sample of a human subject, the method comprising:

(a) non-uniquely tagging a population of double-stranded cfDNA molecules ~~obtained~~ from the sample with ~~at least~~ more than a 10X molar excess of adapters comprising molecular barcodes, relative to the double-stranded cfDNA molecules in the population, ~~of adapters comprising molecular barcodes~~ to generate non-uniquely tagged parent polynucleotides,

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 double-stranded cfDNA molecules that map to the mappable base position, and

wherein at least 20% of the double-stranded cfDNA molecules are ~~attached~~ non-uniquely tagged with the adapters comprising the molecular barcodes at both ends of a molecule of the double-stranded cfDNA molecules;

(b) amplifying a plurality of the non-uniquely tagged parent polynucleotides to produce progeny polynucleotides;

(c) enriching a plurality of the progeny polynucleotides for target regions of interest[[,]] ~~whereby~~ to generate enriched progeny polynucleotides ~~are generated~~;

(d) sequencing ~~at least a subset~~ a plurality of the enriched progeny polynucleotides to produce a set of sequencing reads;

(e) mapping a plurality of sequencing reads from the set of sequencing reads to the reference sequence;

(f) grouping a plurality of the mapped sequencing reads ~~mapped in (e)~~ into families of mapped sequencing reads based at least on (i) sequence information from the molecular barcodes and (ii) [[the]] a beginning base position and an ending base position of the mapped sequencing reads ~~that map to the reference sequence~~;

(g) generating a consensus sequence [[of]] for each family from among ~~a plurality~~ one or more of the families to produce a set of consensus sequences; and

(h) classifying ~~a plurality of~~ one or more consensus sequences from among the set of consensus sequences as (1) paired consensus sequences generated from sequencing reads representing a Watson strand and a Crick strand of a non-uniquely tagged parent polynucleotide or (2) unpaired consensus sequences generated from sequencing reads representing only one of either a Watson strand or a Crick strand of a non-uniquely tagged parent polynucleotide.

32. (Cancelled).

33. (Previously presented): The method of claim 31, wherein the population of double-stranded cfDNA molecules comprises 1 nanogram (ng) to 100 ng of double-stranded cfDNA molecules.

34. (Previously presented): The method of claim 31, wherein the sample is blood, plasma, or serum.

35. (Currently amended): The method of claim 31, wherein the adapters comprising the molecular barcodes are ~~attached~~ non-uniquely tagged to the double-stranded cfDNA molecules by blunt-end ligation or sticky-end ligation.

36. (Currently amended): The method of claim 31, wherein at least 40% of the double-stranded cfDNA molecules are non-uniquely tagged with the adapters comprising the molecular barcodes at both ends of a molecule of the double-stranded cfDNA molecules.

37. (Currently amended): The method of claim 31, wherein more than a 90X molar excess of the adapters relative to the double-stranded cfDNA molecules in the population is used to generate the non-uniquely tagged parent polynucleotides.

38. (Previously presented): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes having 2 to 1,000 different molecular barcode sequences.
39. (Previously presented): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes having 5 to 100 different molecular barcode sequences that have a length of 5 to 20 nucleotides.
40. (Previously presented): The method of claim 31, wherein the target regions of interest comprise genetic sequences of 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.
41. (Currently amended): The method of claim 31, further comprising amplifying a plurality of the enriched progeny polynucleotides prior to the sequencing.
42. (Cancelled).
43. (Cancelled).
44. (Cancelled).
45. (Cancelled).
46. (Cancelled).
47. (Currently amended): The method of claim 31, further comprising:
    (i) calculating a first quantitative measure of paired consensus sequences that map to a locus of the reference sequence, and
    (ii) calculating a second quantitative measure of unpaired consensus sequences that map to [[a]] the locus of the reference sequence.
48. (Currently amended): The method of claim 47, further comprising:
    (iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to [[a]] the locus of the reference sequence for which neither complementary strand of the double-stranded cfDNA molecules is detected in [[said]] the set of

consensus sequences, wherein the third quantitative measure is calculated based at least in part on the first quantitative measure and the second quantitative measure[[s]].

49. (Currently amended): The method of claim 48, further comprising:

(iv) calculating a fourth quantitative measure of a ~~total~~ number of non-uniquely tagged double-stranded cfDNA molecules ~~in the population of double-stranded cfDNA molecules in the sample~~, wherein the ~~total~~ number of non-uniquely tagged double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules ~~in the sample~~, and wherein the ~~total~~ number of non-uniquely tagged double-stranded cfDNA molecules is ~~determined~~ calculated based at least in part on the first quantitative measure, the second quantitative measure, and the third quantitative measure[[s]].

50. (Cancelled).

51. (Currently amended): The method of claim 49, comprising ~~determining~~ calculating the first quantitative measure, the second quantitative measure, the third quantitative measure, and the fourth quantitative measure[[s]] with a programmed computer processor.

~~52.~~ (Cancelled).

53. (Cancelled).

54. (Cancelled).

55. (Cancelled).

56. (Cancelled).

57. (Currently amended): A method for classifying unique ~~sequence~~ sequencing reads generated from sequencing reads derived from double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a bodily fluid sample of a human subject, the method comprising:

(a) tagging a population of double-stranded cfDNA molecules ~~obtained~~ from the bodily fluid sample with ~~at least~~ more than a 10X molar excess of adapters comprising molecular barcodes, relative to the double-stranded cfDNA molecules in the population, to generate tagged parent polynucleotides,

wherein at least 20% of the cfDNA molecules are ligated with the adapters comprising the molecular barcodes at 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 plurality of the progeny polynucleotides to produce a set of sequencing reads;

(d) mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence;

(e) determining unique ~~sequence~~ sequencing reads from the set of mapped sequencing reads based at least on the molecular barcode sequences, wherein a unique ~~sequence~~ sequencing read from among the unique sequencing reads is representative of a tagged parent polynucleotide from among the tagged parent polynucleotides; and

(f) classifying ~~a plurality~~ one or more of the unique ~~sequence~~ sequencing reads as either (1) paired sequences generated from sequencing reads representing a Watson strand and a Crick strand of a tagged parent polynucleotide or (2) unpaired sequences generated from sequencing reads representing only one of either a Watson strand or a Crick strand of a tagged parent polynucleotide.

58. (Previously presented): The method of claim 57, further comprising selectively enriching a plurality of the progeny polynucleotides for target regions of interest.

59. (Currently amended): The method of claim 57, wherein determining the unique ~~sequence~~ sequencing reads comprises grouping [[the]] a plurality of the mapped sequencing reads ~~in the set of sequencing reads that map to the reference sequence~~ into families, wherein a family of the families comprises mapped sequencing reads of progeny polynucleotides amplified from [[the]] a same tagged parent polynucleotide from among the tagged parent polynucleotides.

60. (Cancelled).

61. (Currently amended): The method of claim 57, further comprising:

(i) calculating a first quantitative measure of paired sequences that map to a locus of the reference sequence, and

(ii) calculating a second quantitative measure of unpaired sequences that map to [[a]] the locus of the reference sequence.

62. (Currently amended): The method of claim 61, further comprising:

(iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to [[a]] the locus of the reference sequence for which neither complementary strand of the double-stranded cfDNA molecules is detected in [[said]] the set of unique sequence reads, wherein the third quantitative measure is calculated based at least in part on the first quantitative measure and the second quantitative measure[[s]].

63. (Currently amended): The method of claim 62, further comprising:

(iv) calculating a fourth quantitative measure of a ~~total~~ number of non-uniquely tagged double-stranded cfDNA molecules ~~in the population of double-stranded cfDNA molecules in the sample~~, wherein the ~~total~~ number of non-uniquely tagged double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules ~~in the sample~~, and wherein the ~~total~~ number of non-uniquely tagged double-stranded cfDNA molecules is determined based at least in part on the first quantitative measure, the second quantitative measure, and the third quantitative measure[[s]].

64. (Currently amended): The method of claim 63, comprising ~~determining~~ calculating the first quantitative measure, the second quantitative measure, the third quantitative measure, and the fourth quantitative measure[[s]] with a programmed computer processor.

65. (Previously presented): The method of claim 57, wherein the molecular barcodes are from a set of molecular barcodes having 2 to 10,000 different molecular barcode sequences.

66. (Currently amended): The method of claim 57, wherein the molecular barcodes ~~sequences~~ are pre-determined sequences.

67. (Currently amended): The method of claim 57, wherein at least 40% of the ~~double-stranded~~ cfDNA molecules are tagged with the adapters comprising the molecular barcodes at both ends of a molecule of the double-stranded cfDNA molecules.

68. (Currently amended): The method of claim 57, wherein more than a 90X molar excess of the adapters relative to the double-stranded cfDNA molecules in the population is used to generate the tagged parent polynucleotides.

69. (Currently amended): The method of claim 57, further comprising quantifying a number of the unique ~~sequence~~ sequencing reads ~~identified~~ determined from the set of mapped sequencing reads.

70. (Currently amended): The method of claim 57, wherein determining the unique ~~sequence~~ sequencing reads is further based on (1) a start base position of a given sequencing read from among the set of mapped sequencing reads at which the given sequencing read starts aligning to the reference sequence, and (2) a stop base position of the given sequencing read at which the given sequencing read stops aligning to the reference sequence[[s]].

71. (Currently amended): The method of claim 31, further comprising determining a quantity of the set of consensus sequences ~~generated from among a plurality of families~~.

REMARKS

Claims 31, 33-41, 47-49, 51, 57-59, and 61-71 were allowed prior to entry of the abovementioned amendments. Claims 31, 35-37, 41, 47-49, 51, 57, 59, 61-64, 66-71 are amended herein for clarity and/or antecedent basis purposes. Applicant submits that the amendments are fully supported by the application as filed. These amendments do not add any new matter or raise new issues. Accordingly, Applicant respectfully requests entry of these amendments under 37 C.F.R. §1.312.

CONCLUSION

Should the Examiner have any question, the Examiner is respectfully encouraged to telephone the undersigned attorney. The Commissioner is authorized to charge any underpayment or credit any overpayment to Deposit Account No. 60-2231 (Attorney Docket No. GH0004US-CON2).

Respectfully submitted,
GUARDANT HEALTH, INC.

Date: July 23, 2020

By: /Timothy A. Hott/

Timothy A. Hott
Registration No.: 67740

GUARDANT HEALTH, INC.
505 Penobscot Drive
Redwood City, CA 94063
Customer No. 115823

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EFS ID:	40090945
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	23-JUL-2020
Filing Date:	14-OCT-2019
Time Stamp:	17:36:05
Application Type:	Utility under 35 USC 111(a)

Payment information:

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1		2020-07-23_GH0004US-CON2_312Amend.pdf	199576 2f587c21d357b8d56692cc404befeb57be8cebf2	yes	9

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Document Description: Issue Fee Payment (PTO-85B)

Issue Fee Transmittal Form

Application Number	Filing Date	First Named Inventor	Atty. Docket No.	Confirmation No.
16601168	14-Oct-2019	Helmy ELTOUKHY	42534-708.303	1052

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	13-Oct-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 Wilson Sonsini Goodrich & Rosati / Guardant Health 650 Page Mill Road Palo Alto CA 94304 UNITED STATES 650-493-9300 Patents@guardanthealth.com	
☐ Change of correspondence address requested, system generated AIA/122-EFS form attached	☐ 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.

Doc Code: IFEE PTOL/85B-EFS

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 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**	07-24-2020
Name	Timothy A Hott	**Registration Number**	67740

Electronic Patent Application Fee Transmittal

Application Number:	16601168
Filing Date:	14-Oct-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.303

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:	40102079
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	24-JUL-2020
Filing Date:	14-OCT-2019
Time Stamp:	17:10:27
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	E20207NH10253205
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	Issue Fee Payment (PTO-85B)	Web85b.pdf	46238 a9eac2adeb4d9c02cd0decb79e92264737f8d2bd	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	31944 c4149ef16954605284aaf5d36ba1ac48cc46aec0	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	78182		

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.

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.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

115823 7590 07/31/2020

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
07/31/2020	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

Response to Rule 312 Communication	Application No.	Applicant(s)	
	16/601,168	ELTOUKHY et al.	
	Examiner	Art Unit	AIA (FITF) Status
	KENNETH R HORLICK	1637	Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

1. ☑ The amendment filed on 23 July 2020 under 37 CFR 1.312 has been considered, and has been:

a) ☐ entered.

b) ☑ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.

Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Helmy ELTOUKHY et al. Serial Number: 16/601,168 Filing Date: October 14, 2019 Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Confirmation No.: 1052 Customer No.: 115823 Group Art Unit: 1637 Examiner: Kenneth R. HORLICK

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

OK TO ENTER: /K.R.H/

/K.R.H/ (07/27/2020)

AMENDMENT UNDER 37 C.F.R. §1.312

Dear Commissioner:

This communication is submitted under 37 C.F.R. §1.312 after mailing of the Notice of Allowance on July 13, 2020.

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

Remarks and **Conclusion** begin on page 9 of this paper.

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.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

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

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR/ PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
16/601,168	10/14/2019	ELTOUKHY et al.	42534-708.303

	EXAMINER	
Wilson Sonsini Goodrich & Rosati / Guardant Health 650 Page Mill Road Palo Alto, CA 94304	KENNETH R HORLICK	
	ART UNIT	PAPER
	1637	20200805

DATE MAILED: _____

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

See attached IDS filed 06/03/20 with corrected item 11.

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

PTO-90C (Rev.04-03)

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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
/K.R.H/	1	10287631	B2	2019-05-14	Salk et al.	Entire Document
/K.R.H/	2	10370713	B2	2019-08-06	Salk et al.	Entire Document
/K.R.H/	3	10385393	B2	2019-08-20	Salk et al.	Entire Document
/K.R.H/	4	10388403	B2	2019-08-20	Rava et al.	Entire Document
/K.R.H/	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

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	20190271040	A1	2019-09-05	Salk et al.	Entire Document

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

/K.R.H/	2	20190292597	A1	2019-09-26	Salk et al.	Entire Document
/K.R.H/	3	20190338358	A1	2019-11-07	Salk et al.	Entire Document
/K.R.H/	4	20190352714	A1	2019-11-21	Salk et al.	Entire Document

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]
/K.R.H/	1	KAMPS-HUGHES, N. et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data"PLOS One (2018)	
/K.R.H/	2	LENNON, N.J. et al. "Technological considerations for genome-guided diagnosis and management of cancer" Gen Med (2016) 8:112	
/K.R.H/	3	MISHRA, S. et al. "Different Facets of Copy Number Changes: Permanent, Transient, and Adaptive" Mol Cell Biol (2016) 36(7):1050-1063	

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

/K.R.H/	4	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)
/K.R.H/	5	NEWMAN, A. et al. "Integrated digital error suppression for improved detection of circulating tumor DNA" Nature Biotech (2016) 34(5):547-555
/K.R.H/	6	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
/K.R.H/	7	OU, SHI et al. "Liquid Biopsy to Identify Actionable Genomic Alterations" Am Soc Clin Onc (2018) 978
/K.R.H/	8	SATHIRAPONGSASUTI, J.F. et al. "Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV" BioInformatics (2011) 27(19):2648-2654
/K.R.H/	9	TRAPNELL, C. et al. "How to map billions of short reads onto genomes" Nature Biotech (2009) 27(5):455-457
/K.R.H/	10	VAN LOO, P. et al. "Allele-specific copy number analysis of tumors" PNAS (2010) 107(39):16910-16915
/K.R.H/	11	WANG, T.T. et al. "High efficiency error suppression for accurate detection of low-frequency variants" NAR (2019) 47 (15):e87

If you wish to add additional non-patent literature document citation information please click the Add button Add

EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	08/05/2020

*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	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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 record s.

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.

Doc Code: QPIDS.REQ
Document Description: QuickPath Information Disclosure Statement

PTO/SB/09 (09-18)

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: 16/601,168	Filing Date: 2019-10-14
First Named Inventor: Helmy ELTOUKHY	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 2020-08-28
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 _____ forms are submitted.

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.

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

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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
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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
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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							

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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	16601168
	Filing Date	2019-10-14
	First Named Inventor	Helmy ELTOUKHY
	Art Unit	1637
	Examiner Name	Kenneth R. HORLICK
	Attorney Docket Number	42534-708.303

	1	IPR2019-00634, Final Written Decision of U.S. Patent 9,840,743, dated August 18, 2020
	2	IPR2019-00652, 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 Add

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	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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)	2020-08-28
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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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).

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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:	16601168
Filing Date:	14-Oct-2019
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Filer:	Timothy A Hott/Michelle Chan
Attorney Docket Number:	42534-708.303

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	1300	1300
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1440**

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 : August 28, 2020

In re Application of :

Helmy ELTOUKHY

DECISION ON PETITION

UNDER CFR 1.313(c)(2)

Application No : 16601168

Filed : 14-Oct-2019

Attorney Docket No : 42534-708.303

This is an electronic decision on the petition under 37 CFR 1.313(c)(2), filed August 28, 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:	40417702
Application Number:	16601168
International Application Number:	
Confirmation Number:	1052
Title of Invention:	METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS
First Named Inventor/Applicant Name:	Helmy ELTOUKHY
Customer Number:	115823
Filer:	Timothy A Hott/Michelle Chan
Filer Authorized By:	Timothy A Hott
Attorney Docket Number:	42534-708.303
Receipt Date:	28-AUG-2020
Filing Date:	14-OCT-2019
Time Stamp:	17:28:33
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1440
RAM confirmation Number	E20208RH28299291
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	Petition automatically granted by EFS	petition-request.pdf	31578 4f65dc47f6d39b4aeed857935dfd6ad1922ddc43	no	2
Warnings:					
Information:					
2	Request for Continued Examination (RCE)	2020-08-28_GH0004US-CON2_RCE_Conditional.pdf	1364247 4b15ae70f6866b1ccd5d0c93b4f3e634edd7f1e8	no	3
Warnings:					
Information:					
3	Quick Path Information Disclosure Statement	2020-08-28_GH0004US-CON2_QPIDS.pdf	195722 2579695e635e2857198dcb0f7ba33b13cdf99f2d	no	2
Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	2020-08-28_GH0004US-CON2_SB08.pdf	1053191 cb4628fcb66da7eb8286337c06c96d8c9a630c3f	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.					
5	Other Reference-Patent/App/Search documents	IPR2019-00634-FinalWrittenDecision_2020-08-18.pdf	731090 1bc4df5152cada6534ee7ef7772b4fd478febea8	no	96
Warnings:					
Information:					

6	Other Reference-Patent/App/Search documents	IPR2019-00652-FinalWrittenDecision_2020-08-18.pdf	527384 aea9a8cbac118da8e8684665de91a125db412ffa	no	68
Warnings:					
Information:					
7	Fee Worksheet (SB06)	fee-info.pdf	32051 e19d57cd50097bde0d0ee2015123c08900da457b	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	3935263	

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.

Doc Code: PET.AUTO Document Description: Petition automatically granted by EFS-Web	PTO/SB/140 U.S. Patent and Trademark Office Department of Commerce

Electronic Petition Request	**PETITION TO WITHDRAW AN APPLICATION FROM ISSUE AFTER PAYMENT OF THE ISSUE FEE UNDER 37 CFR 1.313(c)**
Application Number	16601168
Filing Date	14-Oct-2019
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	KENNETH HORLICK
Attorney Docket Number	42534-708.303
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

○	Small Entity
○	Micro Entity
◉	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.
16/601,168	10/14/2019	Helmy ELTOUKHY	42534-708.303	1052

115823 7590 09/09/2020

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
09/09/2020	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.** 16/601,168	**Applicant(s)** ELTOUKHY 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 08/28/20.
   ☐ 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 08/28/20.
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: 31,33-41,47-49,51,57-59 and 61-71

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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Application Number	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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
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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							

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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]

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	Attorney Docket Number	42534-708.303

/K.R.H/	1	IPR2019-00634, Final Written Decision of U.S. Patent 9,840,743, dated August 18, 2020
/K.R.H/	2	IPR2019-00652, 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 Add

EXAMINER SIGNATURE

Examiner Signature	/KENNETH R HORLICK/	Date Considered	09/03/2020

*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	16601168
Filing Date	2019-10-14
First Named Inventor	Helmy ELTOUKHY
Art Unit	1637
Examiner Name	Kenneth R. HORLICK
Attorney Docket Number	42534-708.303

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)	2020-08-28
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:

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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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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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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/601,168	10/13/2020	10801063	42534-708.303	1052

115823 7590 09/23/2020

Wilson Sonsini Goodrich & Rosati / Guardant Health
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):

Helmy ELTOUKHY, Atherton, CA;
Guardant Health, Inc., Redwood City, CA;
AmirAli Talasaz, Atherton, CA;
Stefanie Ann Ward Mortimer, Morgan Hill, CA;

AO 120 (Rev. 08/10)

TO:	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	

In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court District of Delaware on the following

☐ Trademarks or ☑ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
	11/23/2020	District of Delaware

PLAINTIFF	DEFENDANT
Guardant Health, Inc.	Foundation Medicine, Inc.

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	See Attached		
2			
3			
4			
5			

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1			
2			
3			
4			
5			

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE

Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director
Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	US 10,501,810 B2	12/10/2019	Guardant Health, Inc.
2	US 10,704,085 B2	7/7/2020	Guardant Health, Inc.
3	US 10,704,086 B2	7/7/2020	Guardant Health, Inc.
4	US 10,793,916 B2	10/6/2020	Guardant Health, Inc.
5	US 10,801,063 B2	10/13/2020	Guardant Health, Inc.
6	US 9,840,743 B2	12/12/2017	Guardant Health, Inc.
7	US 9,834,822 B2	12/5/2017	Guardant Health, Inc.