(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 March 2010 (25.03.2010)

PCT

(10) International Publication Number
WO 2010/033578 A3

(51) International Patent Classification:
C12Q 1/68 (2006.01) *G01N 33/48* (2006.01)

(21) International Application Number:
PCT/US2009/057136

(22) International Filing Date:
16 September 2009 (16.09.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/098,758 20 September 2008 (20.09.2008) US

(71) Applicant *(for all designated States except US)*: **THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY** [US/US]; 1705 El Camino Real, Palo Alto, CA 94306-1106 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FAN, Hei-Mun, Christina** [CN/US]; 248 Pamela Dr., Apt 23, Mountain View, CA 94040 (US). **QUAKE, Stephen, R.** [US/US]; 636 Alvarado Row, Stanford, CA 94305 (US).

(74) Agents: **ASTON, David, J.** et al.; Peters Verny, LLP, 425 Sherman Avenue, Suite 230, Palo Alto, CA 94306 (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, 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, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (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, 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))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
14 May 2010

WO 2010/033578 A3

(54) Title: NONINVASIVE DIAGNOSIS OF FETAL ANEUPLOIDY BY SEQUENCING

(57) Abstract: Disclosed is a method to achieve digital quantification of DNA (i.e., counting differences between identical sequences) using direct shotgun sequencing followed by mapping to the chromosome of origin and enumeration of fragments per chromosome. The preferred method uses massively parallel sequencing, which can produce tens of millions of short sequence tags in a single run and enabling a sampling that can be statistically evaluated. By counting the number of sequence tags mapped to a predefined window in each chromosome, the over- or under- representation of any chromosome in maternal plasma DNA contributed by an aneuploid fetus can be detected. This method does not require the differentiation of fetal versus maternal DNA. The median count of autosomal values is used as a normalization constant to account for differences in total number of sequence tags is used for comparison between samples and between chromosomes.