



- (51) **International Patent Classification:**
C12Q 1/68 (2006.01)
- (21) **International Application Number:**
PCT/US2013/041031
- (22) **International Filing Date:**
14 May 2013 (14.05.2013)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/646,714 14 May 2012 (14.05.2012) US
- (71) **Applicant:** **CB BIOTECHNOLOGIES, INC.** [US/US];
7712 Donegal Drive, SE, Huntsville, Alabama 35802 (US).
- (72) **Inventors:** **WANG, Chunlin**; 735 Roble Avenue #3,
Menlo Park, California 94025 (US). **HAN, Jian**; 7712
Donegal Drive SE, Huntsville, Alabama 35802 (US).
- (74) **Agent:** **RUSSELL, Donna**; 1492 Anthony Way, Mt. Ju-
liet, Tennessee 37122 (US).
- (81) **Designated States** (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,

HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.

(84) **Designated States** (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))*

(88) **Date of publication of the international search report:**
23 January 2014



WO 2013/173394 A3

(54) **Title:** METHOD FOR INCREASING ACCURACY IN QUANTITATIVE DETECTION OF POLYNUCLEOTIDES

(57) **Abstract:** Disclosed is a method for improving the sensitivity and accuracy of quantitative detection of polynucleotides in a sample, such a clinical specimen, by a method that utilizes a two- or three-step process of tagging/labeling target molecules and adding an adapter sequence for adding a universal primer for efficient amplification of targets while decreasing target amplification bias. When combined with the step of statistically correcting for sequencing errors, the method can significantly increase the accuracy of quantitative detection of polynucleotides in a sample.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/41031

<p>A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12Q 1/68 (2013.01) USPC - 435/6.1, 6.12 According to International Patent Classification (IPC) or to both national classification and IPC</p>																	
<p>B. FIELDS SEARCHED</p> <p>Minimum documentation searched (classification system followed by classification symbols) USPC:435/6.1, 6.12</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC:435/6.1, 6.12 (text search)</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, Google Patents, Google Scholar, Google Web, search terms: accuracy quantitative PCR, measure accuracy of qPCR, qPCR universal primer, unique molecular identifier, next generation sequencing, barcode sequence error, sequence accuracy Poisson distribution, error rates universal primer, detection PCR universal primer, sequencing artifact rate</p>																	
<p>C. DOCUMENTS CONSIDERED TO BE RELEVANT</p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X --- Y</td> <td>US 2011/0294689 A1 (NAMSARAEV) 1 December 2011 (01.12.2011). Especially para [0017], [0018], [0022], [0034], [0127], [0135].</td> <td>1, 3-5 ----- 2, 6</td> </tr> <tr> <td>Y</td> <td>US 2011/0081367 A1 (ONG et al.) 7 April 2011 (07.04.2011). Especially para [0109].</td> <td>2</td> </tr> <tr> <td>Y</td> <td>MARGERIDON et al. Ultra-deep pyrosequencing of hepatitis B virus quasiespecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. J Infect Dis 1 May 2009 Vol 199 No 9 Pages 1275-1285. Especially pg 1276 col 2 para 2 and table 1, pg 1277 col 1 para 4 and col 2 para 1.</td> <td>6</td> </tr> <tr> <td>Y</td> <td>WANG et al. Characterization of mutation spectra with ultra-deep pyrosequencing: application to HIV-1 drug resistance. Genome Res August 2007 Vol 17 No 8 Pages 1195-1201. Especially pg 1200 col 1 para 2.</td> <td>6</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X --- Y	US 2011/0294689 A1 (NAMSARAEV) 1 December 2011 (01.12.2011). Especially para [0017], [0018], [0022], [0034], [0127], [0135].	1, 3-5 ----- 2, 6	Y	US 2011/0081367 A1 (ONG et al.) 7 April 2011 (07.04.2011). Especially para [0109].	2	Y	MARGERIDON et al. Ultra-deep pyrosequencing of hepatitis B virus quasiespecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. J Infect Dis 1 May 2009 Vol 199 No 9 Pages 1275-1285. Especially pg 1276 col 2 para 2 and table 1, pg 1277 col 1 para 4 and col 2 para 1.	6	Y	WANG et al. Characterization of mutation spectra with ultra-deep pyrosequencing: application to HIV-1 drug resistance. Genome Res August 2007 Vol 17 No 8 Pages 1195-1201. Especially pg 1200 col 1 para 2.	6
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.															
X --- Y	US 2011/0294689 A1 (NAMSARAEV) 1 December 2011 (01.12.2011). Especially para [0017], [0018], [0022], [0034], [0127], [0135].	1, 3-5 ----- 2, 6															
Y	US 2011/0081367 A1 (ONG et al.) 7 April 2011 (07.04.2011). Especially para [0109].	2															
Y	MARGERIDON et al. Ultra-deep pyrosequencing of hepatitis B virus quasiespecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. J Infect Dis 1 May 2009 Vol 199 No 9 Pages 1275-1285. Especially pg 1276 col 2 para 2 and table 1, pg 1277 col 1 para 4 and col 2 para 1.	6															
Y	WANG et al. Characterization of mutation spectra with ultra-deep pyrosequencing: application to HIV-1 drug resistance. Genome Res August 2007 Vol 17 No 8 Pages 1195-1201. Especially pg 1200 col 1 para 2.	6															
<p><input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/></p>																	
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed						
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family																
"P" document published prior to the international filing date but later than the priority date claimed																	
<p>Date of the actual completion of the international search 6 November 2013 (06.11.2013)</p>		<p>Date of mailing of the international search report 02.Dec. 2013</p>															
<p>Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201</p>		<p>Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774</p>															