



(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2015/034724

(22) International Filing Date:
8 June 2015 (08.06.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/009,047 6 June 2014 (06.06.2014) US
62/136,093 20 March 2015 (20.03.2015) US

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(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, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, 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, SA, 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, ST, 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, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished
upon receipt of that report (Rule 48.2(g))*

(54) Title: METHOD FOR IDENTIFICATION AND ENUMERATION OF NUCLEIC ACID SEQUENCE, EXPRESSION, COPY, OR DNA METHYLATION CHANGES, USING COMBINED NUCLEASE, LIGASE, POLYMERASE, AND SEQUENCING REACTIONS

(57) Abstract: The present invention relates to a method for the highly specific, targeted capture of regions of human genomes and transcriptomes from the blood, i.e. from cell free circulating DNA, exosomes, microRNA, circulating tumor cells, or total blood cells, to allow for the highly sensitive detection of mutation, expression, copy number, translocation, alternative splicing, and methylation changes using combined nuclease, ligation, polymerase, and massively parallel sequencing reactions. The method generates a collection of different circular chimeric single-stranded nucleic acid constructs, suitable for sequencing on multiple platforms. In some embodiments, each construct of the collection comprised a first single stranded segment of original genomic DNA from a host organism and a second single stranded synthetic nucleic acid segment that is linked to the first single stranded segment and comprises a nucleotide sequence that is exogenous to the host organism. These chimeric constructs are suitable for identifying and enumerating mutations, copy changes, translocations, and methylation changes. In other embodiments, input mRNA, lncRNA, or miRNA is used to generate circular DNA products that reflect the presence and copy number of specific mRNA's, lncRNA's splice-site variants, translocations, and miRNA.



WO 2015/188192 A2