



US010407459B2

(12) **United States Patent**
Ju et al.

(10) **Patent No.:** **US 10,407,459 B2**
(45) **Date of Patent:** ***Sep. 10, 2019**

(54) **MASSIVE PARALLEL METHOD FOR
DECODING DNA AND RNA**

C12Q 1/6876 (2018.01)
C40B 40/00 (2006.01)

(71) Applicant: **The Trustees of Columbia University
in the City of New York, New York,
NY (US)**

(52) **U.S. Cl.**
CPC *C07H 19/14* (2013.01); *C07H 19/10*
(2013.01); *C07H 21/00* (2013.01); *C12Q 1/68*
(2013.01); *C12Q 1/686* (2013.01); *C12Q*
1/6869 (2013.01); *C12Q 1/6872* (2013.01);
C12Q 1/6874 (2013.01); *C12Q 1/6876*
(2013.01); *C07B 2200/11* (2013.01); *C12Q*
2525/117 (2013.01); *C12Q 2525/186*
(2013.01); *C12Q 2535/101* (2013.01); *C12Q*
2535/122 (2013.01); *C12Q 2563/107*
(2013.01); *C12Q 2565/501* (2013.01); *C40B*
40/00 (2013.01)

(72) Inventors: **Jingyue Ju, Englewood Cliffs, NJ (US);
Zengmin Li, Flushing, NY (US); John
Robert Edwards, St. Louis, MO (US);
Yasuhiro Itagaki, New York, NY (US)**

(73) Assignee: **THE TRUSTEES OF COLUMBIA
UNIVERSITY IN THE CITY OF
NEW YORK, New York, NY (US)**

(58) **Field of Classification Search**
CPC C07H 19/04; C12Q 1/6869
USPC 536/4.1; 435/6.1
See application file for complete search history.

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-
claimer.

(56) **References Cited**

U.S. PATENT DOCUMENTS

(21) Appl. No.: **16/149,114**

4,711,955 A 12/1987 Ward et al.
4,772,691 A 9/1988 Herman
4,804,748 A 2/1989 Seela
4,824,775 A 4/1989 Dattagupta et al.
4,863,849 A 9/1989 Melamede
4,888,274 A 12/1989 Radding et al.
5,043,272 A 8/1991 Hartley
5,047,519 A 9/1991 Hobbs, Jr. et al.
5,118,605 A 6/1992 Urdea
5,151,507 A 9/1992 Hobbs, Jr. et al.
5,174,962 A 12/1992 Brennan

(22) Filed: **Oct. 1, 2018**

(65) **Prior Publication Data**

US 2019/0085014 A1 Mar. 21, 2019

Related U.S. Application Data

(60) Continuation of application No. 15/915,983, filed on
Mar. 8, 2018, which is a continuation of application
No. 14/670,748, filed on Mar. 27, 2015, which is a
continuation of application No. 13/959,660, filed on
Aug. 5, 2013, now Pat. No. 9,133,511, which is a
continuation of application No. 13/672,437, filed on
Nov. 8, 2012, now abandoned, which is a
continuation of application No. 13/339,089, filed on
Dec. 28, 2011, now abandoned, which is a
continuation of application No. 12/804,284, filed on
Jul. 19, 2010, now Pat. No. 8,088,575, which is a
continuation of application No. 11/810,509, filed on
Jun. 5, 2007, now Pat. No. 7,790,869, which is a
continuation of application No. 10/702,203, filed on
Nov. 4, 2003, now Pat. No. 7,345,159, which is a
division of application No. 09/972,364, filed on Oct.
5, 2001, now Pat. No. 6,664,079, which is a
continuation-in-part of application No. 09/684,670,
filed on Oct. 6, 2000, now abandoned.

(Continued)

FOREIGN PATENT DOCUMENTS

CA 2425112 4/2002
CA 2408143 11/2002

(Continued)

OTHER PUBLICATIONS

Krečmerová (1990) "Synthesis of 5'-O-Phosphonomethyl Deriva-
tives of Pyrimidine 2'-Deoxynucleosides." Coll. Czech. Chem.
Commun., 55:2521-2536.

(Continued)

Primary Examiner — Jezia Riley

(74) *Attorney, Agent, or Firm* — John P. White; Cooper &
Dunham LLP

(60) Provisional application No. 60/300,894, filed on Jun.
26, 2001.

(57) **ABSTRACT**

This invention provides methods for attaching a nucleic acid
to a solid surface and for sequencing nucleic acid by
detecting the identity of each nucleotide analogue after the
nucleotide analogue is incorporated into a growing strand of
DNA in a polymerase reaction. The invention also provides
nucleotide analogues which comprise unique labels attached
to the nucleotide analogue through a cleavable linker, and a
cleavable chemical group to cap the —OH group at the
3'-position of the deoxyribose.

2 Claims, 28 Drawing Sheets

(56)

References Cited

U.S. PATENT DOCUMENTS

5,175,269	A	12/1992	Stavrianopoulos	6,255,475	B1	7/2001	Kwiatkowski
5,242,796	A	9/1993	Prober et al.	6,274,320	B1	8/2001	Rothberg et al.
5,302,509	A	4/1994	Cheeseman	6,277,607	B1	8/2001	Tyagi et al.
5,308,990	A	5/1994	Takahashi et al.	6,287,821	B1	9/2001	Shi et al.
5,328,824	A	7/1994	Ward et al.	6,294,324	B1	9/2001	Bensimon et al.
5,332,666	A	7/1994	Prober et al.	6,309,829	B1	10/2001	Livak et al.
5,383,858	A	1/1995	Reilly et al.	6,309,836	B1	10/2001	Kwiatkowski
5,424,186	A	6/1995	Fodor et al.	6,312,893	B1	11/2001	Van Ness et al.
5,436,143	A	7/1995	Hyman	6,316,230	B1	11/2001	Egholm et al.
5,437,975	A	8/1995	McClelland et al.	6,335,155	B1	1/2002	Wells et al.
5,449,767	A	9/1995	Ward et al.	6,361,940	B1	3/2002	Van Ness et al.
5,476,928	A	12/1995	Ward et al.	6,380,378	B1	4/2002	Kitamura et al.
5,516,664	A	5/1996	Hyman	6,432,360	B1	8/2002	Church
5,534,424	A	7/1996	Uhlen et al.	6,495,680	B1	12/2002	Gong
5,547,839	A	8/1996	Dower et al.	6,524,829	B1	2/2003	Seeger
5,547,859	A	8/1996	Goodman et al.	6,555,349	B1	4/2003	O'Donnell
5,556,748	A	9/1996	Douglas	6,613,508	B1	9/2003	Ness et al.
5,599,675	A	2/1997	Brenner	6,613,513	B1	9/2003	Parce et al.
5,602,000	A	2/1997	Hyman	6,627,436	B2	9/2003	Sorge et al.
5,614,365	A	3/1997	Tabor et al.	6,627,748	B1	9/2003	Ju et al.
5,637,469	A	6/1997	Wilding et al.	6,632,655	B1	10/2003	Mehta et al.
5,654,419	A	8/1997	Mathies et al.	6,639,088	B2	10/2003	Kwiatkowski
5,658,736	A	8/1997	Wong	6,664,079	B2	12/2003	Ju et al.
5,709,999	A	1/1998	Shattuck-Eidens et al.	6,664,399	B1	12/2003	Sabesan
5,714,330	A	2/1998	Brenner et al.	6,713,255	B1	3/2004	Makino et al.
5,728,528	A	3/1998	Mathies et al.	6,780,591	B2	8/2004	Williams et al.
5,763,594	A	6/1998	Hiatt et al.	6,787,308	B2	9/2004	Balasubramanian et al.
5,770,365	A	6/1998	Lane et al.	6,818,395	B1	11/2004	Quake et al.
5,770,367	A	6/1998	Southern et al.	6,833,246	B2	12/2004	Balasubramanian
5,789,167	A	8/1998	Konrad	6,858,393	B1	2/2005	Anderson et al.
5,798,210	A	8/1998	Canard et al.	6,864,052	B1	3/2005	Drmanac et al.
5,804,386	A	9/1998	Ju	6,911,345	B2	6/2005	Quake et al.
5,808,045	A	9/1998	Hiatt et al.	6,934,636	B1	8/2005	Skierczynski et al.
5,814,454	A	9/1998	Ju	6,982,146	B1	1/2006	Schneider et al.
5,821,356	A	10/1998	Khan et al.	7,037,687	B2	5/2006	Williams et al.
5,834,203	A	11/1998	Katzir et al.	7,056,661	B2	6/2006	Korlach et al.
5,844,106	A	12/1998	Seela et al.	7,056,666	B2	6/2006	Dower et al.
5,849,542	A	12/1998	Reeve et al.	7,057,026	B2	6/2006	Barnes et al.
5,853,992	A	12/1998	Glazer et al.	7,057,031	B2	6/2006	Olejniak et al.
5,856,104	A	1/1999	Chee et al.	7,074,597	B2	7/2006	Ju
5,858,671	A	1/1999	Jones	7,078,499	B2	7/2006	Odedra et al.
5,869,255	A	2/1999	Mathies et al.	7,105,300	B2	9/2006	Parce et al.
5,872,244	A	2/1999	Hiatt et al.	7,270,951	B1	9/2007	Stemple et al.
5,876,936	A	3/1999	Ju	7,279,563	B2	10/2007	Kwiatkowski
5,885,775	A	3/1999	Haff et al.	7,329,496	B2	2/2008	Dower et al.
5,885,813	A	3/1999	Davis et al.	7,345,159	B2	3/2008	Ju et al.
5,908,755	A	6/1999	Kumar et al.	7,393,533	B1	7/2008	Crotty et al.
5,945,283	A	8/1999	Kwok et al.	7,414,116	B2	8/2008	Milton et al.
5,948,648	A	9/1999	Khan et al.	7,427,673	B2	9/2008	Balasubramanian et al.
5,952,180	A	9/1999	Ju	7,459,275	B2	12/2008	Dower et al.
5,959,089	A	9/1999	Hannessian	7,541,444	B2	6/2009	Milton et al.
5,962,228	A	10/1999	Brenner	7,566,537	B2	7/2009	Balasubramanian et al.
6,001,566	A	12/1999	Canard et al.	7,622,279	B2	11/2009	Ju
6,001,611	A	12/1999	Will	7,635,578	B2	12/2009	Ju et al.
6,008,379	A	12/1999	Benson et al.	7,713,698	B2	5/2010	Ju et al.
6,013,445	A	1/2000	Albrecht et al.	7,771,973	B2	8/2010	Milton et al.
6,028,190	A	2/2000	Mathies et al.	7,785,790	B1	8/2010	Church et al.
6,046,005	A	4/2000	Ju et al.	7,790,869	B2	9/2010	Ju et al.
6,074,823	A	6/2000	Koster	7,883,869	B2	2/2011	Ju et al.
6,087,095	A	7/2000	Rosenthal et al.	7,982,029	B2	7/2011	Ju et al.
6,111,116	A	8/2000	Benson et al.	8,088,575	B2	1/2012	Ju et al.
6,136,543	A	10/2000	Anazawa et al.	8,158,346	B2	4/2012	Balasubramanian et al.
6,175,107	B1	1/2001	Juvinall	8,298,792	B2	10/2012	Ju et al.
6,197,557	B1	3/2001	Makarov et al.	8,399,188	B2	3/2013	Zhao et al.
6,207,831	B1	3/2001	Auer et al.	8,796,432	B2	8/2014	Ju et al.
6,210,891	B1	4/2001	Nyren et al.	8,889,348	B2	11/2014	Ju
6,214,987	B1	4/2001	Hiatt et al.	9,115,163	B2	8/2015	Ju et al.
6,218,118	B1	4/2001	Sampson et al.	9,133,511	B2	9/2015	Ju et al.
6,218,530	B1	4/2001	Rothschild et al.	9,159,610	B2	10/2015	Zhang et al.
6,221,592	B1	4/2001	Schwartz et al.	9,175,342	B2	11/2015	Ju et al.
6,232,465	B1	5/2001	Hiatt et al.	9,255,292	B2	2/2016	Ju et al.
6,242,193	B1	6/2001	Anazawa et al.	9,297,042	B2	3/2016	Ju et al.
6,245,507	B1	6/2001	Bogdanov	9,708,358	B2	7/2017	Ju et al.
				9,718,852	B2	8/2017	Ju et al.
				9,719,139	B2	8/2017	Ju et al.
				9,725,480	B2	8/2017	Ju et al.
				9,868,985	B2	1/2018	Ju et al.

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO WO 2015/148402 10/2015
 WO WO 2015/179284 11/2015

OTHER PUBLICATIONS

- Kurata et al. (2001) "Fluorescent quenching-based quantitative detection of specific DNA/RNA using BODIPY® FL-labeled probe of primer," *Nucleic Acids Research*, vol. 29, No. 6, p. e34.
- Kvam et al., (1994) "Characterization of singlet oxygen-induced guanine residue damage after photochemical treatment of free nucleosides and DNA," *Biochimica et Biophysica Acta.*, 1217:9-15.
- Lee, L.G., et al. (1992) "DNA sequencing with dye labeled terminators and T7 DNA polymerase effect of dyes and dNTPs on incorporation of dye terminators and probability analysis of termination fragments," *Nucleic Acids Res.* 20:2471-2483.
- Lee L.G. et al. (1997) "New energy transfer dyes for DNA sequencing," *Nucleic Acids Res.* 25:2816-2822.
- Leroy, E.M. et al. (2000) "Diagnosis of Ebola Haemorrhagic Fever by RT-PCR in an Epidemic Setting," *Journal of Medical Virology* 60:463-467.
- Lewis et al. (2002) "Click Chemistry in Situ: Acetylcholinesterase as a Reaction Vessel for the Selective Assembly of a Femtomolar Inhibitor from an Array of Building Blocks," *Angew. Chem. Int. Ed.* 41(6):1053-1057.
- Li, J. (1999) "Single Oligonucleotide Polymorphism Determination Using Primer Extension and Time-of-Flight Mass Spectrometry," *Electrophoresis* 20:1258-1265.
- Li et al. (2003) "A photocleavable Fluorescent Nucleotide for DNA Sequencing and Analysis," *PNAS* 100(2):414-419.
- Liu, H. et al. (2000) "Development of Multichannel Devices with an Array of Electrospray Tips for High-Throughput Mass Spectrometry," *Anal. Chem.* 72:3303-3310.
- Loubinoux, B. et al. "Protection Des Phenols Par Le Groupement Azidomethylene Application A La Synthèse De Phenols Instables," *Tetrahedron*, 1998, 44(19): 6055 (English Abstract Only).
- Lu, G. and Burgess, K. (2006) "A Diversity Oriented Synthesis of 3'-O-Modified Nucleoside Triphosphates for DNA Sequencing by Synthesis" *Bioorg. Med. Chem. Lett.*, 16:3902-3905.
- Lyamichev, V. et al. (1999) "Polymorphism Identification and Quantitative Detection of Genomic DNA by Invasive Cleavage of Oligonucleotide Probes," *Nat. Biotech* 17:292-296.
- Maier et al. (1995) "Synthesis and Properties of New Fluorescein-Labeled Oligonucleotides," *Nucleosides and Nucleotides*, 14:961-965.
- Margulies, M.; Egholm, M.; Altman, W. E. (2005) "Genome Sequencing in Microfabricated High-Density Picolitre Reactors." *Nature*, 437:376-380.
- Markiewicz et al. (1997) "A new method of synthesis of fluorescently labeled oligonucleotides and their application in DNA sequencing," *Nucleic Acids Research*, 25:3672-3690.
- Marquez et al. (2003) "Selective Fluorescence Quenching of 2,3-Diazabicyclo[2.2.2]oct-2-ene by Nucleotides," *Organic Letters*, 5:3911-3914.
- Mathews C.K. et al. (1985) "Chemical Synthesis of Oligonucleotides," *Biochemistry*, 2nd Edition, pp. 127-128.
- Meng et al. (2006) "Design and Synthesis of a Photocleavable Fluorescent Nucleotide 3'-O-Allyl-dGTP-PC-Biodipy-FL-510 as a Reversible Terminator for DNA Sequencing by Synthesis," *J. Org. Chem* 71:3248-3252.
- Metzker, M.L. et al. (1994) "Termination of DNA synthesis by novel 3' modified deoxyribonucleoside 5' triphosphates," *Nucleic Acids Res.* 22: 4259-4267.
- Metzker M.L. (2005) "Emerging Technologies in DNA Sequencing," *Genome Res.*, 15:1767-1776.
- Mitra, R. D.; Shendure J.; Olejnik, J.; et al. (2003) "Fluorescent in Monforte, J.A. and Becker, C.H. (1997) "High-throughput DNA analysis by time-of-flight mass spectrometry," *Nat. Med.* 3(3):360-362.
- Nazarenko et al. (2002) "Effect of primary and secondary structure of oligodeoxyribonucleotides on the fluorescent properties of conjugated dyes," *Nucleic Acids Research*, 30:2089-2095.
- Nickel et al. (1992) "Interactions of Azidothymidine triphosphate with the Cellular DNA polymerases alpha, delta, and epsilon and with DNA Primase," *J. Biol. Chem.* 267 (2): 848-854.
- Nielsen et al. (2004) "Multiplexed Sandwich Assays in Microarray Format," *Journal of Immunological Methods*, vol. 290, pp. 107-120.
- Nishino et al. (1991) "Efficient Deamidation of Phosphoranilidates by the Use of Nitrites and Acetic Anhydride." *Heteroatom Chemistry*, vol. 2, pp. 187-196.
- Olejnik, J. et al. (1995) "Photocleavable biotin derivatives: a versatile approach for the isolation of biomolecules," *Proc. Natl. Acad. Sci. USA.* 92:7590-7594.
- Olejnik, J. et al. (1999) "Photocleavable peptide DNA conjugates: synthesis and applications to DNA analysis using MALDI MS," *Nucleic Acids Res.* 27:4626-4631.
- Pastinen et al. (1997) "Minisequencing: A Specific Tool for DNA Analysis and Diagnostics on Oligonucleotide Arrays," *Genomic Res.*, 7:606-614.
- Pelletier, H. et al. (1994) "Structures of ternary complexes of rat DNA polymerase β , a DNA template-primer and ddCTP," *Science* 264:1891-1903.
- Prober, J.M. et al. (1987) "A system for rapid DNA sequencing with fluorescent chain-terminating dideoxynucleotides," *Science* 238:336-341.
- Quaedflieg et al. (1992) "An Alternative Approach Toward the Synthesis of (3'→5') Methylene Acetal Linked Dinucleosides." *Tetrahedron Letters*, vol. 33, pp. 3081-3084.
- Rao et al. (2001) "Four Color FRET Dye Nucleotide Terminators for DNA Sequencing," *Nucleosides, Nucleotides and Nucleic Acids*, 20:673-676.
- Rasolonjatovo et al. (1998) "g-N-(N-Methanthranlylamido)-4-Oxo-Hexanoic Acid: A New Fluorescent Protecting Group Applicable to a New DNA Sequencing Method," *Nucleosides and Nucleotides*, 17:2021-2025.
- Ronaghi, (1998) "PCR-Introduced Loop Structure as Primer in DNA Sequencing," *BioTechniques*, 25:876.
- Ronaghi, M., Uhlen, M., and Nyren, P. (1998) "A Sequencing Method Based on Real-time Pyrophosphate," *Science* 281:364-365.
- Rosenblum, B.B. et al. (1997) "New dye-labeled terminators for improved DNA sequencing patterns," *Nucleic Acids Res.* 25:4500-4504.
- Roskey, M.T., Juhasz, P., Smirnov, I.P., Takach, E. J., Martin, S.A., and Haff, L.A. (1996) "DNA sequencing by delayed extraction-matrix-assisted laser desorption/ionization time of flight mass spectrometry," *Proc. Natl. Acad. Sci. USA.* 93:4724-4729.
- Ross, P.L. et al. (1997) "Discrimination of Single-Nucleotide Polymorphisms in Human DNA Using Peptide Nucleic Acid Probes Detected by MALDI-TOF Mass Spectrometry," *Anal. Chem.* 69:4197-4202.
- Ross, P. et al. (1998) High Level Multiplex Genotyping by MALDI-TOF Mass Spectrometry. *Nat. Biotech* 16:1347-1351.
- Ruparel et al. (2005) "Design and Synthesis of a 3'-O-Allyl Photocleavable Fluorescent Nucleotide as a Reversible Terminator for DNA Sequencing by Synthesis," *PNAS* 102(17):5932-5937.
- Sarfati et al., (1995) "Synthesis of fluorescent derivatives of 3'-O-(6-aminohexanoyl)-pyrimidine nucleosides 5'-triphosphates that act as DNA polymerase substrates reversibly tagged at C-3'," *JCS Perkin Trans.* 1163-1171.
- Saxon, E. and Bertozzi, C.R. (2000) "Cell surface engineering by a modified Staudinger reaction," *Science* 287:2007-2010.
- Schena, M., Shalon, D. and Davis, R. Brown P.O. (1995) "Quantitative monitoring of gene expression patterns with a cDNA microarray," *Science* 270: 467-470.
- Seeger (1998) "Single Molecule Fluorescence: High-Performance Molecular Diagnosis and Screening," *Bioforum*, Git Verlag, Darmstadt, DE vol. 21, (German text).

(56)

References Cited

OTHER PUBLICATIONS

- Seo et al. (2004) "Photocleavable Fluorescent Nucleotides for DNA Sequencing on a Chip Constructed by Site-Specific Coupling Chemistry," PNAS 101 (15):5488-5493.
- Seo et al. (2005) "Four-Color DNA Sequencing by Synthesis on a Chip Using Photocleavable Fluorescent Nucleotides," PNAS 102 (17): 5926-593.
- Shendure, J.; Porreca, G. J.; Reppas, N.B.; et al. (2005) "Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome." Science 309:1728-1732.
- Smith, L.M., Sanders, J.Z., Kaiser, R.J., et al. (1986) "Fluorescence Detection in Automated DNA Sequence Analysis," Nature 321:674-679.
- Speicher, M.R., Ballard, S.G., and Ward, D.C. (1996) "Karyotyping human chromosomes by combinatorial multi-fluor FISH," Nature Genetics 12: 368-375.
- Stoerker, J. et al. (2000) "Rapid Genotyping by MALDI-monitored nuclease selection from probe Libraries," Nat. Biotech 18:1213-1216.
- Tang, K., Fu, D.J., Julien, D., Braun, A., Cantor, C.R., and Koster, H. (1999) "Chip-based genotyping by mass spectrometry," Proc. Natl. Acad. Sci. USA. 96:10016-10020.
- Tong, X. and Smith, L.M. (1992) "Solid-Phase Method for the Purification of DNA Sequencing Reactions," Anal. Chem. 64:2672-2677.
- Torimura et al. (2001) "Fluorescence-Quenching Phenomenon by Photoinduced Electron Transfer between a Fluorescent Dye and Nucleotide Base," Analytical Sciences, 17:155-160.
- Tuncel et al. (1999) "Catalytically Self-Threading Polyrotaxanes," Chem. Comm. 1509-1510.
- Veeneman et al. (1991) "An Efficient Approach to the Synthesis of Thymidine Derivatives containing Phosphate-Isoteric Methylene Acetyl Linkages," Tetrahedron, 47:1547-1562.
- Wada et al. (2001) "2-(Azidomethyl)benzoyl as a new protecting group in nucleosides," Tetrahedron Letters, 42:1069-1072.
- Weiss (1999) "Fluorescent Spectroscopy of Single Biomolecules." Science, 283:1676.
- Welch et al. (1999) "Synthesis of Nucleosides Designed for Combinatorial DNA Sequencing," Chemistry, European Journal, 5:951-960.
- Welch MB, Burgess K, (1999) "Synthesis of fluorescent, photolabile 3'-O-protected nucleoside triphosphates for the base addition sequencing scheme," Nucleosides and Nucleotides 18:197-201.
- Wendy, Jen. Et al. (2000) "New Strategies for Organic Catalysis: The First Enantioselective Organocatalytic 1,3-Dipolar Cycloaddition," J. Am. Chem. Soc. 122:9874-9875.
- Woolley, A. T. et al. (1997) "High-Speed DNA Genotyping Using Microfabricated Capillary Array Electrophoresis Chips," Anal. Chem. 69:2181-2186.
- Yamashita et al. (1987) "Studies on Antitumor Agents VII. Antitumor Activities of O-Alkoxyalkyl Derivatives of 2'-Deoxy-5-trifluoromethyluridine." Chem Pharm. Bull., vol. 35, pp. 2373-2381.
- Zavgorodny, S. et al. (1991) "1-Alkylthioalkylation of Nucleoside Hydroxyl Functions and its synthetic Applications: A New Versatile Method in Nucleoside Chemistry," Tetrahedron Letters, 32(51): 7593-7596.
- Zavgorodny et al. (2000) "S,X-Acetals in Nucleoside Chemistry. III. Synthesis of 2'- and 3'-O-Azidomethyl Derivatives of Ribonucleosides" Nucleosides, Nucleotides and Nucleic Acids, 19(10-12):1977-1991.
- Zhang et al. (2002) "Synthesis of Releasable Electrophore Tags for Applications in Mass Spectrometry," Bioconjugate Chem., vol. 13, pp. 1002-1012.
- Zhu, Z., Chao, J., Yu, H., et al. (1994) "Directly Labeled DNA Probes Using Fluorescent Nucleotides with Different Length Linkers," Nucleic Acids Res., 22:3418-3422.
- U.S. Appl. No. 09/266,187, filed Mar. 10, 1999, Stemple et al. Sep. 16, 2012 Motion to Waive Page Limit and Proposed Petition in connection with Petition for Inter Partes Review of U.S. Pat. No. 7,713,698, issued May 11, 2010.
- Dec. 20, 2012 Preliminary Response under 37 C.F.R. 42.107 in connection with IPR2012-00006.
- Mar. 12, 2013 Decision on Petition for Inter Partes Review in connection with IPR2012-00006.
- Mar. 26, 2013 Request for Reconsideration in connection with IPR2012-00006.
- Apr. 26, 2013 Opposition to Request for Reconsideration (Rehearing) Under 37 C.F.R. 42.71.(C) in connection with IPR2012-00006.
- Sep. 27, 2013 Petitioner Opposition to Motion to Amend in connection with IPR2012-00006.
- Sep. 27, 2013 Petitioner Reply to Response to Petition in connection with IPR2012-00006.
- Nov. 18, 2013 Substitute Patent Owner Reply on Motion to Amend in connection with IPR2012-00006.
- Prober et al. (1987), "A System for Rapid DNA Sequencing with Fluorescent Chain-Terminating Dideoxynucleotides", *Science* vol. 238, Oct. 16, 1987, pp. 336-341 (Exhibit 1003, filed Sep. 16, 2012 in connection with IPR2012-00006).
- Sep. 15, 2012 Declaration of George Weinstock Under Rule 37 C.F.R. §1.132 (Exhibit 1021, filed Sep. 16, 2012 in connection with IPR2012-00006).
- Excerpts of File History of U.S. Pat. No. 7,713,698 (Exhibit 1022, filed Sep. 16, 2012 in connection with IPR2012-00006).
- Columbia's Amended Complaint from *The Trustees of Columbia University in the City of New York v. Illumina, Inc.*, D. Del C.A. No. 12-376 (GMS), filed Apr. 11, 2012 (Exhibit 1025, filed Apr. 30, 2013 in connection with IPR2012-00006).
- Illumina's Answer to Amended Complaint from *The Trustees of Columbia University in the City of New York v. Illumina, Inc.*, D. Del C.A. No. 12-376 (GMS), filed Dec. 21, 2012 (Exhibit 1026, filed Apr. 30, 2013 in connection with).
- Rosenblum et al., "New Dye-Labeled Terminators for Improved DNA Sequencing Patterns," *Nucleic Acid Research*, 1997, vol. 25, No. 22, pp. 4500-4504 (Exhibit 1030, filed Jun. 18, 2013 in connection with IPR2012-00006).
- Jun. 8, 2013 Videotaped Deposition Transcript of George M. Weinstock, Ph.D. (Exhibit 1034, filed Jun. 18, 2013 in connection with IPR2012-00006).
- "Next Generation Genomics: World Map of High-throughput Sequencers," Sep. 1, 2013 (Exhibit 1036, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Videotaped. Deposition Transcript of Dr. Xiaohai Liu, Mar. 20, 2013 (Exhibit 1039, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Excerpt from videotaped Deposition Transcript of George M. Weinstock, Ph.D., Jun. 8, 2013 (Exhibit 1040, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Seela et al., "Oligonucleotide Duplex Stability Controlled by the 7-Substituents of 7-Deazaguanine Bases," *Bioorganic & Medical Chemistry Letters*, vol. 5, No. 24, pp. 3049-3052, 1995 (Exhibit 1041, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Ramzaeva et al., "123. 7-Deazaguanine DNA: Oligonucleotides with Hydrophobic or Cationic Side Chains," *Helvetica Chimica Acta*, vol. 80, pp. 1809-1822, 1997 (Exhibit 1042, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Ramzaeva et al., "88. 7-Substituted 7-Deaza-2'-deoxyguanosines: Regioselective Halogenation of Pyrrolo[2,3-d]pyrimidine Nucleosides," *Helvetica Chimica Acta*, vol. 78, pp. 1083-1090, 1995 (Exhibit 1043, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Seela et al., "Duplex Stability of Oligonucleotides Containing 7-Substituted 7-Deaza- and 8-Aza-7-Deazapurine Nucleosides," *Nucleosides & Nucleotides*, 16(7-9), pp. 963-966, 1997 (Exhibit 1044, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Burgess et al., "Syntheses of Nucleosides Designed for Combinatorial DNA Sequencing," *Chemistry—A European Journal*, vol. 5, No. 3, pp. 951-960, 1999 (Exhibit 1045, filed Sep. 27, 2013 in connection with IPR2012-00006).
- Jan. 28, 2013 Declaration of Dr. Bruce P. Branchaud in Support of

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.