(19) DANMARK

(10) DK/EP 3649257 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 12 Q 1/6806 (2018.01)* *C 12 Q 1/6883 (2018.01)* *C 12 Q 1/6886 (2018.01)*

(45) Oversættelsen bekendtgjort den: **2022-07-04**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2022-03-30**

(86) Europæisk ansøgning nr.: **18745508.4**

(86) Europæisk indleveringsdag: **2018-07-06**

(87) Den europæiske ansøgnings publiceringsdag: **2020-05-13**

(86) International ansøgning nr.: **EP2018068402**

(87) Internationalt publikationsnr.: **WO2019008148**

(30) Prioritet: **2017-07-07 US 201762529667 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **BERIGELSE AF MÅLRETTEDE GENOMISKE REGIONER TIL MULTIPLEKSET PARALLEL ANALYSE**

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DESCRIPTION

Field of the Invention

[0001] The invention is in the field of biology, medicine and chemistry, more in particular in the field of molecular biology and more in particular in the field of molecular diagnostics.

Background of the Invention

[0002] The discovery of free fetal DNA (ffDNA) in maternal circulation (Lo, Y.M. et al. (1997) Lancet 350:485-487) was a landmark towards the development of non-invasive prenatal testing for chromosomal abnormalities and has opened up new possibilities in the clinical setting. However, direct analysis of the limited amount of ffDNA in the presence of an excess of maternal DNA is a great challenge for Non-Invasive Prenatal Testing (NIPT) of chromosomal abnormalities. The implementation of next generation sequencing (NGS) technologies in the development of NIPT has revolutionized the field. In 2008, two independent groups demonstrated that NIPT of trisomy 21 could be achieved using next generation massively parallel shotgun sequencing (MPSS) (Chiu, R. W. et a/.(2008) Proc. Natl. Acad. Sci. USA 105:20458-20463; Fan, H.C. et a/.(2008) Proc. Natl. Acad. Sci. USA 105:16266-162710). The new era of NIPT for chromosomal abnormalities has opened new possibilities for the implementation of these technologies into clinical practice. Biotechnology companies that are partly or wholly dedicated to the development of NIPT tests have initiated large-scale clinical studies towards their implementation (Palomaki, G.E. et al. (2011) Genet. Med. 13:913-920; Ehrich, M. et al. (2011) Am. J. Obstet. Gynecol. 204:205e1-11; Chen, E.Z. et al. (2011) PLoS One 6:e21791; Sehnert, A.J. et al. (2011) Clin. Chem. 57:1042-1049; Palomaki, G.E. et al. (2012); Genet. Med. 14:296-305; Bianchi, D.W. et al. (2012) Obstet. Gynecol. 119:890-901; Zimmerman, B. et al. (2012) Prenat. Diag. 32:1233-1241; Nicolaides, K.H. et al. (2013) Prenat. Diagn. 33:575-579; Sparks, A.B. et al. (2012) Prenat. Diagn. 32:3-9).

[0003] Initial NIPT approaches used massively parallel shotgun sequencing (MPSS) NGS methodologies (see e.g., US Patent No. 7,888,017; US Patent No. 8,008,018; US Patent No. 8,195,415; US Patent No. 8,296,076; US Patent No. 8,682,594; US Patent Publication 20110201507; US Patent Publication 20120270739). Thus, these approaches are whole genome-based, in which the entire maternal sample containing both maternal DNA and free fetal DNA is subjected to amplification, sequencing and analysis.

[0004] More recently, targeted-based NGS approaches for NIPT, in which only specific sequences of interest are sequenced, have been developed. For example, a targeted NIPT approach using TArget Capture Sequences (TACS) for identifying fetal chromosomal abnormalities using a maternal blood sample has been described (PCT Publication WO 2016/189388; US Patent Publication 2016/0340733; Koumbaris, G. et al. (2015) Clinical

chemistry, 62(6), pp.848-855.).

[0005] Such targeted approaches require significantly less sequencing than the MPSS approaches, since sequencing is only performed on specific loci on the target sequence of interest rather than across the whole genome. Additional methodologies for NGS-based approaches are still needed, in particular approaches that can target specific sequences of interest, thereby greatly reducing the amount of sequencing needed as compared to whole genome-based approaches, as well as increasing the read-depth of regions of interest, thus enabling detection of low signal to noise ratio regions. In particular, additional methodologies are still needed that allow for genetic aberrations present in diminutive amounts in a sample to be reliably detected.

Summary of the Invention

[0006] This invention provides improved methods for enriching targeted genomic regions of interest to be analyzed by multiplexed parallel sequencing. The methods of the invention utilize a pool of TArget Capture Sequences (TACS) designed such that the sequences within the pool have features that optimize the efficiency, specificity and accuracy of genetic assessment. More specifically, the size of the TACS, the number of TACS, their placement on the chromosome(s) of interest and their GC content all have been optimized. Furthermore, the pool of TACS comprises a plurality of TACS families, wherein each member of a TACS family binds to the same target sequence of interest but with different start/stop positions on the sequence with respect to a reference coordinate system (i.e., binding of TACS family members to the target sequence is staggered) to thereby enrich for target sequences of interest, followed by massive parallel sequencing and statistical analysis of the enriched population. The use of families of TACS with the TACS pool that bind to each target sequence of interest, as compared to use of a single TACS within the TACS pool that binds to each target sequence of interest, significantly increases enrichment for the target sequences of interest, as evidenced by a greater than 50% average increase in read-depth for the family of TACS versus a single TACS.

[0007] The methods of the invention for genetic assessment using highly enriched target sequences of interest can be used for a variety of clinical purposes. In one embodiment, the methods are used in non-invasive prenatal testing (NIPT), for example in detecting fetal chromosomal abnormalities (e.g., using a maternal plasma sample containing maternal and fetal DNA, or using a DNA sample obtained from a pre-implantation IVF embryo or from a maternal pap smear). The methods for NIPT can also be used for assessment of maternal and paternal carrier status for inherited genetic disorders to thereby determine risk of fetal inheritance of genetic disorders. In another embodiment, the methods are used for detection of tumor biomarkers for a wide variety of purposes in the oncology field, including initial cancer diagnosis, selection of appropriate therapeutic regimens based on tumor biomarkers (personalized medicine) and monitoring of treatment efficacy (reduction of tumor load based on changes in tumor biomarkers). For oncology purposes, the method can be used with a

tissue sample (e.g., tumor tissue biopsy) or can be used with a blood or plasma sample (e.g., liquid biopsy) or other suitable biological sample as described herein. Kits for carrying out the methods of the invention are also provided.

[0008] Accordingly, in one aspect the invention pertains to a method of testing for risk of a genetic abnormality in a DNA sample comprising genomic sequences of interest, the method comprising:

1. (a) preparing a sequencing library from the DNA sample;
2. (b) hybridizing the sequencing library to a pool of double-stranded TArget Capture Sequences (TACS), wherein the pool of TACS comprises a plurality of TACS families directed to different genomic sequences of interest, wherein each TACS family comprises a plurality of member sequences, wherein each member sequence binds to the same genomic sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest, and further wherein:
    1. (i) each member sequence within each TACS family is between 150-260 base pairs in length, each member sequence having a 5' end and a 3' end;
    2. (ii) each member sequence binds to the same genomic sequence of interest at least 50 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and
    3. (iii) the GC content of the pool of TACS is between 19% and 80%, as determined by calculating the GC content of each member within each family of TACS;
3. (c) isolating members of the sequencing library that bind to the pool of TACS to obtain an enriched library;
4. (d) amplifying and sequencing the enriched library; and
5. (e) performing statistical analysis on the enriched library sequences to thereby determine risk of a genetic abnormality in the DNA sample, wherein the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by 5 to 10 base pairs.

[0009] In certain embodiments, each TACS family comprises at least 2 member sequences or at least 5 member sequences. Alternative numbers of member sequences in each TACS family are described herein. In one embodiment, the pool of TACS comprises at least 50 different TACS families. Alternative numbers of different TACS families within the pool of TACS are described herein. In certain embodiments, the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by at least 3 base pairs or by 5 to 10 base pairs. Alternative lengths (sizes) for the number of base pairs within the stagger are described herein but are not according to the invention and are present for illustration purposes only

[0010] In one embodiment, the genomic abnormality is a chromosomal aneuploidy. In other embodiments, the genomic abnormality is a structural abnormality, including but not limited to copy number changes including microdeletions and microduplications, insertions, deletions, translocations, inversions and small-size mutations including point mutations and mutational signatures.

[0011] In one embodiment, the pool of TACS is fixed to a solid support. For example, in one embodiment, the TACS are biotinylated and are bound to streptavidin-coated magnetic beads.

[0012] In certain embodiments, the GC content of the pool of TACS is between 19% and 80% or is between 19% and 46%. Alternative % ranges for the GC content of the pool of TACS are described herein.

[0013] In one embodiment, sequencing of the enriched library provides a read-depth for the genomic sequences of interest and read-depths for reference loci and the statistical analysis comprises applying an algorithm that tests sequentially the read-depth of the loci of from the genomic sequences of interest against the read-depth of the reference loci, the algorithm comprising steps for: (a) removal of inadequately sequenced loci; (b) GC-content bias alleviation; and (c) ploidy status determination. In one embodiment, GC-content bias is alleviated by grouping together loci of matching GC content. In one embodiment, sequencing of the enriched library provides the number and size of sequenced fragments for TACS-specific coordinates and the statistical analysis comprises applying an algorithm that tests sequentially the fragment-size proportion for the genomic sequence of interest against the fragment-size proportion of the reference loci, the algorithm comprising steps for: (a) removal of fragment-size outliers; (b) fragment-size proportion calculation; and (c) ploidy status determination.

[0014] In one embodiment, the DNA sample is a maternal plasma sample comprising maternal DNA and cell-free fetal DNA (cffDNA).

[0015] In one embodiment, the DNA sample comprises cell free tumor DNA (cftDNA) and wherein each member sequence within a TACS family binds to a tumor biomarker sequence of interest. In one embodiment, the DNA sample is selected from the group consisting of a plasma sample, a urine sample, a sputum sample, a cerebrospinal fluid sample, an ascites sample and a pleural fluid sample from a subject having or suspected of having a tumor. In one embodiment, the DNA sample is from a tissue sample from a subject having or suspected of having a tumor. In one embodiment, the plurality of TACS families bind to a plurality of tumor biomarker sequences of interest selected from the group consisting of EGFR_6240, KRAS_521, EGFR_6225, NRAS_578, NRAS_580, PIK3CA_763, EGFR_13553, EGFR_18430, BRAF_476, KIT_1314, NRAS_584, EGFR_12378, and combinations thereof.

[0016] In one embodiment, the maternal plasma sample is screened to determine maternal carrier status for a plurality of variant alleles, wherein each family of TACS binds to a variant allele locus associated with a genetic condition. In one embodiment, each member sequence within each family of TACS is at least 160 base pairs in length.

[0017] In another embodiment, the plurality of variant allele loci of interest are associated with genetic conditions selected from the group consisting of Abetalipoproteinemia; Arthrogryposis Mental Retardation Seizures; Autosomal recessive polycystic kidney disease; Bardet Biedl syndrome 12; Beta thalassemia; Canavan disease; Choreacanthocytosis; Crigler Najjar syndrome, Type I; Cystic fibrosis; Factor V Leiden thrombophilia; Factor XI deficiency; Familial dysautonomia; Familial Mediterranean fever; Fanconi anemia (FANCG-related); Glycine encephalopathy (GLDC-related); Glycogen storage disease, Type 3; Glycogen storage disease, Type 7; GRACILE Syndrome; Inclusion body myopathy, Type 2; Isovaleric acidemia; Joubert syndrome, Type 2; Junctional epidermolysis bullosa, Herlitz type; Leber congenital amaurosis (LCA5-related); Leydig cell hypoplasia [Luteinizing Hormone Resistance]; Limb girdle muscular dystrophy, Type 2E; Lipoamide Dehydrogenase Deficiency [Maple syrup urine disease, Type 3]; Lipoprotein lipase deficiency; Long chain 3-hydroxyacyl-CoA dehydrogenase deficiency; Maple syrup urine disease, Type 1B; Methylmalonic acidemia (MMAA-related); Multiple sulfatase deficiency; Navajo neurohepatopathy [MPV17-related hepatocerebral mitochondrial DNA depletion syndrome]; Neuronal ceroid lipofuscinosis (MFSD8-related); Nijmegen breakage syndrome; Ornithine translocase deficiency [Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH) Syndrome]; Peroxisome biogenesis disorders Zellweger syndrome spectrum (PEX1-related); Peroxisome biogenesis disorders Zellweger syndrome spectrum (PEX2-related); Phenylketonurea; Pontocerebellar hypoplasia, Type 2E; Pycnodysostosis; Pyruvate dehydrogenase deficiency (PDHB-related); Retinal Dystrophy (RLBP1-related) [Bothnia retinal dystrophy]; Retinitis pigmentosa (DHDDS-related); Sanfilippo syndrome, Type D [Mucopolysaccharidosis IIID]; Sickle-cell disease; Sjogren-Larsson syndrome; Tay-Sachs disease; Usher syndrome, Type 1F; 3 Methylcrotonyl CoA Carboxylase Deficiency 1; 3 Methylcrotonyl CoA Carboxylase Deficiency 2, and combinations thereof.. In one embodiment, the method further comprises, for a sample with a positive maternal carrier status, obtaining a paternal DNA sample and performing steps (a)-(e) of the method on the paternal DNA sample to determine paternal carrier status, to thereby compute a fetal risk score for inheriting the genetic condition.

[0018] In one embodiment, the DNA sample is from a group comprising of a fetal or embryonic DNA sample. In one embodiment, the fetal or embryonic DNA sample is from a single or a few cells of a pre-implantation embryo. In one embodiment, the fetal or embryonic DNA sample is from a single or a few fetal cells obtained from a maternal pap smear. In one embodiment, the pool of TACS comprise a plurality of sequences whose binding encompasses all chromosomes of the human genome.

[0019] In one embodiment, amplification of the enriched library is performed in the presence of blocking sequences that inhibit amplification of wild-type sequences.

[0020] In one embodiment, members of the sequencing library that bind to the pool of TACS are partially complementary to the TACS.

Brief Description of the Figures

[0021] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 is a schematic diagram of multiplexed parallel analysis of targeted genomic regions for non-invasive prenatal testing using TArget Capture Sequences (TACS).

Figure 2 is a listing of exemplary chromosomal regions for amplifying TACS that bind to for example chromosomes 13, 18, 21 or X. A more extensive list is shown in Table 1 below.

Figure 3 is a schematic diagram of TACS-based enrichment of a sequence of interest (bold line) using a single TACS (left) versus TACS-based enrichment using a family of TACS (right).

Figures 4A-4B are graphs showing enrichment using families of TACS versus a single TACS, as illustrated by increase in the average read-depth. Figure 4A shows loci enriched using a family of TACS (red dots) as compared to loci enriched using a single TACS (blue dots), with different target sequences shown on the X-axis and the fold change in read-depth shown on the Y-axis. Figure 4B is a bar graph illustrating the average fold-increase in read-depth (54.7%) using a family of TACS (right) versus a single TACS (left).

Figure 5 shows bar graphs illustrating detection of known genetic mutations that are tumor biomarkers in certified reference material harboring the mutations. Two replicates of the reference material are shown. The line illustrates the expected minor allele frequency (MAF) for each of the assessed tumor loads. The bars (x-axis) illustrate the detected MAF (y-axis) for the indicated genetic mutations in the certified reference material.

Figure 6 shows bar graphs illustrating detection of tumor biomarkers in cancer patient samples. Results are shown for two patients, one harboring mutation PIK3CA E545K (top bars) and one harboring mutation TP53 K139 (bottom bars). Both tumor tissue samples ("Tissue Rep. 1" and "Tissue Rep. 2") and plasma samples ("Plasma") are shown. The y-axis shows % variant allele frequency (VAF) detected in the samples.

Figure 7 is a bar graph showing the observed pattern of somatic SNVs in breast cancer, as found in the COSMIC database. The x-axis shows a single base mutation observed in cancer in the context of its neighboring sequences. For example A[C>A]T describes the mutation of Cytosine (C) to Adenine (A) where the upstream sequence is Adenine and the downstream sequence is Thymine. The y-axis shows the frequency of occurrence of this mutation in breast cancer.

Figure 8 is a bar graph showing results of a simulations study where simulated sequencing data includes mutational motifs. The data were subjected to mutational motif detection. The bars indicate the average estimated frequency of the known mutational breast cancer motifs computed from a data set of 10000 simulations. Results illustrate that detection of mutational motifs is possible using the developed algorithm.

Figure 9 is a dot plot graph showing results of a fragments-based test for detecting increased numbers of smaller-size fragments in a mixed sample. An abnormal, aneuploid sample, with an estimated fetal fraction of 2.8%, was correctly detected using this method. The black dots are individual samples. The x-axis shows the sample index. The y-axis shows the score result of the fragments-size based method. A score result greater than the threshold shown by the grey line indicates a deviation from the expected size of fragments illustrating the presence of aneuploidy.

Figure 10 is a plot graph illustrating variant allele frequencies (VAFs) of various loci associated with the indicated genetic conditions, as computed from a mixed sample containing maternal and fetal DNA. The x-axis is an index of samples. The y-axis shows the % VAF. The VAF value is dependent on the maternal fraction present in the mixed sample. VAF values above a certain threshold illustrate the presence of a genetic condition in the maternal sample (i.e., the maternal sample is assigned as a maternal carrier).

Figure 11 is a graph of results from fetal DNA samples that underwent ploidy status determination using likelihood-based segmentation analysis and whole-genome sequencing data. The horizontal blue line indicates the average read-depth of each segment. The red lines indicate threshold intervals of expected diploids. Data above the top red line indicate a state of more than diploid and data below the red line indicate a state of less than diploid. The top panel illustrates the results of a euploid female sample (i.e., a female fetus with diploid X chromosome, no Y chromosome, and without any ploidy abnormalities present). The bottom panel illustrates the results of a female aneuploid sample (i.e., a female fetus with diploid X chromosome and no Y chromosome) with monosomy 18 and monosomy 20. Values on the y-axis are log of read-depth.

Figure 12 is a graph of results from fetal DNA samples that underwent ploidy status determination by whole genome sequencing, followed by segmentation analysis using small overlapping windows analysis. The horizontal blue line indicates the average read-depth of each chromosome. The red lines indicate threshold intervals of expected diploids. The top panel illustrates the results of a euploid male sample (i.e., a male fetus with a single copy of X and Y chromosomes and without any ploidy abnormalities present). The bottom panel illustrates the results of an aneuploid male sample (i.e., a male fetus with a single copy of X and Y chromosomes) and with aneuploidies on chromosomes 13 and 19 (trisomy 13 and mosaicism on chromosome 19). Values are log of read-depth.

Figure 13 is a graph of results from fetal DNA samples that underwent ploidy status determination by whole genome sequencing, followed by segmentation analysis using parallel pairwise testing. The top panel illustrates the results of a normal (euploid) sample and the bottom panel illustrates the results of an aneuploidy sample with aneuploidies on chromosomes 1, 2, 13, 15, 16, 19, and 20.

Figure 14 is a graph depicting results from fetal DNA samples that underwent ploidy status determination using TACS-based enrichment, followed by a score-based classification. As per the key, samples plotted with N indicate normal ploidy status, the sample plotted with P illustrates partial trisomy, the samples plotted with T indicate trisomy and the samples plotted

with M indicate monosomy.

Figure 15 is a graph of results from fetal DNA samples that underwent ploidy status determination using likelihood-based segmentation analysis and TACS-based enrichment whole genome sequencing data. The horizontal blue line indicates the average read-depth of each chromosome. The red lines indicate threshold intervals of expected diploids. Data above the top red line is classified as more than diploid and data below the red line is classified as less than diploid. The top panel illustrates the results of a euploid male sample (i.e., a male fetus with one copy of chromosome X chromosome and one copy of chromosome Y, and without any ploidy abnormalities present). The bottom panel illustrates the results of a male aneuploid sample with trisomy 13 and monosomy 21. Values on the y-axis are log-based transformations of read-depth.

Figure 16 is a graph of results from fetal DNA samples that underwent ploidy status determination using likelihood-based segmentation analysis and TACS-based enrichment data. The horizontal blue line indicates the average read-depth of each chromosome. The red lines indicate threshold intervals of expected diploids. Data above the top red line is classified as more than diploid and data below the red line is classified as less than diploid. The top panel illustrates the results of a euploid male sample (i.e., a male fetus with one copy of chromosome X chromosome and one copy of chromosome Y, and without any ploidy abnormalities present). The bottom panel illustrates the results of a male aneuploid sample with trisomy 13 and monosomy 21. Values on the y-axis are log-based transformations of read-depth.

Figure 17 is a listing of exemplary chromosomal regions for amplifying TACS that bind to exemplary, non-limiting tumor biomarker genes.

Detailed Description

[0022] The invention pertains to a method for analyzing genetic abnormalities that involves hybridization-based enrichment of selected target regions across the human genome in a multiplexed panel assay, followed by quantification, coupled with a novel bioinformatics and mathematical analysis pipeline. An overview of the method is shown schematically in Figure 1.

[0023] In-solution hybridization enrichment has been used in the past to enrich specific regions of interest prior to sequencing (see e.g., Meyer, M and Kirchner, M. (2010) Cold Spring Harb. Protoc. 2010(6):pdbprot5448; Liao, G.J. et al. (2012) PLoS One 7:e38154; Maricic, T. et al. (2010) PLoS One 5:e14004; Tewhey, R. et al.(2009) Genome Biol. 10:R116; Tsangaras, K. et al. (2014) PLoS One 9:e109101; PCT Publication WO 2016/189388; US Patent Publication 2016/0340733; Koumbaris, G. et al. (2016) Clinical chemistry, 62(6), pp.848-855). However, for the methods of the invention, the target sequences (referred to as TArget Capture Sequences, or TACS) used to enrich for specific regions of interest have been optimized for maximum efficiency, specificity and accuracy and, furthermore, are used in families of TACS,

comprising a plurality of members that bind to the same genomic sequence but with differing start and/or stop positions, such that enrichment of the genomic sequences of interest is significantly improved compared to use of a single TACS binding to the genomic sequence. The configuration of such families of TACS is illustrated schematically in Figure 3, showing that the different start and/or stop positions of the members of the TACS family when bound to the genomic sequence of interest results in a staggered binding pattern for the family members.

[0024] The use of families of TACS with the TACS pool that bind to each target sequence of interest, as compared to use of a single TACS within the TACS pool that binds to each target sequence of interest, significantly increases enrichment for the target sequences of interest, as evidenced by a greater than 50% average increase in read-depth for the family of TACS versus a single TACS. Comparison of use of a family of TACS versus a single TACS, and the significantly improved read-depth that was observed, is described in detail in Example 5.

[0025] Accordingly, in one aspect, the invention pertains to a method of testing for risk of a genetic abnormality in a DNA sample comprising genomic sequences of interest, the method comprising:

1. (a) preparing a sequencing library from the DNA sample;
2. (b) hybridizing the sequencing library to a pool of double-stranded TArget Capture Sequences (TACS), wherein the pool of TACS comprises a plurality of TACS families directed to different genomic sequences of interest, wherein each TACS family comprises a plurality of member sequences, wherein each member sequence binds to the same genomic sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest, and further wherein:
    1. (i) each member sequence within each TACS family is between 150-260 base pairs in length, each member sequence having a 5' end and a 3' end;
    2. (ii) each member sequence binds to the same genomic sequence of interest at least 50 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and
    3. (iii) the GC content of the pool of TACS is between 19% and 80% as determined by calculating the GC content of each member within each family of TACS;
3. (c) isolating members of the sequencing library that bind to the pool of TACS to obtain an enriched library;
4. (d) amplifying and sequencing the enriched library; and
5. (e) performing statistical analysis on the enriched library sequences to thereby determine risk of a genetic abnormality in the DNA sample, wherein the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by 5 to 10 base pairs.

[0026] Each TACS family comprises a plurality of members that bind to the same genomic sequence of interest but having different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest. Typically, the reference coordinate system that is used for analyzing human genomic DNA is the human reference genome built hg19, which is publically available in the art, although other versions may be used. Alternatively, the reference coordinate system can be an artificially created genome based on built hg19 that contains only the genomic sequences of interest. Exemplary non-limiting examples of start/stop positions for TACS that bind to chromosome 13, 18, 21, X or Y are shown in Figure 2. Exemplary non-limiting examples of start/stop positions for TACS that bind to NRAS on chromosome 1, PI3KCA on chromosome 3, EGFR on chromosome 7 or KRAS on chromosome 12 (as non-limiting examples of tumor biomarkers) are shown in Figure 17.

[0027] Each TACS family comprises at least 2 members that bind to the same genomic sequence of interest. In various embodiments, each TACS family comprises at least 2 member sequences, or at least 3 member sequences, or at least 4 member sequences, or at least 5 member sequences, or at least 6 member sequences, or at least 7 member sequences, or at least 8 member sequence, or at least 9 member sequences, or at least 10 member sequences. In various embodiments, each TACS family comprises 2 member sequences, or 3 member sequences, or 4 member sequences, or 5 member sequences, or 6 member sequences, or 7 member sequences, or 8 member sequences, or 9 member sequences, or 10 member sequences. In various embodiments, the plurality of TACS families comprises different families having different numbers of member sequences. For example, a pool of TACS can comprise one TACS family that comprises 3 member sequences, another TACS family that comprises 4 member sequences, and yet another TACS family that comprises 5 member sequences, and the like. In one embodiment, a TACS family comprises 3-5 member sequences. In another embodiment, the TACS family comprises 4 member sequences.

[0028] The pool of TACS comprises a plurality of TACS families. Thus, a pool of TACS comprises at least 2 TACS families. In various embodiments, a pool of TACS comprises at least 3 different TACS families, or at least 5 different TACS families, or at least 10 different TACS families, or at least 50 different TACS families, or at least 100 different TACS families, or at least 500 different TACS families, or at least 1000 different TACS families, or at least 2000 TACS families, or at least 4000 TACS families, or at least 5000 TACS families.

[0029] Each member within a family of TACS binds to the same genomic region of interest but with different start and/or stop positions, with respect to a reference coordinate system for the genomic sequence of interest, such that the binding pattern of the members of the TACS family is staggered (see Figure 3).

[0030] Typically, the start and/or stop positions are staggered by 5-10 base pairs. In one embodiment, the start and/or stop positions are staggered by 5 base pairs. In another embodiment, the start and/or stop positions are staggered by 10 base pairs.

[0031] The TACS-enrichment based method of the disclosure can be used in the detection of a

wide variety of genetic abnormalities. In one embodiment, the genetic abnormality is a chromosomal aneuploidy (such as a trisomy, a partial trisomy or a monosomy). In other embodiments, the genomic abnormality is a structural abnormality, including but not limited to copy number changes including microdeletions and microduplications, insertions, translocations, inversions and small-size mutations including point mutations and mutational signatures. In another embodiment, the genetic abnormality is a chromosomal mosaicism.

[0032] Further aspects and features of the methods of the disclosure are described in the subsections below.

[0033] The methods of the disclosure can be used with a wide variety of types of DNA samples and in a wide variety of clinical circumstances, including for non-invasive prenatal testing and for in the oncology field for cancer diagnosis and treatment. Such uses are described in further detail in the subsections below.

TArget Capture Sequence Design

[0034] As used herein, the term "TArget Capture Sequences" or "TACS" refers to short DNA sequences that are complementary to the region(s) of interest on a genomic sequence(s) of interest (e.g., chromosome(s) of interest) and which are used as "bait" to capture and enrich the region of interest from a large library of sequences, such as a whole genomic sequencing library prepared from a biological sample. In addition to the features of the families of TACS described above (e.g., staggered binding to the genomic sequence of interest), a pool of TACS is used for enrichment wherein the sequences within the pool have been optimized with regard to: (i) the length of the sequences; (ii) the distribution of the TACS across the region(s) of interest; and (iii) the GC content of the TACS. The number of sequences within the TACS pool (pool size) has also been optimized.

[0035] It has been discovered that TACS having a length between 150-260 base pairs are optimal to maximize enrichment efficiency. In various other embodiments, each sequence within the pool of TACS is between 150-260 or 200-260 base pairs.

[0036] In preferred embodiments, the length of the TACS within the pool is at least 250 base pairs, or is 250 base pairs or is 260 base pairs.

[0037] The distribution of the TACS across each region or chromosome of interest has been optimized to avoid high copy repeats, low copy repeats and copy number variants, while at the same time also being able to target informative single nucleotide polymorphisms (SNPs) in order to enable both aneuploidy, or structural copy number change detection, and fetal fraction (ff) estimation. Accordingly, each sequence within the TACS pool is designed such that the 5' end and the 3' end are each at least 50 base pairs away from regions in the genome that are known to harbour one or more of the following genomic elements: Copy Number Variations (CNVs), Segmental duplications and/or repetitive DNA elements (such as transposable

elements or tandem repeat areas). In various other embodiments, each sequence within the TACS pool is designed such that the 5' end and the 3' end are each at least 50, 100, 150, 200, 250, 300, 400 or 500 base pairs away from regions in the genome that are known to harbour one or more of the aforementioned elements.

[0038] The term "Copy Number Variations" is a term of art that refers to a form of structural variation in the human genome in which there can be alterations in the DNA of the genome in different individuals that can result in a fewer or greater than normal number of a section(s) of the genome in certain individuals. CNVs correspond to relatively large regions of the genome that may be deleted (e.g., a section that normally is A-B-C-D can be A-B-D) or may be duplicated (e.g., a section that normally is A-B-C-D can be A-B-C-C-D). CNVs account for roughly 13% of the human genome, with each variation ranging in size from about 1 kilobase to several megabases in size.

[0039] The term "Segmental duplications" (also known as "low-copy repeats") is also a term of art that refers to blocks of DNA that range from about 1 to 400 kilobases in length that occur at more than one site within the genome and typically share a high level (greater than 90%) of sequence identity. Segmental duplications are reviewed in, for example, Eichler. E.E. (2001) Trends Genet. 17:661-669.

[0040] The term "repetitive DNA elements" (also known as "repeat DNA" or "repeated DNA") is also a term of art that refers to patterns of DNA that occur in multiple copies throughout the genome. The term "repetitive DNA element" encompasses terminal repeats, tandem repeats and interspersed repeats, including transposable elements. Repetitive DNA elements in NGS is discussed further in, for example, Todd, J. et al. (2012) Nature Reviews Genet. 13:36-46.

[0041] The TACS are designed with specific GC content characteristics in order to minimize data GC bias and to allow a custom and innovative data analysis pipeline. It has been determined that TACS with a GC content of 19-80% achieve optimal enrichment and perform best with cell free fetal DNA. Within the pool of TACS, different sequences can have different % GC content, although to be selected for inclusion with the pool, the % GC content of each sequence is chosen as between 19-80%, as determined by calculating the GC content of each member within each family of TACS. That is, every member within each family of TACS has a % GC content within the given percentage range (e.g., between 19-80% GC content).

[0042] In some instances, the pool of TACS (i.e., each member within each family of TACS) may be chosen so as to define a different % GC content range, deemed to be more suitable for the assessment of specific genetic abnormalities. Non-limiting examples of various % GC content ranges, can be between 19% and 80%, or between 19% and 79%, or between 19% and 78%, or between 19% and 77%, or between 19% and 76%, or between 19% and 75%, or between 19% and 74%, or between 19% and 73%, or between 19% and 72%, or between 19% and 71%, or between 19% and 70%, or between 19% and 69%, or between 19% and 68%, or between 19% and 67%, or between 19% and 66%, or between 19% and 65%, or between 19% and 64%, or between 19% and 63%, or between 19% and 62%, or between 19% and 61%, or

between 19% and 60%, or between 19% and 59%, or between 19% and 58%, or between 19% and 57%, or between 19% and 56%, or between 19% and 55%, or between 19% and 54%, or between 19% and 53%, or between 19% and 52%, or between 19% and 51%, or between 19% and 50%, or between 19% and 49%, or between 19% and 48%, or between 19% and 47%, or between 19% and 46%, or between 19% and 45%, or between 19% and 44%, or between 19% and 43%, or between 19% and 42%, or between 19% and 41%, or between 19% and 40%.

[0043] As described in further detail below with respect to one embodiment of the data analysis, following amplification and sequencing of the enriched sequences, the test loci and reference loci can then be "matched" or grouped together according to their % GC content (e.g., test loci with a % GC content of 40% is matched with reference loci with a % GC content of 40%). It is appreciated that the % GC content matching procedure may allow slight variation in the allowed matched % GC range. A non-limiting instance, and with reference to the previously described example in text, a test locus with % GC content of 40% could be matched with reference loci of % GC ranging from 39-41%, thereby encompassing the test locus % GC within a suitable range.

[0044] To prepare a pool of TACS having the optimized criteria set forth above with respect to size, placement within the human genome and % GC content, both manual and computerized analysis methods known in the art can be applied to the analysis of the human reference genome. In one embodiment, a semi-automatic method is implemented where regions are firstly manually designed based on the human reference genome build 19 (hg19) ensuring that the aforementioned repetitive regions are avoided and subsequently are curated for GC-content using software that computes the % GC-content of each region based on its coordinates on the human reference genome build 19 (hg19). In another embodiment, custom-built software is used to analyze the human reference genome in order to identify suitable TACS regions that fulfill certain criteria, such as but not limited to, % GC content, proximity to repetitive regions and/or proximity to other TACS.

[0045] The number of TACS in the pool has been carefully examined and adjusted to achieve the best balance between result robustness and assay cost/throughput. The pool typically contains at least 800 or more TACS, but can include more, such as 1500 or more TACS, 2000 or more TACS or 2500 or more TACS or 3500 or more TACS or 5000 or more TACS. It has been found that an optimal number of TACS in the pool is 5000. It will be appreciated by the ordinarily skilled artisan that a slight variation in pool size typically can be used without altering the results (e.g., the addition or removal of a small number of TACS); accordingly, the number sizes of the pool given herein are to be considered "about" or "approximate", allowing for some slight variation (e.g., 1-5%) in size. Thus, for example, a pool size of "1600 sequences" is intended to refer to "about 1600 sequences" or "approximately 1600 sequences", such that, for example, 1590 or 1610 sequences is also encompassed.

[0046] In view of the foregoing, in another aspect, the invention provides a method for preparing a pool of TACS for use in the method of the invention for detecting risk of a chromosomal and/or other genetic abnormality, wherein the method for preparing the pool of

TACS comprises: selecting regions in one or more chromosomes of interest having the criteria set forth above (e.g., at least 50 base pairs away on either end from the aforementioned repetitive sequences and a GC content of between 19% and 80%, as determined by calculating the GC content of each member within each family of TACS), preparing primers that amplify sequences that hybridize to the selected regions, and amplifying the sequences, wherein each sequence is between 150-260 base pairs in length.

[0047] For use in the methods of the disclosure, the pool of TACS typically is fixed to a solid support, such as beads (such as magnetic beads) or a column. In one embodiment, the pool of TACS are labeled with biotin and are bound to magnetic beads coated with a biotin-binding substance, such as streptavidin or avidin, to thereby fix the pool of TACS to a solid support. Other suitable binding systems for fixing the pool of TACS to a solid support (such as beads or column) are known to the skilled artisan and readily available in the art. When magnetic beads are used as the solid support, sequences that bind to the TACS affixed to the beads can be separated magnetically from those sequences that do not bind to the TACS.

Sample Collection and Preparation

[0048] The methods of the invention can be used with a variety of biological samples. Essentially any biological sample containing DNA, and in particular cell-free DNA (cfDNA), can be used as the sample in the methods, allowing for genetic analysis of the DNA therein. For example, in one embodiment, the DNA sample is a plasma sample containing cell-free DNA (cfDNA). In particular for prenatal testing, the DNA sample contains fetal DNA (e.g., cell-free fetal DNA). In one embodiment for NIPT, the sample is a mixed sample that contains both maternal DNA and fetal DNA (e.g., cell-free fetal DNA (cffDNA)), such as a maternal plasma sample obtained from maternal peripheral blood. Typically for mixed maternal/fetal DNA samples, the sample is a maternal plasma sample, although other tissue sources that contain both maternal and fetal DNA can be used. Maternal plasma can be obtained from a peripheral whole blood sample from a pregnant woman and the plasma can be obtained by standard methods. As little as 2-4 ml of plasma is sufficient to provide suitable DNA material for analysis according to the method of the disclosure. Total cell free DNA can then be extracted from the sample using standard techniques, non-limiting examples of which include a Qiasymphony protocol (Qiagen) suitable for free fetal DNA isolation or any other manual or automated extraction method suitable for cell free DNA isolation.

[0049] In another embodiment for NIPT, the sample contains predominantly fetal or embryonic DNA. As used herein, a sample containing "predominantly fetal or embryonic DNA" is one that contains more than 50% fetal or embryonic DNA, and typically contains more than 90%, or 95% or 99% fetal or embryonic DNA. In one embodiment, the source of the sample that contains predominantly fetal or embryonic DNA is fetal or embryonic cells obtained from embryo biopsy of *in vitro* fertilized (IVF) pre-implantation embryos. It has been demonstrated that intact cells can be obtained from IVF pre-implantation embryos for Pre-implantation Genetic Screening (PGS) and Pre-implantation Genetic Diagnosis (PGD) processes. An ovum

is fertilized through IVF and resulting cells are collected during *in vitro* growth of the embryo. For example, cells can be collected from a day 3 embryo or a day 5 embryo. Typically, if cell harvesting is performed at day 3 a single fetal cell is obtained, also known as a blastomere, and if harvesting is performed at day 5 a few cells are obtained, also known as trophectoderm cells. Typically, the genetic integrity of the grown fetal cells is interrogated using array Comparative Genomic Hybridization (aCGH), a technology that can detect genetic abnormalities of a certain genomic size and above. The method of the disclosure provides an alternative means for detecting genomic abnormalities in fetal or embryonic cells obtained from an embryo.

[0050] In another embodiment, the source of the sample that contains predominantly fetal or embryonic DNA is fetal or embryonic cells obtained non-invasively from collecting intact cells (trophoblasts) from a maternal Papanicolaou smear (pap test). Recently it has been shown that this is a simple and safe approach for obtaining fetal or embryonic genetic material non-invasively and that the cells obtained from the pap test had an abundance (near 100%) of fetal genetic material (Jain, C.V. et al. (2016) Science Translational Medicine 8(363):363re4-363re4).

[0051] In another embodiment, the sample containing predominantly fetal or embryonic DNA is a DNA sample from one or a few fetal cells found in maternal plasma. In yet other embodiments, the sample containing predominantly fetal or embryonic DNA is a DNA sample that is obtained directly from fetal tissue, or from amniotic fluid, or from chorionic villi or from medium where products of conception were grown.

[0052] In yet another embodiment for oncology purposes, the sample is a biological sample obtained from a patient having or suspected of having a tumor. In one embodiment, the DNA sample comprises cell free tumor DNA (cftDNA). In one embodiment, the oncology sample is a sample of tissue (e.g., from a tumor biopsy). In another embodiment the sample is a patient's urine, sputum, ascites, cerebrospinal fluid or pleural effusion. In another embodiment, the oncology sample is a patient plasma sample, prepared from patient peripheral blood. Thus, the sample can be a liquid biopsy sample that is obtained non-invasively from a patient's blood sample, thereby potentially allowing for early detection of cancer prior to development of a detectable or palpable tumor.

[0053] For the biological sample preparation, typically cells are lysed and DNA is extracted using standard techniques known in the art, a non-limiting example of which is the Qiasymphony (Qiagen) protocol.

[0054] Following isolation, the cell free DNA of the sample is used for sequencing library construction to make the sample compatible with a downstream sequencing technology, such as Next Generation Sequencing. Typically this involves ligation of adapters onto the ends of the cell free DNA fragments, followed by amplification. Sequencing library preparation kits are commercially available. A non-limiting exemplary protocol for sequencing library preparation is described in detail in Example 1.

Enrichment by TACS Hybridization

[0055] The region(s) of interest on the chromosome(s) of interest is enriched by hybridizing the pool of TACS to the sequencing library, followed by isolation of those sequences within the sequencing library that bind to the TACS. To facilitate isolation of the desired, enriched sequences, typically the TACS sequences are modified in such a way that sequences that hybridize to the TACS can be separated from sequences that do not hybridize to the TACS. Typically, this is achieved by fixing the TACS to a solid support. This allows for physical separation of those sequences that bind the TACS from those sequences that do not bind the TACS. For example, each sequence within the pool of TACS can be labeled with biotin and the pool can then be bound to beads coated with a biotin-binding substance, such as streptavidin or avidin. In a preferred embodiment, the TACS are labeled with biotin and bound to streptavidin-coated magnetic beads. The ordinarily skilled artisan will appreciate, however, that other affinity binding systems are known in the art and can be used instead of biotin-streptavidin/avidin. For example, an antibody-based system can be used in which the TACS are labeled with an antigen and then bound to antibody-coated beads. Moreover, the TACS can incorporate on one end a sequence tag and can be bound to a solid support via a complementary sequence on the solid support that hybridizes to the sequence tag. Furthermore in addition to magnetic beads, other types of solid supports can be used, such as polymer beads and the like.

[0056] In certain embodiments, the members of the sequencing library that bind to the pool of TACS are fully complementary to the TACS. In other embodiments, the members of the sequencing library that bind to the pool of TACS are partially complementary to the TACS. For example, in certain circumstances it may be desirable to utilize and analyze data that are from DNA fragments that are products of the enrichment process but that do not necessarily belong to the genomic regions of interest (i.e. such DNA fragments could bind to the TACS because of part homologies (partial complementarity) with the TACS and when sequenced would produce very low coverage throughout the genome in non-TACS coordinates).

[0057] Following enrichment of the sequence(s) of interest using the TACS, thereby forming an enriched library, the members of the enriched library are eluted from the solid support and are amplified and sequenced using standard methods known in the art. Next Generation Sequencing is typically used, although other sequencing technologies can also be employed, which provides very accurate counting in addition to sequence information. To detect genetic abnormalities, such as but not limited to, aneuploidies or structural copy number changes requires very accurate counting and NGS is a type of technology that enables very accurate counting. Accordingly, for the detection of genetic abnormalities, such as but not limited to, aneuploidies or structural copy number changes, other accurate counting methods, such as digital PCR and microarrays can also be used instead of NGS. Non-limiting exemplary protocols for amplification and sequencing of the enriched library are described in detail in Example 3.

Data Analysis

[0058] The information obtained from the sequencing of the enriched library can be analyzed using an innovative biomathematical/biostatistical data analysis pipeline. Details of an exemplary analysis using this pipeline are described in depth in Example 4, and in further detail below. Alternative data analysis approaches for different purposes are also provided herein. For example, data analysis approaches for analyzing oncology samples are described in detail in Example 6-9 and in the oncology section below. Additionally, data analysis approaches for analyzing fetal and/or embryonic DNA samples for genetic abnormalities are described in detail in Example 11 and in the fetal DNA section below.

[0059] The analysis pipeline described in Example 4 exploits the characteristics of the TACS, and the high-efficiency of the target capture enables efficient detection of aneuploidies or structural copy number changes, as well as other types of genetic abnormalities. In the analysis, first the sample's sequenced DNA fragments are aligned to the human reference genome. QC metrics are used to inspect the aligned sample's properties and decide whether the sample is suitable to undergo classification. These QC metrics can include, but are not limited to, analysis of the enrichment patterns of the loci of interest, such as for example the overall sequencing depth of the sample, the on-target sequencing output of the sample, TACS performance, GC bias expectation, fraction of interest quantification. For determining the risk of a chromosomal abnormality in the fetal DNA of the sample, an innovative algorithm is applied. The steps of the algorithm include, but are not limited to, removal of inadequately sequenced loci, read-depth and fragment-size information extraction at TACS-specific coordinates, genetic (GC-content) bias alleviation and ploidy status classification.

[0060] Ploidy status determination is achieved using one or more statistical methods, non-limiting examples of which include a t-test method, a bootstrap method, a permutation test and/or a binomial test of proportions and/or segmentation-based methods and/or combinations thereof. It will be appreciated by the ordinarily skilled artisan that the selection and application of tests to be included in ploidy status determination is based on the number of data points available. As such, the suitability of each test is determined by various factors such as, but not limited to, the number of TACS utilized and the respective application for GC bias alleviation, if applicable. Thus, the aforementioned methods are to be taken as examples of the types of statistical analysis that may be employed and are not the only methods suitable for the determination of ploidy status. Typically, the statistical method results in a score value for the mixed sample and risk of the chromosomal abnormality in the fetal DNA is detected when the score value for the mixed sample is above a reference threshold value.

[0061] In particular, one aspect of the statistical analysis involves quantifying and alleviating GC-content bias. In addition to the challenge of detecting small signal changes in fetal DNA in the mixed sample, and/or other components of DNA of interest part of a mixed sample (for example, but not limited to, additional or less genetic material from certain chromosomal

regions), the sequencing process itself introduces certain biases that can obscure signal detection. One such bias is the preferential sequencing/amplification of genetic regions based on their GC-content. As such, certain detection methods, such as but not limited to, read-depth based methods, need to account for such bias when examining sequencing data. Thus, the bias in the data needs to be quantified and, subsequently, suitable methods are applied to account for it such that genetic context dependencies cannot affect any statistical methods that may be used to quantify fetal genetic abnormality risk.

[0062] For example, one method of quantifying the GC-content bias is to use a locally weighted scatterplot smoothing (LOESS) technique on the sequencing data. Each targeted locus may be defined by its sequencing read-depth output and its' GC-content. A line of best fit through these two variables, for a large set of loci, provides an estimate of the expected sequencing read-depth given the GC-content. Once this GC-bias quantification step is completed, the next step is to use this information to account for possible biases in the data. One method is to normalize the read-depth of all loci by their expected read-depth (based on each locus' GC-content). In principle, this unlinks the read-depth data from their genetic context and makes all data comparable. As such, data that are retrieved from different GC-content regions, such as for example, but not limited, to different chromosomes, can now be used in subsequent statistical tests for detection of any abnormalities. Thus, using the LOESS procedure, the GC bias is unlinked from the data prior to statistical testing. In one embodiment, the statistical analysis of the enriched library sequences comprises alleviating GC bias using a LOESS procedure.

[0063] In an alternative preferred embodiment, the GC-content bias is quantified and alleviated by grouping together loci of similar (matching) GC-content. Thus, conceptually this method for alleviating GC-content bias comprises of three steps, as follows:

1. 1) identification and calculation of GC-content in the TACS;
2. 2) alleviation/accounting of GC-content bias using various matching/grouping procedures of the TACS; and
3. 3) calculation of risk of any genetic abnormalities that may be present in the fetus utilizing statistical and mathematical methods on datasets produced from step 2.

[0064] For the t-test method, the dataset is split into two groups; the test loci and the reference loci. For each group, subsets of groups are created where loci are categorized according to their GC-content as illustrated in a non-limiting example in the sample Table 1 below:

Table 1

GC	Reference loci read-depth	Test loci read-depth
40%	$x_1^{40}, x_2^{40}, \ldots, x_{nx40}^{40}$	$y_1^{40}, y_2^{40}, \ldots, y_{ny40}^{40}$
41%	$x_1^{41}, x_2^{41}, \ldots, x_{nx41}^{41}$	$y_1^{41}, y_2^{41}, \ldots, y_{ny41}^{41}$

GC	Reference loci read-depth	Test loci read-depth
42%	$x_1^{42}, x_2^{42}, \ldots, x_{nx42}^{42}$	$y_1^{42}, y_2^{42}, \ldots, y_{ny42}^{42}$
...	...	...

[0065] It is appreciated by the ordinarily skilled artisan that subgroup creation may involve encompassing a range of appropriate GC-content and/or a subset of loci that are defined by a given GC-content and/or GC-content range. Accordingly, the % GC content given in the non-limiting example of Table 1 are to be considered "about" or "approximate", allowing for some slight variation (e.g., 1-2%). Thus, for example, a % GC content of "40%" is intended to refer to "about 40%" or "approximately 40%", such that, for example, "39%-41%" GC-content loci may also be encompassed if deemed appropriate.

[0066] Hence, when referring to a particular GC-content it is understood that the reference and test loci subgroups may comprise of any number of loci related to a particular % GC content and/or range.

[0067] Subsequently, for each GC-content subgroup, a representative read-depth is calculated. A number of methods may be utilized to choose this such as, but not limited to, the mean, median or mode of each set. Thus, two vectors of representative read-depth are created where one corresponds to the reference loci and the other to the test loci (e.g., Xm, Ym). In one embodiment, the two vectors may be tested against each other to identify significant differences in read-depth. In another embodiment, the difference of the two vectors may be used to assess if there are significant discrepancies between the test and reference loci. The sample is attributed the score of the test.

[0068] For statistical analysis using a bootstrap approach, the dataset is split into two groups, the test loci and the reference loci. The GC-content of each locus is then calculated. Then the following procedure is performed:
A random locus is selected from the reference loci; its read-depth and GC-content are recorded. Subsequently, a random locus from the test loci is selected, with the only condition being that its' GC-content is similar to that of the reference locus. Its read-depth is recorded. It is appreciated by the ordinarily skilled artisan that GC-content similarity may encompass a range of suitable GC-content. As such, referral to a specific % GC content may be considered as "approximate" or "proximal" or "within a suitable range" (e.g., 1%-2%) encompassing the specific % GC content under investigation. Thus, a reference-test locus pair of similar GC-content is created. The difference of the reference-test pair is recorded, say E1. The loci are then replaced to their respective groups. This process is repeated until a bootstrap sample of the same size as the number of test TACS present is created. A representative read-depth of the bootstrap sample is estimated, say E_mu, and recorded. A number of methods may be utilized to do so, such as but not limited to, the mean, mode or median value of the vector, and/or multiples thereof.

[0069] The process described above is repeated as many times as necessary and a distribution of E_mu is created. The sample is then attributed a score that corresponds to a percentile of this distribution.

[0070] For statistical analysis using a permutation test, the dataset is sorted firstly into two groups, the test-loci and the reference loci. For each group, subsets of groups are created, where loci are categorized according to their GC-content similarity (see columns 2 and 3 of the non-limiting sample Table 2 below). The number of loci present in each test subgroup is also recorded. The loci of the test group are utilized to calculate an estimate of the test-group's read-depth, say Yobs. A representative number from each GC-content subgroup may be selected to do so. Any number of methods may be used to provide a read-depth estimate, such as but not limited to, the mean, median or mode of the chosen loci.

Table 2

GC	Reference loci read-depth	Test loci read-depth	test loci num	Merging of loci
40%	$x_1^{40}, x_2^{40}, \ldots, x_{nx40}^{40}$	$y_1^{40}, y_2^{40}, \ldots, y_{ny40}^{40}$	ny40	$x_1^{40}, \ldots, x_{nx40}^{40}, y_1^{40}, \ldots, y_{ny40}^{40}$
41%	$x_1^{41}, x_2^{41}, \ldots, x_{nx41}^{41}$	$y_1^{41}, y_2^{41}, \ldots, y_{ny41}^{41}$	ny41	$x_1^{41}, \ldots, x_{nx41}^{41}, y_1^{41}, \ldots, y_{ny41}^{41}$
42%	$x_1^{42}, x_2^{42}, \ldots, x_{nx42}^{42}$	$y_1^{42}, y_2^{42}, \ldots, y_{ny42}^{42}$	ny42	$x_1^{42}, \ldots, x_{nx42}^{42}, y_1^{42}, \ldots, y_{ny42}^{42}$
...	...	...	...	...

[0071] A distribution to test Yobs is then built utilizing loci irrespective of their test or reference status as follows. The test and reference loci of each GC-content subgroup (see last column of sample Table 2) are combined to allow for calculation of a new read-depth estimate. From each merged subgroup a number of loci are chosen at random, where this number is upper-bounded by the number of test-loci utilized in the original calculation of Yobs (e.g., for GC content 40%, and in the context of the non-limiting sample Table 2, this number of loci may be in the range [1,ny40]). The new read-depth estimate is calculated from all the chosen loci. The procedure is iterated as many times as necessary in order to build a distribution of observed means. A sample is then attributed a score that corresponds to the position of Yobs in this distribution using a suitable transformation that accounts for the moments of the built distribution. As with the already described methods, it is appreciated that slight variation in % GC content is allowed (e.g., 1%-2%), if deemed appropriate. Hence, reference to a specific GC-content could be taken as "about" or "approximate", so that for example when referring to a 40% GC-content, loci that are "approximately" or "about" 40% (e.g., 39%-41%) may be utilized in the method.

[0072] For statistical analysis using a binomial test of proportions, fragment-sizes aligned to

TACS-specific genomic coordinates are used. It has been shown that fragments of cell free genetic material originating from the placenta tend to be smaller in length when compared to other cell free genetic material (Chan, K.C. (2004) Clin. Chem. 50:88-92). Hence, the statistic of interest is whether the proportion of small-size fragments aligned to a TACS-specific test-region deviates significantly from what is expected when comparing it to the respective proportion of other TACS-specific reference-regions, as this would indicate fetal genetic abnormalities.

[0073] Thus, fragment-sizes are assigned into two groups. Sizes related to the test loci are assigned to one group and fragment-sizes related to the reference loci are assigned to the other group. Subsequently, in each group, fragment sizes are distributed into two subgroups, whereby small-size fragments are assigned into one subgroup and all remaining fragments are designated to the remaining subgroup. The last step computes the proportion of small-sized fragments in each group and uses these quantities in a binomial test of proportions. The score of the test is attributed to the sample under investigation.

[0074] The final result of a sample may be given by combining one or more scores derived from the different statistical methods, non-limiting examples of which are given in Example 4.

[0075] For statistical analysis using segmentation methods, the read-depth and sequence composition of non-overlapping genomic regions of interest of fixed-size is obtained. On the obtained dataset, GC-content read-depth bias alleviation may be performed, but is not limited to, using a local polynomial fitting method in order to estimate the expected read-depth of regions based on their GC content. The expected value, dependent on GC-content, is then used to normalize regions using suitable methods known to those skilled in the art. The normalized dataset is subsequently processed using one or more segmentation-based classification routines. To do so the algorithms process consecutive data points to detect the presence of read-depth deviations which manifest in the form of a "jump/drop" from their surrounding data points. Depending on the segmentation routine used, data points are given a score which is used towards assigning membership into segments of similar performing read-depths. For example, consecutive data points with score values within a suitable range may be classified as one segment, whereas consecutive data points with score values which exceed the set thresholds may be assigned to a different segment. Details of segmentation-based routines are given in Example 11.

[0076] The following aspects and embodiments referring to kits are not according to the invention and are present for illustration purposes only

[0077] In another aspect, the invention provides kits for carrying out the methods of the disclosure. In one embodiment, the kit comprises a container consisting of the pool of TACS and instructions for performing the method. In one embodiment, the TACS are provided in a form that allows them to be bound to a solid support, such as biotinylated TACS. In another embodiment, the TACS are provided together with a solid support, such as biotinylated TACS provided together with streptavidin-coated magnetic beads.

[0078] In one embodiment, the kit comprises a container comprising the pool of TACS and instructions for performing the method, wherein the pool of TACS comprises a plurality of TACS families, wherein each TACS family comprises a plurality of member sequences, wherein each member sequence binds to the same genomic sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest, and further wherein:

1. (i) each member sequence within each TACS family is between 100-500 base pairs in length, each member sequence having a 5' end and a 3' end;
2. (ii) each member sequence binds to the same genomic sequence of interest at least 50 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and
3. (iii) the GC content of the pool of TACS is between 19% and 80%, as determined by calculating the GC content of each member within each family of TACS.

[0079] Furthermore, any of the various features described herein with respect to the design and structure of the TACS can be incorporated into the TACS that are included in the kit.

[0080] In various other embodiments, the kit can comprise additional components for carrying out other aspects of the method. For example, in addition to the pool of TACS, the kit can comprise one or more of the following (i) one or more components for isolating cell free DNA from a biological sample (e.g., as described in Example 1); (ii) one or more components for preparing the sequencing library (e.g., primers, adapters, buffers, linkers, restriction enzymes, ligation enzymes, polymerase enzymes and the like as described in detail in Example 1); (iii) one or more components for amplifying and/or sequencing the enriched library (e.g., as described in Example 3); and/or (iv) software for performing statistical analysis (e.g., as described in Examples 4 and 6-11).

Oncology Uses

[0081] In various embodiments, the TACS-based enrichment method of the disclosure can be used for a variety of purposes in the oncology field. As described in detail in Examples 6-8, the method allows for detection of tumor biomarkers in biological samples. Accordingly, in another aspect, the invention pertains to a method of detecting a tumor biomarker in a DNA sample from a subject having or suspected of having a tumor, the method comprising:

1. (a) preparing a sequencing library from the DNA sample;
2. (b) hybridizing the sequencing library to a pool of double-stranded TArget Capture Sequences (TACS), wherein the pool of TACS comprises a plurality of TACS families each directed to a different tumor biomarker sequence of interest, wherein each TACS family comprises a plurality of member sequences, wherein each member sequence

binds to the same tumor biomarker sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the tumor biomarker sequence of interest, and further wherein:

1. (i) each member sequence within each TACS family is between 150-260 base pairs in length, each member sequence having a 5' end and a 3' end;
2. (ii) each member sequence binds to the same tumor biomarker sequence of interest at least 50 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and
3. (iii) the GC content of the pool of TACS is between 19% and 80%, as determined by calculating the GC content of each member within each family of TACS;

3. (c) isolating members of the sequencing library that bind to the pool of TACS to obtain an enriched library;
4. (d) amplifying and sequencing the enriched library; and
5. (e) performing statistical analysis on the enriched library sequences to thereby detect a tumor biomarker in the DNA sample, wherein the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by 5 to 10 base pairs.

[0082] In one embodiment, the DNA sample comprises cell free tumor DNA (cftDNA). In one embodiment, the DNA sample is a plasma, or urine, or cerebrospinal fluid, or sputum, or ascites, or pleural effusion sample from subject having or suspected of having a tumor (e.g., a liquid biopsy). In another embodiment, the DNA sample is from a tissue sample from a subject having or suspected of having a tumor.

[0083] The method can be applied to the analysis of essentially any known tumor biomarker. An extensive catalogue of known cancer-associated mutations is known in the art, referred to as COSMIC (Catalogue of Somatic Mutations in Cancer), described in, for example, Forbes, S.A. et al. (2016) Curr. Protocol Hum. Genetic 91:10.11.1-10.11.37; Forbes, S.A. et al. (2017) Nucl. Acids Res. 45:D777-D783; and Prior et al. (2012) Cancer Res. 72:2457-2467. The COSMIC database is publically available at www.cancer.sanger.ac.uk. The database includes oncogenes that have been associated with cancers, any of which can be analyzed using the method of the disclosure. In addition to the COSMIC catalogue, other compilations of tumor biomarker mutations have been described in the art, such as the ENCODE Project, which describes mutations in the regulatory sites of oncogenes (see e.g., Shar, N.A. et al. (2016) Mol. Canc. 15:76).

[0084] For detection of tumor biomarkers TACS are designed based on the design criteria described herein and the known sequences of tumor biomarker genes and genetic mutations therein associated with cancer. In one embodiment, a plurality of TACS families used in the method bind to a plurality of tumor biomarker sequences of interest selected from the group comprising of ABL, AKT, AKT1, ALK, APC, AR, ARAF, ATM, BAP1, BARD1, BCL, BMPR1A,

BRAF, BRCA, BRCA1, BRCA2, BRIP1, CDH1, CDKN, CHEK2, CTNNB1, DDB2, DDR2, DICER1, EGFR, EPCAM, ErbB, ErcC, ESR1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FBXW7, FGFR, FLT, FLT3, FOXA1, FOXL2, GATA3, GNA11, GNAQ, GNAS, GREM1, HOX, HOXB13, HRAS, IDH1, JAK, JAK2, KEAP1, KIT, KRAS, MAP2Ks, MAP3Ks, MET, MLH1, MPL, MRE11A, MSH2, MSH6, MTOR, MUTYH, NBN, NPM1, NRAS, NTRK1, PALB2, PDGFRs, PI3KCs, PMS2, POLD1, POLE, POLH, PTEN, RAD50, RAD51C, RAD51D, RAF1, RB1, RET, RUNX1, SLX4, SMAD, SMAD4, SMARCA4, SPOP, STAT, STK11, TP53, VHL, XPA and XPC, and combinations thereof.

[0085] In one embodiment, the plurality of TACS families used in the method bind to a plurality of tumor biomarker sequences of interest selected from the group consisting of, but not limited to, EGFR_6240, KRAS_521, EGFR_6225, NRAS_578, NRAS_580, PIK3CA_763, EGFR_13553, EGFR_18430, BRAF_476, KIT_1314, NRAS_584, EGFR_12378, and combinations thereof.

[0086] Representative, exemplary and non-limiting examples of chromosomal start and stop positions for amplifying TACS that bind to exemplary, non-limiting tumor biomarker genes are shown in Figure 17, for NRAS on chromosome 1, for PI3KCA on chromosome 3, for EGFR on chromosome 7 and for KRAS on chromosome 12. Alternative suitable chromosomal start and stop positions, for these oncogenes and/or for other oncogenes, for amplifying TACS are readily identifiable by one of ordinary skill in the art based on the teachings herein.

[0087] In one embodiment of the method, following sequencing of the library preparation and enrichment for the sequences of interest through TACS hybridization, the subsequent step of amplifying the enriched library is performed in the presence of blocking sequences that inhibit amplification of wild-type sequences. Thus, amplification is biased toward amplification of the mutant tumor biomarker sequences.

[0088] The pool of TACS and families of TACS used in the method of detecting tumor biomarkers can include any of the design features described herein with respect to the design of the TACS. For example, in various embodiments, each TACS family comprises at least 2, at least 3, at least 4 or at least 5 different member sequences. In one embodiment, each TACS family comprises 4 different member sequences. In various embodiments, the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by at least 5 base pairs, or at least 10 base pairs, or by 5-10 base pairs. In various embodiments, the pool of TACS comprises at least 5, or at least 10 or at least 50 or at least 100 different TACS families, or more.

[0089] Suitable statistical analysis approaches for use with oncology samples and detection of tumor biomarkers are described further in Examples 6-8.

[0090] The method for detecting tumor biomarkers can be used in a variety of different clinical circumstances in the oncology field. For example, the method can be used for making an initial

cancer diagnosis in a subject suspected of having cancer. Accordingly in one embodiment, the method further comprises making a diagnosis of the subject based on detection of at least one tumor biomarker sequence.

[0091] Additionally, the method can be used to select an appropriate treatment regimen for a patient diagnosed with cancer, wherein the treatment regimen is designed to be effective against a tumor having the tumor biomarkers detected in the patient's tumor (i.e., known in the art as personalized medicine). Accordingly, in another embodiment, the method further comprises selecting a therapeutic regimen for the subject based on detection of at least one tumor biomarker sequence.

[0092] Still further, the method can be used to monitor the efficacy of a therapeutic regiment, wherein changes in tumor biomarker detection are used as an indicator of treatment efficacy. Accordingly, in another embodiment, the method further comprises monitoring treatment efficacy of a therapeutic regimen in the subject based on detection of at least one tumor biomarker sequence.

Parental Carrier Status and Fetal Risk of Inheritance of Genetic Conditions

[0093] In another aspect, the methods of the disclosure can be used to determine parental carrier status of inheritable genetic abnormalities associated with genetic conditions (e.g., maternal carrier status and, if necessary based on the maternal status, also paternal carrier status), and from this information the fetal risk of inheriting the genetic condition can be determined. An exemplification of this method is described in Example 10. Accordingly, in another aspect, the invention pertains to a method of determining fetal risk of inheriting a genetic condition, the method comprising:

1. (a) preparing a sequencing library from a sample comprising maternal and fetal DNA;
2. (b) hybridizing the sequencing library to a pool of double-stranded TArget Capture Sequences (TACS), wherein the pool of TACS comprises a plurality of TACS families directed to variant allele loci of interest associated with different genetic conditions, wherein each TACS family comprises a plurality of member sequences, wherein each member sequence binds to the same locus of interest but has different start and/or stop positions with respect to a reference coordinate system for the locus of interest, and further wherein:
    1. (i) each member sequence within each TACS family is between 150-260 base pairs in length, each member sequence having a 5' end and a 3' end;
    2. (ii) each member sequence binds to the same locus of interest at least 50 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and
    3. (iii) the GC content of the pool of TACS is between 19% and 80%, as determined by calculating the GC content of each member within each family of TACS;

3. (c) isolating members of the sequencing library that bind to the pool of TACS to obtain an enriched library;
4. (d) amplifying and sequencing the enriched library;
5. (e) performing statistical analysis on the enriched library sequences to thereby determine maternal carrier status at the loci of interest associated with different genetic conditions, wherein for a sample with a positive maternal carrier status, the method further comprises:
6. (f) obtaining a paternal DNA sample and performing steps (a)-(e) on the paternal DNA sample to determine paternal carrier status at those loci for which there is a positive maternal carrier status; and
7. (g) determining fetal risk of inheriting a genetic condition based on maternal carrier status and, when (f) is performed, paternal carrier status, wherein the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by 5 to 10 base pairs.

[0094] In one embodiment, the sample is a maternal plasma sample.

[0095] In one embodiment, each member sequence within each family of TACS is at least 160 base pairs in length.

[0096] The pool of TACS and families of TACS used in the method of carrier determination and fetal inheritance risk can include any of the design features described herein with respect to the design of the TACS. For example, in various embodiments, each TACS family comprises at least 2, at least 3, at least 4 or at least 5 different member sequences. In one embodiment, each TACS family comprises 4 different member sequences. In various embodiments, the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by 5-10 5-10 base pairs. In various embodiments, the pool of TACS comprises at least 5, or at least 10 or at least 50 or at least 100 different TACS families or more.

[0097] The method of carrier determination and fetal inheritance risk can be combined with detecting chromosomal and structural abnormalities in the fetal DNA, as described in Examples 1-4, in the same sample containing the maternal and fetal DNA (e.g., the maternal plasma sample). That is, maternal carrier determination and detection of fetal chromosomal abnormalities can be assessed simultaneously using the same sample (e.g., maternal plasma sample) through the inclusion of the appropriate TACS in the pool of TACS used in the method. Accordingly, in one embodiment of the method, the pool of TACS further comprises sequences that bind to chromosomes of interest for detecting fetal chromosomal abnormalities and step (e) further comprises performing statistical analysis on the enriched library sequences to thereby determine fetal risk of a chromosomal abnormality at the chromosome of interest. In one embodiment, the chromosomal abnormality is an aneuploidy, such as a trisomy or a

monosomy. Other types of chromosomal abnormalities that can be detected are described herein. In one embodiment, the chromosomes of interest include chromosomes 13, 18, 21, X and Y.

[0098] For determining parental carrier status, TACS are designed to bind to variant allele loci of interest that are associated with inheritable genetic conditions. In one embodiment, the sample (e.g., maternal plasma sample) is screened to determine maternal carrier status for a plurality of variant alleles, wherein each family of TACS binds to a variant allele locus associated with a genetic condition. In one embodiment the variant allele loci of interest are associated with genetic conditions selected from the group consisting of, but not limited to, Achondroplasia, Alpha-1 Antitrypsin Deficiency, Antiphospholipid Syndrome, Autism, Autosomal Dominant Polycystic Kidney Disease, Autosomal Recessive Polycystic Kidney Disease, Inheritable Breast Cancer Gene, Charcot-Marie-Tooth, Inheritable Colon Cancer Gene, Crohn's Disease, Cystic Fibrosis, Dercum Disease, Duane Syndrome, Duchenne Muscular Dystrophy, Factor V Leiden Thrombophilia, Familial Hypercholesterolemia, Familial Mediterranean Fever, Fragile X Syndrome, Gaucher Disease, Hemochromatosis, Hemophilia, Holoprosencephaly, Huntington's Disease, Marfan Syndrome, Myotonic Dystrophy, Neurofibromatosis, Noonan Syndrome, Osteogenesis Imperfecta, Phenylketonuria, Poland Anomaly, Porphyria, Prostate Cancer, Retinitis Pigmentosa, Severe Combined Immunodeficiency (SCID), Sickle Cell Disease, Spinal Muscular Atrophy, Tay-Sachs, Thalassemia, WAGR Syndrome, Wilson Disease, and combinations thereof.

[0099] For samples in which the mother has been determined to be a carrier of a variant allele associated with an inheritable genetic condition (positive maternal carrier status), a sample of paternal DNA can also be assessed using the method to thereby determine the parental carrier status, thus allowing for calculation of the fetal risk of inheritance of the genetic condition. Accordingly, in one embodiment, the method further comprises, for a sample with a positive maternal carrier status, obtaining a paternal DNA sample and performing steps (a)-(e) of the above-described method on the paternal DNA sample to determine paternal carrier status, to thereby compute a fetal risk score for inheriting the genetic condition.

[0100] A non-limiting example of computation of a fetal risk score is described in Example 10, in which both the maternal sample and the paternal sample are carriers for a recessive disease-associated allele (heterozygous for the recessive disease-associated allele) and thus the fetus is calculated to have a 25% chance of inheriting a homozygous recessive disease-associated genotype. Alternative fetal risk scores based on the maternal and/or paternal carrier status and the recessiveness or dominance of the disease-associated allele can readily be calculated by the ordinarily skilled artisan using Mendelian Genetics reasoning well established in the art.

Analysis of Fetal/Embryonic DNA Samples

[0101] In another aspect, the methods of the disclosure can be used in the analysis of fetal or

embryonic DNA samples, e.g., for the presence of genetic abnormalities, for example for purposes of IVF Pre-implantation Genetic Screening (PGS) and Diagnosis (PGD). The methods can be used with samples from a single or only a few fetal or embryonic cells. As used herein "a few" fetal or embryonic cells refers to 10 fetal or embryonic cells or less. Accordingly, the methods allow for analysis of very small amounts of fetal or embryonic DNA. The fetal/embryonic DNA sample contains predominantly or only fetal/embryonic DNA, as described above in the subsection on sample preparation. An exemplification of use of the method with samples from 3-day and 5-day biopsy embryos is described in Example 11. Accordingly, in another aspect, the invention pertains to a method of testing for risk of a genetic abnormality in a DNA sample comprising predominantly fetal or embryonic DNA and comprising genomic sequences of interest, the method comprising:

1. (a) preparing a sequencing library from the DNA sample comprising predominantly fetal or embryonic DNA;
2. (b) hybridizing the sequencing library to a pool of double-stranded TArget Capture Sequences (TACS), wherein the pool of TACS comprises a plurality of TACS families directed to different genomic sequences of interest, wherein each TACS family comprises a plurality of member sequences, wherein each member sequence binds to the same genomic sequence of interest but has different start and/or stop positions with respect to a reference coordinate system for the genomic sequence of interest, and further wherein:
    1. (i) each member sequence within each TACS family is between 150-260 base pairs in length, each member sequence having a 5' end and a 3' end;
    2. (ii) each member sequence binds to the same genomic sequence of interest at least 50 base pairs away, on both the 5' end and the 3' end, from regions harboring Copy Number Variations (CNVs), Segmental duplications or repetitive DNA elements; and
    3. (iii) the GC content of the pool of TACS is between 19% and 80%, as determined by calculating the GC content of each member within each family of TACS;
3. (c) isolating members of the sequencing library that bind to the pool of TACS to obtain an enriched library;
4. (d) amplifying and sequencing the enriched library; and
5. (e) performing statistical analysis on the enriched library sequences to thereby determine risk of a genetic abnormality in the DNA sample, wherein the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by 5 to 10 base pairs.

[0102] In one embodiment, the DNA sample is from a pre-implantation embryo (e.g., a day-3 or day-5 IVF pre-implantation embryo). In another embodiment, the DNA sample is from intact trophoblasts collected from a maternal Papanicolaou smear (Jain, C.V. et al (2016) Science Translational Medicine 8(363):363re4-363re4).

[0103] The method can be used to assess for chromosomal and structural abnormalities, as well as point mutations, in the fetal DNA across the entire human genome in a single sample, through the use of TACS families that encompass the entire human genome. Accordingly, in one embodiment, the plurality of TACS families comprises members that bind to chromosomes 1-22, X and Y of the human genome.

[0104] In one embodiment, each member sequence within each family of TACS is at least 160 base pairs in length.

[0105] The pool of TACS and families of TACS used in the method of analyzing fetal DNA can include any of the design features described herein with respect to the design of the TACS. For example, in various embodiments, each TACS family comprises at least 2, at least 3, at least 4 or at least 5 different member sequences. In one embodiment, each TACS family comprises 4 different member sequences. In various embodiments, the start and/or stop positions for the member sequences within a TACS family, with respect to a reference coordinate system for the genomic sequence of interest, are staggered by by 5-10 base pairs. In various embodiments, the pool of TACS comprises at least 5, or at least 10 or at least 50 or at least 100 different TACS families or more.

[0106] Statistical analysis approaches suitable for applying to the analysis of the fetal DNA samples are described in detail in Example 11. In one embodiment, the statistical analysis comprises a segmentation algorithm. In one embodiment, the segmentation algorithm is selected from the group consisting of likelihood-based segmentation, segmentation using small overlapping windows, segmentation using parallel pairwise testing, and combinations thereof. In one embodiment, the statistical analysis comprises a score-based classification system.

Fragment-Based Analysis

[0107] In another aspect, the invention pertains to fragment based analysis of samples, described further in Example 9. There is evidence from the literature that specific types of cancer can be characterized by and/or associated with fragments in the plasma having a smaller size than the expected size of fragments originating from healthy tissues (Jiang et al, (2015), Proceedings of the National Academy of Sciences, 112(11), ppE1317-E1325). The same hypothesis holds true for fragments originating from the placenta/fetus. Specifically, placenta derived fragments are generally of smaller size when compared to fragments originating from maternal tissues/cells. Accordingly, a fragment size-based test was developed and assessed, demonstrating its ability to identify samples harboring chromosomal abnormalities.

[0108] Thus, the fragments-based detection may be used to detect abnormalities in mixed samples with low signal-to-noise ratio (e.g., as is the case in detection of cancer).

[0109] Accordingly, in one embodiment, a fragments-based test is utilized to detect the

presence of somatic copy number aberrations in a sample from a patient suspected of having cancer. For example, a binomial test of proportions, as described Example 4, can be used for the detection of increased presence of nucleic acid material originating from non-healthy tissue (e.g., tumor tissue) based on fragment size. In particular, under the null hypothesis that the distribution of fragment sizes originating from both healthy and cancerous cells is the same, a binomial test for proportions (as described in Example 4) using continuity correction can be utilized to quantify any evidence against it.

EXAMPLES

Example 1: Maternal Sample Collection and Library Preparation

[0110] The general methodology for the TACS-based multiplexed parallel analysis approach for genetic assessment is shown schematically in Figure 1. In this example, methods for collecting and processing a maternal plasma sample (containing maternal and fetal DNA), followed by sequencing library preparation for use in the methodology of Figure 1 are described.

Sample collection

[0111] Plasma samples were obtained anonymously from pregnant women after the 10^{th} week of gestation. Protocols used for collecting samples for our study were approved by the Cyprus National Bioethics Committee, and informed consent was obtained from all participants.

Sample extraction

[0112] Cell Free DNA was extracted from 2-4ml plasma from each individual using a manual or automated extraction method suitable for cell free DNA isolation such as for example, but not limited to, Qiasymphony protocol suitable for cell free fetal DNA isolation (Qiagen) (Koumbaris, G. et al. (2015) Clinical chemistry, 62(6), pp.848-855).

Sequencing library preparation

[0113] Extracted DNA from maternal plasma samples was used for sequencing library construction. Standard library preparation methods were used with the following modifications. A negative control extraction library was prepared separately to monitor any contamination introduced during the experiment. During this step, 5' and 3' overhangs were filled-in, by

adding 12 units of T4 polymerase (NEB) while 5' phosphates were attached using 40 units of T4 polynucleotide kinase (NEB) in a 100µl reaction and subsequent incubation at 25°C for 15 minutes and then 12° C for 15 minutes. Reaction products were purified using the MinElute kit (Qiagen). Subsequently, adaptors P5 and P7 (see adaptor preparation) were ligated at 1:10 dilution to both ends of the DNA using 5 units of T4 DNA ligase (NEB) in a 40µl reaction for 20 minutes at room temperature, followed by purification using the MinElute kit (Qiagen). Nicks were removed in a fill-in reaction with 16 units of Bst polymerase (NEB) in a 40 µl reaction with subsequent incubation at 65 °C for 25 minutes and then 12 °C for 20 minutes. Products were purified using the MinElute kit (Qiagen). Library amplification was performed using a Fusion polymerase (Herculase II Fusion DNA polymerase (Agilent Technologies) or Pfusion High Fidelity Polymerase (NEB)) in 50 µl reactions and with the following cycling conditions, 95°C for 3 minutes; followed by 10 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds and finally 72°C for 3 minutes (Koumbaris, G. et al. (2015) Clinical chemistry, 62(6), pp.848-855). The final library products were purified using the MinElute Purification Kit (Qiagen) and measured by spectrophotometry.

Adaptor preparation

[0114] Hybridization mixtures for adapter P5 and P7 were prepared separately and incubated for 10 seconds at 95° C followed by a ramp from 95° C to 12° C at a rate of 0.1° C /second. P5 and P7 reactions were combined to obtain a ready-to-use adapter mix (100 µM of each adapter). Hybridization mixtures were prepared as follows: P5 reaction mixture contained adaptor P5_F (500 µM) at a final concentration of 200 µM, adaptor P5+P7_R (500 µM) at a final concentration of 200 µM with 1X oligo hybridization buffer. In addition, P7 reaction mixture contained adaptor P7_F (500 µM) at a final concentration of 200 µM, adapter P5+P7_R(500 µM) at a final concentration of 200 µM with 1X oligo hybridization buffer (Koumbaris, G. et al. (2015) Clinical chemistry, 62(6), pp.848-855.). Sequences were as follows, wherein * = a phosphorothioate bond (PTO) (Integrated DNA Technologies):

adaptor P5_F:

A*C*A*C*TCTTTCCCTACACGACGCTCTTCCG*A*T*C*T (SEQ ID NO: XX)

adaptor P7_F:

G*T*G*A*CTGGAGTTCAGACGTGTGCTCTTCCG*A*T*C*T (SEQ ID NO: YY),

adaptor_P5+P7_R:

A*G*A*T*CGGAA*G*A*G*C (SEQ ID NO: ZZ)

Example 2: TArget Capture Sequences (TACS) Design and Preparation

[0115] This example describes preparation of custom TACS for the detection of whole or partial chromosomal abnormalities for chromosomes 13, 18, 21, X, Y or any other chromosome, as well as other genetic abnormalities, such as but not limited to, microdeletion/microduplication syndromes, translocations, inversions, insertions, and other point or small size mutations. The genomic target-loci used for TACS design were selected based on their GC content and their distance from repetitive elements (minimum 50 bp away). TACS size can be variable. In one embodiment of the method the TACS range from 100-500 bp in size and are generated through a PCR-based approach as described below. The TACS were prepared by simplex polymerase chain reaction using standard Taq polymerase, primers designed to amplify the target-loci, and normal DNA used as template. The chromosomal regions used to design primers to amplify suitable loci on chromosomes 13, 18, 21 and X, to thereby prepare the pool of TACS for analysis of chromosomes 13, 18, 21 and X, are shown in Figure 2.

[0116] All custom TACS were generated using the following cycling conditions: 95°C for 3 minutes; 40 cycles at 95°C for 15 seconds, 60°C for 15 seconds, 72°C for 12 seconds; and 72°C for 12 seconds, followed by verification via agarose gel electrophoresis and purification using standard PCR clean up kits such as the Qiaquick PCR Purification Kit (Qiagen) or the NucleoSpin 96 PCR clean-up (Mackerey Nagel) or the Agencourt AMPure XP for PCR Purification (Beckman Coulter). Concentration was measured by Nanodrop (Thermo Scientific).

Example 3: TACS Hybridization and Amplification

[0117] This example describes the steps schematically illustrated in Figure 1 of target capture by hybridization using TACS, followed by quantitation of captured sequences by Next Generation Sequencing (NGS).

TACS Biotinylation

[0118] TACS were prepared for hybridization, as previously described (Koumbaris, G. et al. (2015) Clinical chemistry, 62(6), pp.848-855), starting with blunt ending with the Quick Blunting Kit (NEB) and incubation at room temperature for 30 minutes. Reaction products were subsequently purified using the MinElute kit (Qiagen) and were ligated with a biotin adaptor using the Quick Ligation Kit (NEB) in a 40µl reaction at RT for 15 minutes. The reaction products were purified with the MinElute kit (Qiagen) and were denatured into single stranded DNA prior to immobilization on streptavidin coated magnetic beads (Invitrogen).

TACS Hybridization

[0119] Amplified libraries were mixed with blocking oligos (Koumbaris, G. et al. (2105) Clinical

chemistry, 62(6), pp.848-855) (200 µM), 5µg of Cot-1 DNA (Invitrogen), 50 µg of Salmon Sperm DNA (Invitrogen), Agilent hybridization buffer 2x, Agilent blocking agent 10X, and were heated at 95°C for 3 minutes to denature the DNA strands. Denaturation was followed by 30 minute incubation at 37°C to block repetitive elements and adaptor sequences. The resulting mixture was then added to the biotinylated TACS. All samples were incubated in a rotating incubator for 12- 48 hours at 66°C. After incubation, the beads were washed as described previously and DNA was eluted by heating (Koumbaris, G. et al. (2105) Clinical chemistry, 62(6), pp.848-855). Eluted products were amplified using outer-bound adaptor primers. Enriched amplified products were pooled equimolarly and sequenced on a suitable platform.

[0120] If appropriate, amplification may be biased toward amplification of specific/desired sequences. In one embodiment of the method, this is performed when amplification is performed in the presence of sequences that hybridize to the undesired sequence of interest, and as such block the action of the polymerase enzyme during the process. Hence, the action of the amplification enzyme is directed toward the sequence of interest during the process.

Example 4: Bioinformatics Sample Analysis

[0121] This example describes representative statistical analysis approaches for use in the methodology illustrated in Figure 1 ("analysis pipeline" in Figure 1).

Human Genome Alignment

[0122] For each sample, the bioinformatic pipeline routine described below was applied in order to align the sample's sequenced DNA fragments to the human reference genome. Targeted paired-end read fragments obtained from NGS results were processed to remove adaptor sequences and poor quality reads (Q-score<25) using the cutadapt software (Martin, M. et al. (2011) EMB.netJournal 17.1). The quality of the raw and/or processed reads as well as any descriptive statistics which aid in the assessment of quality check of the sample's sequencing output were obtained using the FastQC software (Babraham Institute (2015) *FastQC*) and/or other custom-built software. Processed reads which were at least 25 bases long were aligned to the human reference genome built hg19 (UCSC Genome Bioinformatics) using the Burrows-Wheel Alignment algorithm (Li, H. and Durbin, R. (2009) Bioinformatics 25:1754-1760) but other algorithms known to those skilled in the art may be used as well. If relevant, duplicate reads were removed post-alignment. Where applicable, sequencing output pertaining to the same sample but processed on separate sequencing lanes, was merged to a single sequencing output file. The removal of duplicates and merging procedures were performed using the Picard tools software suite (Broad Institute (2015) *Picard*) and/or the Sambamba tools software suite (Tarasov, Artem, et al. "Sambamba: fast processing of NGS alignment formats." Bioinformatics 31.12 (2015): 2032-2034).

[0123] The above software analysis resulted in a final aligned version of a sequenced sample against the human reference genome and all subsequent steps were based on this aligned version. Information in terms of Short Nucleotide Polymorphisms (SNPs) at loci of interest was obtained using bcftools from the SAMtools software suite (Li, H. et al. (2009) Bioinformatics 25:2078-2079) and/or other software known to those skilled in the art. The read-depth per base, at loci of interest, was obtained using the mpileup option of the SAMtools software suite, from here on referred to as the mpileup file. Information pertaining to the size of the aligned fragments was obtained using the view option of the SAMtools software suite, from here on referred to as the fragment-sizes file and/or other software known to those skilled in the art.

[0124] The mpileup file and the fragment-sizes file were processed using custom-build application programming interfaces (APIs) written in the Python and R programming languages (Python Software Foundation (2015) *Python*; The R Foundation (2015) *The R Project for Statistical Computing*). The APIs were used to determine the ploidy state of chromosomes of interest, and/or other genetic abnormalities in regions of interest across the human genome, using a series of steps (collectively henceforth referred to as the "algorithm") and to also collect further descriptive statistics to be used as quality check metrics, such as but not limited to fetal fraction quantification (collectively henceforth referred to as the "QC metrics").The APIs can also be used for the assessment of genetic abnormalities from data generated when applying the described method in cases of multiple gestation pregnancies, as well as other genetic abnormalities such as, but not limited to, microdeletions, microduplications, copy number variations, translocations, inversions, insertions, point mutations and mutational signatures.

QC Metrics

[0125] QC metrics were used to inspect an aligned sample's properties and decide whether the sample was suitable to undergo classification. These metrics were, but are not limited to:

1. (a) The enrichment of a sample. The patterns of enrichment are indicative of whether a sample has had adequate enrichment across loci of interest in a particular sequencing experiment (herein referred to as a "run"). To assess this, various metrics are assessed, non-limiting examples of which are:
    1. (i) overall sample on-target read depth,
    2. (ii) sample on-target sequencing output with respect to total mapped reads,
    3. (iii) individual TACS performance in terms of achieved read-depth,
    4. (iv) kurtosis and skewness of individual TACS enrichment,
    5. (v) kurtosis and skewness moments that arise from all TACS,
    6. (vi) fragment size distribution,
    7. (vii) percentage of duplication,
    8. (viii) percentage of paired reads and,
    9. (ix) percentage of aligned reads,

    if applicable.

[0126] The above checks are also taken into consideration with regards to GC-bias enrichment. Samples that fail to meet one or more of the criteria given above are flagged for further inspection, prior to classification.

[0127] (b) A sample's fetal fraction or fraction of interest. Samples with an estimated fetal fraction, or fraction of interest, that is below a specific threshold are not classified. Furthermore, if applicable the fraction of interest may be calculated using more than one method and concordance of results between estimation methods may be used as an additional QC prior to classification.

The Algorithm

[0128] The algorithm is a collection of data processing, mathematical and statistical model routines arranged as a series of steps. The algorithm's steps aim in deciding the relative ploidy state of a chromosome of interest with respect to all other chromosomes of the sequenced sample and is used for the detection of whole or partial chromosomal abnormalities for chromosomes 13, 18, 21, X, Y or any other chromosome, as well as other genetic abnormalities such as, but not limited to, microdeletion/microduplication syndromes and other point or small size mutations. As such the algorithm can be used, but is not limited to, the detection of whole or partial chromosomal abnormalities for chromosomes 13, 18, 21, X,Y or any other chromosome, as well as other genetic abnormalities such as, but not limited to, microdeletions, microduplications, copy number variations, translocations, inversions, insertions, point mutations and other mutational signatures. The algorithm carries out, but is not limited to, two types of assessments, one pertaining to the read-depth information of each sample and the other to the distribution of fragment-sizes, across TACS-specific regions. One or more statistical tests may be associated with each type of assessment, non-limiting examples of which are given in the statistical methods described herein.

[0129] In the case of read-depth associated tests, the algorithm compares sequentially the read-depth of loci from each chromosome of interest (herein referred to as the test chromosome) against the read-depth of all other loci (herein referred to as the reference loci) to classify its ploidy state. For each sample, these steps were, but are not limited to:

1. (a) Removal of inadequately sequenced loci. The read-depth of each locus was retrieved. Loci that have not achieved a minimum number of reads, were considered as inadequately enriched and were removed prior to subsequent steps.
2. (b) Genetic (GC-content) bias alleviation. The sequencing procedure may introduce discrepancies in read-depth across the loci of interest depending on their GC content. To account for such bias, a novel sequence-matching approach that increases both sensitivity and specificity to detect chromosomal aneuploidies was employed. The GC content of each locus on the test chromosome was identified and similar genetic loci were grouped together to form genetically matched groups. The procedure was

repeated for the reference loci. Then, genetically matched groups from the test chromosome were conditionally paired with their genetically matched group counterparts on the reference chromosome(s). The groups may have any number of members.

[0130] The conditionally matched groups were then used to assess the ploidy status of test chromosomes.

[0131] (c) Genetic abnormality determination. Ploidy status determination, or other genetic abnormalities of interest such as but not limited to microdeletions, microduplications, copy number variations, translocations, inversions, insertions, point mutations and other mutational signatures was achieved using a single statistical method and/or a weighted score approach on the result from the following, but not limited to, statistical methods:

Statistical Method 1: The differences in read-depth of the conditionally paired groups were tested for statistical significance using the t-test formula:

$$t = \frac{\hat{x} - \mu}{s/\sqrt{n}}$$

where t is the result of the t-test, $\hat{x}$is the average of the differences of the conditionally paired groups, μ is the expected read-depth and is set to a value that represents insignificant read-depth differences between the two groups, s the standard deviation of the differences of the conditionally paired groups and n the length of the vector of the conditionally paired differences. The magnitude of the t-score was then used to identify evidence, if any, against the null hypothesis of same ploidy between reference and test chromosomes. Specifically, t>=c1 (where c1 is a predefined threshold belonging to the set of all positive numbers) shows evidence against the null hypothesis of no difference.

Statistical Method 2: Bivariate nonparametric bootstrap. The bootstrap method depends on the relationship between the random variables X (read-depth of reference loci) and Y (read-depth of test loci). Here, the read depth of baits on the reference group (random variable denoted by X) were treated as the independent covariate. The first step of the iterative procedure involved random sampling with replacement (bootstrapping) of the read-depths of loci on the reference chromosomes, i.e., (x1,g1),...,(xn,gn), where the parameter g is known and denotes the GC-content of the chosen bait. Then, for each randomly selected reference bait (xi,gi), a corresponding read depth was generated for a genetically matched locus i.e., (y1,g1),..., (yn,gn). Thus, the bivariate data (x1,y1), (x2,y2),...,(xn,yn) was arrived at, which was conditionally matched on their GC-content (parameter gi). The differences between the read depths of the genetically matched bootstrapped values xi and yi were used to compute the statistic of interest in each iteration. In one embodiment this statistical measure can be, but is not limited to, the mode, mean or median of the recorded differences, and/or multiples thereof. The procedure was repeated as necessary to build up the distribution of the statistic of interest from these differences. The sample was assigned a score that corresponds to a specific percentile of the built distribution (e.g. 5^{th} percentile). Under the null hypothesis the ploidy between chromosomes in the reference and test groups is not different. As such, samples

whose score for a particular chromosome, was greater than a predefined threshold, say c2, were classified as statistically unlikely to have the same ploidy. Other statistical measures may be employed.

Statistical Method 3: Stratified permutation test. The statistic of interest is the read-depth estimate of the test chromosome, denoted by $\hat{Y}_{obs}$, which is calculated using all loci of the test chromosome's genetically matched groups as follows:

$$\hat{Y}_{obs} = \frac{\sum_{j=1}^{j=T} \sum_{i=1}^{i=Nj} y_{ij}}{\sum_{j=1}^{j=T} Nj}$$

where y_{ij} is the read-depth of locus *i* part of the genetically matched group *j* (i.e. loci belonging to a specific group based on their GC-content), *Nj* is the number of test loci part of the genetically matched group j and T is the number of genetically matched groups.

[0132] Subsequently, a null distribution to test $\hat{Y}_{obs}$ was built. To do so, for each group j, the test and reference loci were combined (exchangeability under the null hypothesis), and each group *j* was sampled randomly up to *Nj* times without replacement (stratified permutation). This created a vector of values, say yi, and from this the vector's average value, say $\acute{Y}_i$,was calculated. The procedure was repeated as necessary to build the null distribution. Finally $\hat{Y}_{obs}$, was studentised against the null distribution using the formula:

$$Z_{Yobs} = \frac{\hat{Y_{obs}} - \hat{Y}}{\sigma_Y}$$

wherefand σ_Y are the first and square root of the second moment of all permuted$\acute{y}_i$ statistic values. Samples whoseZ_{Yobs} was greater than a predefined threshold, say c3, were statistically less likely to have the same ploidy in the reference and test groups.

[0133] In the case of fragment-size associated tests, the algorithm computes the proportion of small-size fragments found in test-loci and compares it with the respective proportion in reference-loci as described in Statistical Method 4 below.

[0134] Statistical Method 4: Fragment Size Proportions. For each sample the number and size of fragments aligned onto the human reference genome at the corresponding TACS coordinates, is extracted. The data is subsequently filtered so as to remove fragment-sizes considered statistical outliers using the median outlier detection method. Specifically, outliers are defined as those fragments whose size is above or below the thresholds, F_{thr}, set by equation :

$$F_{thr} = F_{median} \pm (X \times IQR)$$

where F_{median} is the median fragment-size of all fragments of a sample, X is a variable that can take values from the set of R +, and IQR is the interquartile range of fragment sizes. Thereafter, a binomial test of proportions is carried out to test for supporting evidence against the null hypothesis, H0, where this is defined as:

H0: The proportion of small fragments of the test-region is not different from the proportion of small-fragments of the reference region.

[0135] In various embodiments of the invention, small fragments are defined as those fragments whose size is less than or equal to a subset of Z +, that is upper-bounded by 160bp. If the set of all TACS are defined as T, then the test region can be any proper subset S which defines the region under investigation, and the reference region is the relative complement of S in T. For example, in one embodiment of the invention, the set S is defined by all TACS-captured sequences of chromosome 21 and thus the reference set is defined by all TACS-captured fragments on the reference chromosomes, and/or other reference loci

[0136] The alternative hypothesis, H1, is defined as:
H1: The proportion of small fragments of the test-region is not equal to the proportion of test fragments of the reference region.

[0137] As such, and taking into account continuity correction, the following score is computed (Brown et. Al, Harrel):

$$W_{test} = \left(\acute{p} - p_{ref}\right) \Big/ \sqrt{\frac{\acute{p}(1-\acute{p})}{N_{test}}}$$

where

$$\acute{p} = \frac{\left(\acute{F} + 0.5\right)}{\left(N_{test} + 1\right)}$$

$$p_{ref} = \frac{\left(F_{ref} + 0.5\right)}{\left(N_{ref} + 1\right)}$$

[0138] $\acute{F}$ is the number of small-size fragments on the test-region, F_{ref} the number of small size fragments on the reference region, N_{test} the number of all fragments on the test region and N_{ref} the number of all fragments on the reference region.

[0139] For each sample, the algorithm tests sequentially the proportion of fragment sizes of regions under investigation (for example, but not limited to, chromosome 21, chromosome 18, chromosome 13 or other (sub)chromosomal regions of interest) against reference regions; those not under investigation at the time of testing. For each sample a score is assigned for each test. Scores above a set-threshold, say c4, provide evidence against the null hypothesis.

[0140] Weighted Score method 1: In one embodiment of the method, a weighted score was attributed to each sample s, computed as a weighted sum of all statistical methods using the formula:

$$V_s(R,F) = z_1 max\{R_s, F_s\} + (1 - z_1) min\{R_s, F_s\}$$

where R_S is the run-specific corrected score arising from a weighted contribution of each read-depth related statistical method for sample s and is defined as:

$$R_s = \frac{\left(\sum_i w_i S_{is} - \acute{R_r}\right)}{\sigma_r}$$

and $\acute{R}_r$ is the run-specific median value calculated from the vector of all unadjusted read-depth related weighted scores that arise from a single sequencing run, and σ_r is a multiple of the standard deviation of R scores calculated from a reference set of 100 euploid samples. The terms $max\{R_s, F_s\}$and $min\{R_s, F_s\}$ denote the maximum and minimum values of the bracketed set, respectively.

[0141] F_s is the run-specific corrected score arising from the fragment-size related statistical method and is defined as:

$$F_s = \frac{(W_{test} - \acute{R}_f)}{\sigma_f}$$

where W_{test} is as defined earlier, $\acute{R}_f$ is the run specific median calculated from the vector of all unadjusted fragment-related statistical scores that arise from a single sequencing run, and σ_f is a multiple of the standard deviation of F scores calculated from a reference set of 100 euploid samples.

[0142] A unique classification score of less than a predefined value indicates that there is no evidence from the observed data that a sample has a significant risk of aneuploidy.

[0143] Weighted Score method 2: In another embodiment of the method, the weighted score arising from the statistical methods described above was used to assign each sample a unique genetic abnormality risk score using the formula:

$$R(t,c) = \sum_{j=0}^{j=N} w_j \frac{t_j}{c_j}$$

where R is the weighted score result, w_j the weight assigned to method j, t_j the observed score resulting from method j, and c_j the threshold of method j.

[0144] A unique classification score of less than a predefined value indicates that there is no evidence from the observed data that a sample has a significant risk of aneuploidy.

[0145] Since all read depths from baits in the reference group were assumed to be generated from the same population, and in order to have a universal threshold, run-specific adjustments were also employed to alleviate run-specific biases.

[0146] The aforementioned method(s), are also suitable for the detection of other genetic abnormalities, such as but not limited to, subchromosomal abnormalities. A non-limiting example is the contiguous partial loss of chromosomal material leading to a state of microdeletion, or the contiguous partial gain of chromosomal material leading to a state of microduplication. A known genetic locus subject to both such abnormalities is 7q11.23. In one embodiment of statistical method 1, synthetic plasma samples of 5%, 10% and 20% fetal material were tested for increased risk of microdeletion and/or microduplication states for the genetic locus 7q11.23.

[0147] For point mutations various binomial tests are carried out that take into consideration the fetal fraction estimate of the sample, f, the read-depth of the minor allele, r, and the total read-depth of the sequenced base, n. Two frequent, yet non-limiting examples involve assessment of the risk when the genetic abnormality is a recessive point mutation or a dominant point mutation.

[0148] In the non-limiting example of a recessive point mutation the null hypothesis tested is that both the mother and the fetus are heterozygous (minor allele frequency is 0.5) against the alternative in which the fetus is homozygous (minor allele frequency is 0.5-f/2). A small p-value from the corresponding likelihood ratio test would indicate evidence against the null. In the non-limiting example of a dominant point mutation the null hypothesis tested is that the mother and fetus are homozygous at the given position against the alternative in which only the fetus is heterozygous for the given position. A small p-value from the corresponding likelihood ratio test would indicate evidence against the null.

[0149] In addition to the above, fetal sex determination methods were also developed, with non-limiting examples given below. In one embodiment of the invention, fetal sex was assigned to a sample using a Poisson test using the formula:

$$Pr(r_y \leq k) = e^{-\lambda} \sum_{i=0}^{i=k} \frac{\lambda^i}{i!}$$

where

$$\lambda = \frac{fB\mu}{2}$$

and f is the fetal fraction estimate of the sample, B is the number of target sequences on chromosome Y, μ is the read-depth of the sample and k is the sum of reads obtained from all targets B. The null hypothesis of the Poisson test was that the sample is male. A value of $Pr(r_y)$ less than a threshold c_y was considered as enough evidence to reject the null hypothesis, i.e. the sample is not male. If any of the terms for computing $Pr(r_y)$ were unavailable, then the sample's sex was classified as NA (not available).

[0150] In another embodiment of the invention, fetal sex was assigned using the average read-depth of target sequences on chromosome Y. If the average read-depth of the target-sequences was over a predefined threshold, where such threshold may be defined using other sample-specific characteristics such as read-depth and fetal-fraction estimate, the fetal sex was classified as male. If the average read-depth was below such threshold then the sample was classified as female.

Fetal Fraction Estimation/Fraction of Interest Estimation

[0151] Several methods have been developed to estimate fetal fraction that can be applied to singleton and/or to multiple gestation pregnancies. As such, and dependent on the type of pregnancy, the fetal fraction estimate can be obtained from either method or as a weighted estimate from a subset and/or all developed methods. Some non-limiting examples are given

below.

[0152] In one embodiment, a machine learning technique has been developed based on Bayesian inference to compute the posterior distribution of fetal DNA fraction using allelic counts at heterozygous loci in maternal plasma of singleton pregnancies. Three possible informative combinations of maternal/fetal genotypes were utilized within the model to identify those fetal DNA fraction values that get most of the support from the observed data.

[0153] Let *f* denote the fetal DNA fraction. If the mother is heterozygous at a given genomic locus, the fetal genotype can be either heterozygous or homozygous resulting in expected minor allele frequencies at *0.5* and 0.5-*f/2*, respectively. If the mother is homozygous and the fetus is heterozygous then the expected minor allele frequency will be *f/2.* A Markov chain Monte Carlo method (a Metropolis-Hastings algorithm) (The R Foundation (2015) The R Project for Statistical Computing) was used with either a non-informative or an informative prior (i.e. incorporate additional information such as gestational age, maternal weight etc.) to obtain a sequence of random samples from the posterior probability distribution of fetal DNA fraction that is based on a finite mixture model.

[0154] In another embodiment, the fetal fraction estimate is computed only from the fetus-specific minor allele frequency (MAF) cluster, i.e. the cluster formed when the mother is homozygous and the fetus is heterozygous for a given genomic locus. It is assumed that the mean value of the fetal fraction estimate is normally distributed as $N(2\acute{x}, \sigma_{\acute{x}})$, where $\acute{x}$ is the mean of the fetus-specific MAF, and $\sigma_{\acute{x}}$ is the standard deviation of the fetus-specific MAF. The fetal fraction estimate is then obtained from percentiles of the computed distribution, $N(2x, \sigma_{\acute{x}})$.

[0155] For multiple gestation pregnancies, non-limiting examples of which include monozygotic and dizygotic twin pregnancies, triplet pregnancies and various egg and/or sperm donor cases, the fetal fraction can be estimated using information obtained from heterozygous genetic loci whose MAF value is less than a threshold, say M_{thresh}, and derived from potential fetus-specific SNPs. The ordinarily skilled artisan will appreciate that fetus specific SNPs can originate from any fetus, or from any possible combination of the fetuses or from all the fetuses of the gestation. As such, an algorithm that estimates the fetal fraction of the fetus with the smallest contribution to the total fetal content, by taking into account the combinatorial contribution of each fetus to the MAF values that define fetus-specific SNPs, and also allows for inhomogeneous contribution of fetal material to the total fetal content of plasma derived material has been developed. To this effect, a two-step approach is employed by the algorithm.

[0156] In one embodiment of the algorithm, the multiple gestation pregnancy under consideration is a dizygotic twin pregnancy. As a first step, the algorithmic implementation of the model utilizes all informative SNPs and allows for inhomogeneous fetal contribution that can be explained with a fold-difference in fetal fraction estimates of a set threshold, say cf. Specifically, if f1 and f2 represent the fetal fractions of fetus one and fetus two, and f1 <= f2, then the assumption is that f2 <= cf f1, with cf being a positive real number greater than or

equal to 1. Under this assumption, the observed data D, defined as counts of the alternate and reference alleles at informative SNP loci, are believed to be generated from a mixture distribution of three Binomials (defined by parameters, f1/2, f2/2 and (f1+f2)/2), with the posterior distribution p(f1,f2|D) being proportional to the observational model which can be written as p(f1|f2,D) p(f2|D). The posterior distribution p(f1,f2|D) is sampled with an MCMC Metropolis-Hastings algorithm using a uniform prior. The empirical quantile approach is performed on the generated data array to infer the fetal fractions.

[0157] As a second step, the algorithm runs a model-based clustering algorithm (Finite Gaussian mixture modeling fitted via EM algorithm; R-package: mclust) to identify whether there exists a separate outlier SNP cluster which is believed to be centered around f1/2. Existence of such a cluster with a mean invalidating the cf >= f2/f1 assumption , leads to estimation of f1 using only SNPs part of the identified cluster.

[0158] The methods described above are suited to the determination of the fraction of any component of interest part of a mixed sample. As such, the methods are not to be understood as applicable only to the application of fetal fraction estimation and can be applied to the estimation of any component of interest part of a mixed sample.

Example 5: Target Enrichment Using Families of TACS

[0159] In this example, a family of TACS, containing a plurality of members that all bind to the same target sequence of interest, was used for enrichment, compared to use of a single TACS binding to a target sequence of interest. Each member of the family of TACS bound to the same target sequence of interest but had a different start/stop coordinates with respect to a reference coordinate system for that target sequence (e.g., the human reference genome, built hg19). Thus, when aligned to the target sequence, the family of TACS exhibit a staggered binding pattern, as illustrated in Figure 3. Typically, the members of a TACS family were staggered approximately 5-10 base pairs.

[0160] A family of TACS containing four members (i.e., four sequences that bound to the same target sequence but having different start/stop positions such that the binding of the members to the target sequence was staggered) was prepared. Single TACS hybridization was also prepared as a control. The TACS were fixed to a solid support by labelling with biotin and binding to magnetic beads coated with a biotin-binding substance (e.g., streptavidin or avidin) as described in Example 3. The family of TACS and single TACS were then hybridized to a sequence library, bound sequences were eluted and amplified, and these enriched amplified products were then pooled equimolarly and sequenced on a suitable sequencing platform, as described in Example 3.

[0161] The enriched sequences from the family of TACS sample and the single TACS sample were analyzed for read-depth. The results are shown in Figures 4A and 4B. As shown in Figure 4A, target sequences of interest enriched using the family of four TACS (red dots)

exhibited a fold-change in read-depth when compared to control sequences that were subjected to enrichment using only a single TACS (blue dots). Fold-change was assessed by normalizing the read-depth of each locus by the average read-depth of a sample, wherein the average read-depth was calculated from all loci enriched with a single TACS. As shown in Figure 4B, an overall 54.7% average increase in read-depth was observed using the family of four TACS.

[0162] This example demonstrates that use of a family of TACS, as compared to a single TACS, results in significantly improved enrichment of a target sequence of interest resulting in significantly improved read-depth of that sequence.

Example 6: Tumor Biomarker Detection in Reference Material

[0163] In this example, the TACS methodology, illustrated in Figure 1, was used for the detection of tumor biomarkers in certified reference material known to harbour particular genetic mutations that are tumor biomarkers. For detection of the tumor biomarker sequences of interest, families of TACS, as described in Example 5, were used.

[0164] A sample of certified reference material harbouring known tumor-associated genetic mutations was commercially obtained and samples were prepared to simulate tumor loads of 0.1%, 1.0% and 5.0%.

[0165] The samples were subjected to the TACS methodology illustrated in Figure 1 using families of TACS that bound to the following tumor-associated genetic mutations: EGFR_6240, KRAS_521, EGFR_6225, NRAS_578, NRAS_580, PIK3CA_763, EGFR_13553, EGFR_18430.

[0166] Following amplification and sequence of the TACS-enriched products, data analysis was performed as follows. Sequencing products were processed to remove adaptor sequences and poor quality reads. Reads whose length was at least 25 bases long post adaptor-removal were aligned to either:

1. (a) the human reference genome built hg19, or
2. (b) an artificially created genome based on built hg19 which contains only sequences of interest.

[0167] If relevant, duplicate reads were removed post-alignment. Where applicable, sequencing output pertaining to the same sample but processed on separate sequencing lanes was merged to a single sequencing output file. The above software analysis provided a final aligned version of a sequenced sample against the reference genome, defined here as the final BAM file, where information can be extracted from it in terms of Short Nucleotide Polymorphisms (SNPs), Single Nucleotide Variants (SNVs) and other genetic variations with

respect to a reference sequence at loci of interest, read-depth per base and the size of aligned fragments. Various available tools known to those skilled in the art, such as but not limited to bcftools, which is part of the samtools software suite, or varDict can be used to collect SNP information from the final BAM file. Such information concerns the sequence and number of times each SNP present in a sequenced sample was detected and was used to:

1. (a) infer the presence of a genetic mutation, and
2. (b) to estimate the tumor load using the fetal-fraction estimation/fraction of interest estimation method described in Example 4.

[0168] In addition to the detection of the genetic mutation, statistical confidence was ascribed to a detected mutation using the estimated tumor load of the sample and the read-depth of each of the detected variants at a given position using binomial statistics. More than one test may be employed from which one can compute the probability of obtaining the sequenced information, or obtain a 95% confidence interval which describes a range of possible read-depths for the genetic mutation, or whether the obtained proportion of reads which can be ascribed to the genetic mutation is consistent with what would be expected at the given tumor load. A suitable binomial test of proportions is described in Example 4 (in the context of classification of chromosomal abnormalities).

[0169] The results are shown in Figure 5. The line illustrates the expected minor allele frequency (MAF) for each percent (%) tumor load. The bars (x-axis) illustrate the detected MAF (y-axis) for each sample for the indicated genetic mutations. Two technical replicates are shown for the reference material.

[0170] The data demonstrates that the TACS methodology successfully detected the tumor-associated genetic mutations EGFR_6240, KRAS_521, EGFR_6225, NRAS_578, NRAS_580, PIK3CA_763, EGFR_13553 and EGFR_18430 at the expected tumor loads of 1.0% and 5.0%. Mutations EGFR_6240, NRAS_578, PIK3CA_763, EGFR_13553 and EFGR_18430 were also successfully detected at 0.1% tumor load.

[0171] Accordingly, this example demonstrates the successful detection of a large panel of different tumor biomarkers using the TACS methodology at tumor loads as low as 0.1%.

Example 7: Tumor Biomarker Detection in Patient Samples

[0172] In this example, the TACS methodology, illustrated in Figure 1, was used for the detection of tumor biomarkers in tumor tissue and blood plasma samples from untreated cancer patients with confirmed diagnosis. For detection of the tumor biomarker sequences of interest, families of TACS, as described in Example 5, were used.

[0173] Matched pairs of peripheral blood and tumor tissue samples from untreated cancer patients were used to further validate the performance of the TACS methodology for tumor biomarker detection for a patient harbouring mutation PIK3CA E545K (Patient 1) and for a patient harbouring mutation TP53 K139 (Patient 2). The results are shown in Figure 6.

[0174] As shown in Figure 6, application of the TACS methodology to a tissue sample obtained from Patient 1 harbouring mutation PIK3CA E545K (top bars) provided a variant allele frequency (VAF) percentage (i.e., the percentage that the genetic mutation is present instead of the normal allele) of ~62%. Plasma obtained from peripheral blood of Patient 1 was processed according to the method described in Example 1 and provided a 6.05 % VAF. Similarly, application of the TACS methodology to samples obtained from Patient 2 harbouring mutation TP53 K139 (bottom bars) provided a VAF of ~60% for tumor tissue and a VAF of 4.88 % for plasma obtained from a peripheral blood sample.

[0175] Accordingly, this example demonstrates the successful detection of tumor biomarkers in cancer patient samples, in both tumor tissue samples and plasma samples, thereby demonstrating the suitability of the TACS methodology for tissue biopsy and for non-invasive tumor biomarker detection using liquid biopsy.

Example 8: Detection of Mutational Profiles

[0176] Given the ability of the TACS methodology illustrated in Figure 1 to detect a number of somatic single nucleotide variations (SNVs), these can be examined in the context of motifs, also referred to as mutational profiles. Most somatic mutations in tumors can be considered as passengers and may not be associated with pathogenesis if examined individually. Nonetheless, examining the profile of detected mutations as a whole can be useful in determining and/or detecting a pathogenesis-associated mutational profile. Various algorithms have been developed to decompose known mutational motifs operative in many cancer types. Alternatively, other metrics utilizing specific characteristics such as the type of mutations detected in the context of their neighboring bases can be utilized to this effect. The developed algorithms can infer the most likely scenario(s) that explain the observed data. Decomposition of the number and types of known mutational patterns/signatures that have, most likely, generated the observed mutational profile has been achieved using, but not limited to, the Lawson-Hanson non-negative least squares algorithm.

[0177] Figure 7 shows the observed pattern of somatic SNVs for breast cancer using data downloaded from the COSMIC database. The x-axis shows a single base mutation observed in cancer in the context of its neighboring sequences. For example A[C>A]T describes the mutation of Cytosine (C) to Adenine (A) where the upstream sequence is Adenine and the downstream sequence is Thymine. The y-axis shows the frequency of occurrence of this mutation in breast cancer.

[0178] Figure 8 illustrates the results of a simulations study where mutational profiles were randomly generated by sampling a subset of SNVs each time, from data available in the COSMIC database, thereby simulating individuals. The simulated data were then subjected to the decomposition algorithms described above in order to detect the likely underlying mutational motifs. The bars indicate the average estimated frequency of the known mutational breast signatures computed from a data set of 10000 simulations. The developed algorithm shows evidence of detection of the mutational profiles, thereby demonstrating that detection of mutational profiles, or motifs, is possible using the developed algorithms.

Example 9: Fragment Size Based Tests

[0179] There is evidence from the literature that specific types of cancer can be characterized by and/or associated with fragments in the plasma having a smaller size than the expected size of fragments originating from healthy tissues (Jiang et al, (2015), Proceedings of the National Academy of Sciences, 112(11), ppE1317-E1325). Thus, a fragments-size based test can be utilized to detect the presence of somatic copy number variations in individuals suspected of having cancer. To this effect, a binomial test of proportions, as described Example 4, can be used for the detection of increased presence of nucleic acid material originating from non-healthy tissue (e.g., tumor tissue) based on fragment size. In particular, under the null hypothesis that the distribution of fragment sizes originating from both healthy and non-healthy cells (for example, but not limited to cancerous cells) is the same, a binomial test for proportions (as described in Example 4) using continuity correction can be utilized to quantify any evidence against it.

[0180] The same hypothesis holds true for fragments originating from the placenta/fetus. Specifically, placenta derived fragments are generally of smaller size when compared to fragments originating from maternal tissues/cells. Accordingly, assessment of the fragment size-based test was performed using maternal plasma samples (i.e., mixed samples where cell free DNA is of maternal and fetal origin). The size of fragments that have aligned to TACS-enriched regions can be obtained from the aligned data. Subsequently, the proportion of fragments under a specific threshold from a test region is compared respective proportion of fragments from a reference region for evidence against the null hypothesis H0,
H0: The proportion of small fragments of the test-region is not different from the proportion of small-fragments of the reference region.

[0181] Figure 9 shows results when applying the fragment sizes method to the mixed sample containing maternal and fetal DNA. The black dots are individual samples. The x-axis shows the sample index. The y-axis shows the score result of the fragments-based method. A score result greater than the one indicated by the threshold, illustrated as a grey line, indicates a deviation from the expected size of fragments illustrating the presence of aneuploidy. The results demonstrate that an aneuploid sample, having an estimated fetal fraction equal to 2.8%, was correctly identified, illustrating that fragments-based detection may be used to detect abnormalities in mixed samples with low signal-to-noise ratio (e.g., as is the case in

detection of cancer).

[0182] Accordingly, this example demonstrates the successful ability of the fragments-based detection method in detecting genetic abnormalities in mixed samples with low signal-to-noise ratios, thereby demonstrating the suitability of the fragments-based test for analysis of either cancer samples for oncology purposes or maternal samples for NIPT.

[0183] Since small-sized fragments are associated with fragments from non-healthy tissues (Jiang et al, (2015), Proceedings of the National Academy of Sciences, 112(11), ppE1317-E1325) they can also be leveraged for the detection of small-sized mutations, such as point mutations and mutational signatures. For example, one may only use small-sized fragments in Variant Allele Frequency estimation as described in examples 6-9, thereby increasing the signal-to-noise ratio.

Example 10: Quantification of Variant Alleles in Mixed Samples Containing

Maternal DNA at Loci Associated with Genetic Conditions

[0184] Mixed samples, containing both maternal and fetal DNA, were processed as described in Example 1. Families of TACS were designed for the detection of inheritable genetic conditions associated with 5 different genetic abnormalities (β-thalassemia, phenylketonuria, cystic fibrosis, Gauchers' disease and autosomal recessive polycystic kidney disease). The members of the TACS families were designed such that they had staggered start/stop positions for binding to the target sequence of interest, as described in Example 5. Furthermore, the members of the TACS families were designed to have the optimized features with respect to their size, distance from repetitive elements and GC content, as described in Example 2.

[0185] The TACS methodology illustrated in Figure 1 (and described in Examples 1-3) was used with the families of TACS for enhanced enrichment of target sequences of interest containing specific sequences relevant to the determination of maternal carrier status for five inheritable genetic conditions (β-thalassemia, phenylketonuria, cystic fibrosis, Gauchers' disease and autosomal recessive polycystic kidney disease). To determine the maternal carrier status for these genetic conditions, analysis was conducted across 14 different genes, covering a total of 157 loci. Optionally, the maternal sample can be simultaneously interrogated with TACS (or families of TACS) for detecting fetal chromosomal abnormalities (e.g., aneuploidies, such as for chromosomes 13, 18, 21, X and Y, as described herein).

[0186] Targeted sequencing products obtained from Next Generation Sequencing (NGS) results were processed to remove adaptor sequences and poor quality reads. Reads whose length was at least 25 bases long post adaptor-removal were aligned to the human reference genome built hg19. If relevant, duplicate reads are removed post-alignment. Where applicable,

sequencing output pertaining to the same sample but processed on separate sequencing lanes was merged to a single sequencing output file. Software analysis provided a final aligned version of a sequenced sample against the human reference genome from which information can then be extracted in terms of Single Nucleotide Polymorphisms (SNPs), Single Nucleotide Variants (SNVs) and other genetic variations with respect to a reference sequence at loci of interest, read-depth per base and the size of aligned fragments. The maternal sample can be fully processed using the pipeline described in Examples 1-4 to determine the ploidy status of the fetus. In addition to this, information in terms of SNVs and indels at loci of interest concerning the sequence and number of times each SNV is present in a sequenced sample was detected and was used to infer the presence and carrier status the maternal sample using binomial statistics as described herein.

[0187] Data in the form of calculated Variant Allele Frequencies (VAFs) from mixed samples, containing both maternal and fetal DNA, are presented in Figure 10. The Variant Allele Frequency was computed as the number of times the variant allele was sequenced over the number of times the locus was sequenced. The x-axis is an index of the different samples analyzed. The y-axis is the value of the Variant Allele Frequency of a sample (VAF %). The value of the VAF is based on the maternal fraction present in the mixed sample. A carrier of the variant allele would be expected to have a VAF of around 50%. However, a pregnant woman who is a carrier would be expected to have a VAF value around 50% minus half the fetal fraction value since a mixed sample contains both fetal and maternal DNA. Thus, if for example a mixed sample has an estimated fetal fraction of 10% then the maternal fraction is 90%. Thus, it is expected that maternal carrier status for autosomes (i.e. non-sex chromosomes) would have a VAF value near 45%. A similar line of reasoning may be used for sex-linked diseases where one has to take into account the sex of the fetus before estimating expected VAFs. If a sample has a very low VAF value for a given region (illustrated by the very small grey dots at the bottom of the plot in Figure 10), then this likely indicates absence of the allele variant (i.e., the pregnant woman is not a carrier of the genetic condition), or that the VAFs could originate from the fetus or could be a result of sequencing error. Large value VAFs appear at the top of the plot indicating maternal carrier status (colored dots). For those mixed maternal/fetal samples having positive maternal carrier status, a paternal sample is then processed in order to compute the paternal carrier status and determine the fetal risk of inheriting the genetic condition. A paternal sample (e.g., plasma sample) also undergoes the TACS methodology illustrated in Figure 1, as described herein, using families of TACS directed to those loci for which maternal sample has been determined to have positive carrier status. The sequencing data are aligned as described for the maternal sample and information in terms of Short Nucleotide Variants (SNVs) at loci of interest, read-depth per base and the size of aligned fragments is obtained. Using this information the presence and carrier status of the paternal sample is inferred using binomial statistics.

[0188] Finally, a fetal risk score for inheriting the detected genetic conditions is determined from the data using Mendelian genetics reasoning. An example of a fetal risk score is illustrated below in Table 3, where the algorithms used have detected that the mother is a carrier, with allelic sequence Aa, for a given recessive genetic condition and the father also has

been determined to be a carrier, with allelic sequence Aa, for the same given recessive genetic condition.

Table 3: Example of Mendelian Genetics Reasoning for Determining Fetal Risk

Possible Fetal Outcomes		Maternal Status	
		A	a
Paternal Status	A	AA	Aa
	a	Aa	aa

Accordingly, for the allelic combination of Aa, where "A" describes the dominant allele and "a" the recessive disease-associated allele and "Aa" thus implies maternal and paternal carrier of the condition, then the fetus has a 25% chance of having the genetic condition ("aa" homozygous recessive genotype in the lower right corner of Table 3 above).

[0189] In summary, this example demonstrates that the TACS methodology can successfully be used to determine maternal (and, if necessary based on the maternal results, paternal) carrier status for inheritable genetic conditions, thereby allowing for determination of fetal risk of inheriting genetic conditions.

Example 11: Analysis of Fetal DNA Samples from Embryo Biopsy

[0190] In this example, fetal DNA samples obtained from fetal cells from embryo biopsy were analyzed using the TACS-based methodology shown in Figure 1 to detect chromosomal abnormalities in the fetal samples.

Fetal Sample Collection, Library Preparation and TACS Enrichment

[0191] Fetal cell samples were obtained from 3-day and 5-day biopsy embryos respectively were subjected to the TACS methodology shown in Figure 1 to determine the status of genetic abnormalities. All samples were previously referred for Pre-implantation Genetic Screening (PGS) and subjected to array Comparative Genomic Hybridization (aCGH) as part of the routine screening test. Results of aCGH were used as a reference standard for the results obtained.

[0192] Collected fetal cells were initially lysed and DNA extracted using the Rubicon Genomics PicoPLEX WGA Kit (Liang, L. et al. (2013) PLoS One 8(4), p. e61838).

[0193] For certain samples in which whole-genome sequencing was to be performed, the lysed material was subjected to whole genome amplification using commercial whole genome amplification kits. Briefly, following a pre-amplification step, the lysed material was then amplified using amplification enzyme and buffer supplied by the manufacturer. Subsequently, DNA was purified followed by fragmentation using sonication. Fragmented DNA was then

processed using standard sequencing library preparation methods such as described in Example 1, typically involving ligation of adapters onto the ends of the cell free DNA fragments, followed by amplification. In addition to the description provided in Example 1, sequencing library preparation kits are commercially available for this purpose.

[0194] For samples in which TACS-based enrichment was to be performed, then the sequencing library obtained from the above methods underwent TACS hybridization essentially as described in Example 3. The region(s) of interest on the chromosome(s) of interest were enriched by hybridizing the pool of TACS to the sequencing library, followed by isolation of those sequences within the sequencing library that bind to the TACS. To facilitate isolation of the desired, enriched sequences, typically the TACS sequences were modified such that sequences that hybridized to the TACS were separable from sequences that did not hybridize to the TACS. Typically this was achieved by fixing the TACS to a solid support such as described in Example 3, thereby allowing for physical separation of those sequences that bind the TACS from those sequences that do not bind the TACS. The pools of TACS used either can contain a plurality of single TACS that bind to different target sequences of interest or, alternatively, can contain a plurality of families of TACS containing a plurality of members that each bind to the same target sequence of interest but with different start and/or stop positions on the target sequence, as described in Example 5.

[0195] For analysis of fetal DNA samples by TACS-based enrichment, the pool of TACS can contain TACS that target a subset of chromosomes of interest (e.g., chromosomes 13, 18, 21, X and Y). More preferably, however, the pool of TACS contains various TACS that target every chromosome within the human genome (chromosomes 1-22, X and Y) such that the entire genome is encompassed, allowing for determination of chromosomal abnormalities in any chromosome within the human genome.

[0196] Next Generation Sequencing (NGS) typically was used to sequence the TACS-enriched sequences (or the whole genome for samples analyzed by whole genome sequencing), thereby providing very accurate counting as well as sequence information. Library products were pooled equimolarly and then subjected to sequencing.

Data Analysis

[0197] Sequencing data obtained from NGS were processed to remove adaptor sequences and poor quality reads. Reads whose length was at least 25 bases long post adaptor-removal were aligned to the human reference genome built hg19. If relevant, duplicate reads were removed post-alignment. Where applicable, sequencing output pertaining to the same sample but processed on separate sequencing lanes, was merged to a single sequencing output file. Software analysis provides a final aligned version of a sequenced sample against the human reference genome from which information was extracted in terms of Short Nucleotide Polymorphisms (SNPs) at loci of interest, read-depth per base and the size of aligned fragments.

[0198] For whole-genome sequencing and TACS-based whole-genome sequencing, the read-depth of non-overlapping genomic regions of fixed size (e.g. 50kb or 1Mb) was obtained by using the samtools bedcov tool, which provides the sum of all reads across a specified genomic region. The obtained value was divided by the length of the windows. For TACS targeted-based sequencing, the read-depth was obtained by using the samtools mpileup tool, which provides information on the read-depth per base, across specified contiguous sequences or the bedcov tool. The median value of the obtained information was assigned as the read-depth of a given locus. Removal of read-depth outliers was performed using either a median-based or mean-based outlier detection approach. Finally, GC-content read-depth bias alleviation was achieved using a local polynomial fitting method to estimate the expected read-depth of regions based on their GC content and then normalize regions using this expected value accordingly.

[0199] The normalized read-depth from all regions was used as input into

1. (a) various segmentation-based classification algorithms (described further below), and/or
2. (b) score-based classification algorithms (described further below),

which were then used to determine the ploidy status of the interrogated regions, as well as the size of any genetic aneuploidies. Score-based classification algorithms were used only with targeted enrichment sequencing data.

Ploidy Status Determination Using Segmentation Algorithms

[0200] Three different types of segmentation algorithms were developed and applied to fetal DNA sample analysis: (i) Likelihood-based segmentation; (ii) Segmentation using small overlapping windows; and (iii) Segmentation using parallel pairwise testing, each of which is described further below, along with the results for application of the algorithm.

[0201] Each algorithm is a collection of data processing and statistical modeling routines arranged as a series of steps with aim to decide if the observed sequencing data does not support the null hypothesis, H0 defined as:
H0= There are no ploidy deviations from the expected ploidy state.

[0202] For human genomes the expected ploidy state is the diploid state. The segmentation approach aims to discover breakpoints in consecutive data where there is a clear distinction between read-depths, which in turn indicates that there is a change in ploidy state. The algorithms are described below.

A. Likelihood-based segmentation

[0203] Given a set of ordered data points {*x_{1},x_{2},x_{3},x_{4},..,x_{N}*}, that describe read-depth, the aim was to infer at which point *x_{i}* the data changes distribution (i.e. there is a significant and consecutive change in read-depth). This was labeled as the break point *ϑ_{1}*. For example, if the data changes distribution after *x_{3}* then *ϑ_{1}=x_{3}*. If more than one break point exists, then the algorithm will label the next discovered break point as *ϑ_{2}*. The algorithm steps were as follows:

1. (a) Given a sequence of data (*i,x_{i}*), where *i=1..N,* the algorithm estimates the number of modes in the data. To this end, a process known as bivariate kernel density estimation was utilized. For example, if there was a single breakpoint, then the algorithm returned that there were 2 modes in our data distribution.
2. (b) Decide the position of the break point(s) in the data, if such point(s) exist(s). This was achieved with the following algorithm:
    1. (1) Based on the number of breakpoints found in (a) define the probability density function (p.d.f) of the data, which depends on the unknown values of the breakpoints. This may be, but not limited to, a mixture of Normal distributions.
    2. (2) Calculate the maximum likelihood estimate of the p.d.f in (1) for a fixed set of value(s) for the breakpoints.
    3. (3) Repeat (2) for different sets of break point value(s).
    4. (4) Select as estimated break point(s) the values that maximizes step (2).

[0204] It was noted that the algorithm does this by assigning membership in all combinations for all break-points estimated in part (a). As an example, if the probability is maximized when data points *x_{1}* to *x_{3}* come from the first distribution then *ϑ_{1}=x_{3}* and membership of *x_{1}* to *x_{3}* is assigned to the first distribution and *x_{4}* to *x_{N}* to the next identified distribution(s). If the likelihood is maximized with all data points *x_{i}* assigned to the same mode then no breakpoint is defined and all data points are assigned to the same distribution. Various distributions and computational methods known to those skilled in the art can be used to implement this.

[0205] Representative results of fetal DNA analysis using the likelihood-based segmentation algorithm are shown in Figure 11. These results demonstrate that likelihood-based segmentation analysis can classify whole-chromosome aberrations in fetal DNA samples (e.g., from PGD/PGS products of conception). At the top panel of Figure 11, a sample without any ploidy abnormalities subjected to whole-genome sequencing is presented. The expected read-depth of each chromosome (blue horizontal bars) lies within the red lines that indicate the range of values of normal ploidy, as decided from the data. Even if on occasion individual data points (grey dots) deviate from the confidence intervals this is not sufficient evidence of ploidy aberrations according to the probabilistic metric used. Conversely, if enough data points deviate from the confidence intervals then the probabilistic measure used can assign a different ploidy state. Such a case is presented at the bottom of Figure 11, where the sample has been determined to have monosomy 18 and monosomy 20.

[0206] In similar fashion, Figure 15 presents results from the algorithm utilizing data derived from TACS specific coordinates combined with data from products of partial complementarity to the TACS that align to non-TACS coordinates thus producing low coverage throughout the genome. In the top panel of Figure 15 a normal male sample is presented, whereas in the bottom panel the male sample is classified as having trisomy for chromosome 13 and monosomy for chromosome 21.

[0207] Figure 16, presents results from the algorithm utilizing data from TACS specific coordinates only. As with Figure 15, in the top panel of Figure 16 a normal male sample is presented, whereas in the bottom panel the male sample is classified as having trisomy for chromosome 13 and monosomy for chromosome 21.

[0208] Thus, it can be seen that the algorithm successfully classifies TACS-based enrichment and TACS-based whole genome sequencing data, allowing for correct classification of chromosomal abnormalities and at the same time requiring significantly less sequencing than massively parallel shotgun sequencing approaches.

B. Segmentation using small overlapping windows

[0209] Given a set of data points the aim was to decide membership of each data point into a set of clusters, based on a thresholding scheme. The algorithm does so as follows:

1. (a) Given a set of consecutive read-depth data *x_{i}* (*i=1* to N) the data are divided into overlapping windows of fixed size. For example let *w_{1}* = {*x_{1}* , ..., *x{10 }*} denote the first window, then *w_{2}* = {*x_{2}, ...*, *x-_{11}*}, *w_{3}* = {*x_{3}, ...*, *x_{12}*} etc.
2. (b) For each window *w_{k},* a score *S(k)* = *(X_{k}* - *m)/m* is computed, where X_{k} is the median of *w_{k}* and m is the median from all *x_{i}* from all chromosomes.
3. (c) Assign cluster membership based on a thresholding value s, whereby:

   if *S(k)* < *s ,* assign to cluster1

   if s <= *S(k)* < *C_{1}s* are assigned to cluster 2,

   if *2s* <= *S(k)* < *C_{2}s* are assigned to cluster 3 etc.

where *C_{j}.* are positive real numbers greater than one. For example, if s is a particular threshold value then all consecutive *w_{k}* where *S(k)* < s are assigned to cluster 1. All consecutive *w_{k}* where s <= *S(k)* < *C_{1}s* are assigned to cluster 2. All consecutive *w_{k}* where 2s <= *S(k)* <*C_{2}s* are assigned to cluster 3 etc. The threshold s can be either decided from the data or treated as a tuning parameter.

[0210] Representative results of ploidy determination for fetal DNA samples (e.g., PGS/PGD products of conception) using whole genome sequencing and small overlapping windows segmentation are shown in Figure 12. The top panel illustrates a normal sample. As with

Figure 11, the expected read-depth of each chromosome (blue horizontal bars) lies within the red lines, which indicate the range of values of normal ploidy. The expected read-depth is calculated from the individual data points (grey dots). The average read-depth and data points of chromosomes X and Y lie below the bottom red-line, indicating that there is only a single copy of each chromosome, as expected from a male sample. An aneuploid sample is presented at the bottom of Figure 12 where the sample is classified with trisomy 13 and mosaicism on chromosome 19.

C. Segmentation using Parallel Pairwise Testing

[0211] This segmentation approach firstly performs full chromosome ploidy determination and then a sub-chromosomal ploidy determination as follows:

1. (a) Read-depth data from one candidate chromosome are compared with read-depth data from other chromosomes using non-parametric statistical tests. The process is repeated until all candidate chromosomes are tested.
2. (b) Perform a multiple comparisons adjustment on the results of the statistical tests to avoid false positive results.
3. (c) Depending on the statistical test result from the adjusted data, assign the relevant ploidy to candidate chromosomes that illustrate significant evidence against the null hypothesis
4. (d) Once full-chromosomal ploidy is determined then sub-chromosomal ploidy is tested by randomly splitting regions of each chromosome into smaller sizes. Each sub-chromosomal region is then tested for significant deviations from its expected full-chromosomal read-depth using similar statistical tests as in steps (a)-(c).

[0212] Representative results of ploidy determination for fetal DNA samples (e.g., PGS/PGD products of conception) using whole genome sequencing and small overlapping windows segmentation are presented in Figure 13. The top panel illustrates a normal sample. As with Figures 11, 12, 15 and 16, the expected read-depth of each chromosome is illustrated using blue horizontal bars. In this instance, confidence interval bars have been omitted. A normal sample is presented at the top Figure 13 whilst a sample presenting many abnormalities is presented at the bottom panel.

Ploidy Status Determination Using Score-Based Classification

[0213] Additionally or alternatively to the segmentation-based algorithms described above, fetal DNA samples can be analyzed using score-based classification. The read-depth data were firstly transformed using square root or logarithmic transformation in order to minimize variance biases. Then methods such as those described in Example 4 were performed to

decide on the ploidy status of each tested region (chromosomal and sub-chromosomal regions may be tested).

[0214] Representative results using a score-based classification system on the fetal DNA samples (e.g., PGS/PGD products of conception) are shown in Figure 14. Green dots illustrate normal ploidy samples whilst all others that lie above or below the normal ploidy thresholds illustrate some type of abnormality. Specifically, blue dots illustrate trisomy samples, cyan dots illustrate partial trisomy samples and red dots illustrate monosomy samples.

[0215] In summary, this example demonstrates the successful analysis of fetal DNA samples (e.g., PGS/PGD products of conception) for chromosomal abnormalities using either whole genome sequencing or TACS-based enrichment and using a variety of statistical analysis approaches

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<213> Homo sapiens

<400> 2549
tccctctgta ctatgtagca tg 22

<210> 2550
<211> 20
<212> DNA
<213> Homo sapiens

<400> 2550
ttcatttccc tttgttgccc 20

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Fremgangsmåde til at teste risikoen for en genetisk abnormitet i en DNA-prøve der omfatter genomiske sekvenser af interesse, hvilken fremgangsmåde omfatter:

(a) at forberede et sekventeringsbibliotek fra DNA-prøven;

(b) at hybridisere sekventeringsbiblioteket til en pulje af dobbeltstrengede targetcapturesekvenser (TACS), hvor puljen af TACS omfatter et antal TACS-familier, der er rettet mod forskellige genomiske sekvenser af interesse, hvor hver TACS-familie omfatter et antal medlemssekvenser, hvor hver medlemssekvens binder til den samme genomiske sekvens af interesse, men har forskellige start- og/eller stoppositioner i forhold til et referencekoordinatsystem for den genomiske sekvens af interesse, og yderligere hvor:

(i) hver medlemssekvens inden for hver TACS-familie er mellem 150-260 basepar i længden, hvor hver medlemssekvens har en 5'-ende og en 3'-ende;

(ii) hver medlemssekvens binder til den samme genomiske sekvens af interesse mindst 50 basepar væk, på både 5'-enden og 3'-enden, fra regioner, der huser kopinummervariationer (CNV'er), segmentduplikationer eller gentagne DNA-elementer; og

(iii) GC-indholdet i puljen af TACS er mellem 19 % og 80 %, som bestemt ved at beregne GC-indholdet for hvert medlem inden for hver familie af TACS;

(c) at isolere medlemmer af sekventeringsbiblioteket der binder til puljen af TACS for at opnå et beriget bibliotek;

(d) at amplificere og sekventere det berigede bibliotek; og

e) at udføre statistisk analyse af de berigede biblioteksekvenser for derved at bestemme risikoen for en genetisk abnormitet i DNA-prøven,

hvor start- og/eller stoppositionerne for medlemssekvenserne inden for en TACS-familie, med hensyn til et referencekoordinatsystem for den genomiske sekvens af interesse, er forskudt med 5 til 10 basepar.

2. Fremgangsmåden ifølge krav 1, hvor DNA-prøven er en plasmaprøve, der indeholder cellefrit DNA (cfDNA), eller hvor DNA-prøven er en maternel plasmaprøve, der omfatter maternel DNA og cellefrit føtalt DNA (cffDNA).

3. Fremgangsmåden ifølge krav 1, hvor DNA-prøven omfatter cellefrit tumor-DNA (cftDNA), og hvor hver medlemssekvens inden for en TACS-familie binder til en tumorbiomarkørsekvens af interesse, eventuelt hvor DNA-prøven er valgt fra en gruppe, der består af: en plasmaprøve, en urinprøve, en sputumprøve, en cerebrospinalvæskeprøve, en ascitesprøve og en pleural væskeprøve fra et individ, der har eller er mistænkt for at have en tumor.

4. Fremgangsmåde ifølge et hvilket som helst af kravene 1-3, hvor puljen af TACS binder til et antal tumorbiomarkørsekvenser af interesse, der er udvalgt fra en gruppe, som omfatter: AKT1, ALK, APC, AR, ARAF, ATM, BAP1, BARD1, BMPR1A, BRAF, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, CTNNB1, DDB2, DDR2, DICER1, EGFR, EPCAM, ESR1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FBXW7, FLT3, FOXA1, FOXL2, GATA3, GNA11, GNAQ, GNAS, GREM1, HOXB13, IDH1, JAK2, KEAP1, KIT, KRAS, MET, MLH1, MPL, MRE11A, MSH2, MSH6, MTOR, MUTYH, NBN, NPM1, NRAS, NTRK1, PALB2, PMS2, POLD1, POLE, POLH, PTEN, RAD50, RAD51C, RAD51D, RAF1, RB1, RET, RUNX1, SLX4, SMAD4, SMARCA4, SPOP, STAT, STK11, TP53, VHL, XPA, XPC, og kombinationer der.

5. Fremgangsmåde til bestemmelse af føtal risiko for at nedarve en genetisk tilstand, hvilken fremgangsmåde omfatter:

(a) at fremstille et sekventeringsbibliotek ud fra en prøve, der omfatter maternel og føtalt DNA;

(b) at hybridisere sekventeringsbiblioteket til en pulje af dobbeltstrengede targetcaptursekvenser (TACS), hvor puljen af TACS omfatter et antal TACS-familier der er rettet mod forskellige genomiske sekvenser af interesse, hvor hver TACS-familie omfatter et antal medlemssekvenser, hvor hver medlemssekvens binder til den samme genomiske sekvens af interesse, herunder variant-allel-loci af interesse der er forbundet med forskellige genetiske tilstande, men omfatter forskellige start- og/eller stoppositioner i forhold til et referencekoordinatsystem for locusset af interesse, og yderligere hvor:

(i) hver medlemssekvens inden for hver TACS-familie er mellem 150-260 basepar i længden, hvor hver medlemssekvens har en 5'-ende og en 3'-ende;

(ii) hver medlemssekvens binder til det samme locus af interesse mindst 50 basepar væk, på både 5'-enden og 3'-enden, fra regioner, der huser kopiantalsvariationer (CNV'er), segmentduplikationer eller gentagne DNA-elementer; og

(iii) GC-indholdet i puljen af TACS er mellem 19 % og 80 %, som bestemt ved at beregne GC-indholdet for hvert medlem inden for hver TACS-familie;

(c) at isolere medlemmer af sekventeringsbiblioteket der binder til puljen af TACS for at opnå et beriget bibliotek;

(d) at amplificere og sekventere det berigede bibliotek;

(e) at udføre statistisk analyse på de berigede biblioteksekvenser for derved at bestemme maternel bærerstatus på de loci af interesse, der er forbundet med forskellige genetiske forhold, hvor fremgangsmåden for en prøve med en positiv maternel bærerstatus yderligere omfatter:

(f) at opnå en faderlig DNA-prøve og at udføre trin (a)-(e) på den faderlige DNA-prøve for at bestemme faderlig bærerstatus for de sygdomme, for hvilke der foreligger en positiv maternel bærerstatus; og

g) at bestemme føtal risiko for at arve en genetisk tilstand der er baseret på maternel bærerstatus og, når (f) udføres, baseret på faderlig bærerstatus,

hvor start- og/eller stoppositionerne for medlemssekvenserne inden for en TACS-familie, med hensyn til et referencekoordinatsystem for den genomiske sekvens af interesse, er forskudt med 5 til 10 basepar.

6. Fremgangsmåden ifølge krav 5, hvor allel-loci-varianter af interesse er forbundet med genetiske tilstande, der er valgt fra en gruppe, som omfatter: abetalipoproteinæmi; arthrogryposis mental retardationsanfald; autosomal recessiv polycystisk nyresygdom; Bartlet-Biedl-syndrom 12; beta-thalassæmi; Canavansygdom; choreacantocytose; Crigler-Najjar-syndrom, type I; cystisk fibrose; Faktor-V-Leiden-trombofili; Faktor-XI-mangel; familiær dysautonomi; familiær middelhavsfeber; Fanconi-anæmi (FAN CG-relateret); glycin-encefalopati (GLDC-relateret); Gierkes sygdom, type 3; Gierkes sygdom, type 7; GRACILE syndrom; inklusionslegememyopati, type 2; isovalerinsyreanæmi; Jouberts syndrom, type 2; junctional epidermolysis bullosa, Berlitz type; Lebers medfødt amaurose (LCA5-relateret); Leydig-cellehypoplasi [luteiniserende hormonresistens]; limb-girdle muskeldystrofi, type 2E; lipoamid-dehydrogenase-mangel [ahornsirup-urinsygdom, type 3]; lipoprotein-lipase-mangel; langkædet 3-hydroxyacyl-CoA-dehydrogenase-mangel; ahornsirup urinsygdom, type IB; methylmalonsyreæmi (MMAA-relateret); multipel sulfatase-mangel; Navajo-neurohepatopati [MPVI 7-relateret hepatocerebralt mitokondrielt DNA-depletionssyndrom]; neuronal ceroid-lipofuscinose (MFSD8-relateret); Nijmegen-breakage-syndrom; ornithin-translokase-mangel [hyperomithinemia-hyperammonemia-homocitrullinuria (HHH) syndrom]; Peroxisom-biogenese-lidelser Zellwegers syndromspektrum (PEXI-relateret); Peroxisom-biogenese-lidelser Zellwegers syndromspektrum (PEX2-relateret); phenylketonurinstof; pontocerebellær hypoplasi, type 2E; pyknodysostose; pyruvat-dehydrogenase-mangel (PDHB-relateret); retinal dystrofi (RLBPI-relateret) [Bothnia retinal dystrofi]; retinitis pigmentosa (DHDDS-relateret); Sanfilippos syndrom, type D [muco-polysaccharidosis IIID]; seglcellesygdom; Sjögren-Larsson-syndrom; Tay-Sachs sygdom; Ushers syndrom, type IF; 3-methylcrotonyl-CoA-Carboxylase-mangel 1; 3-methylcrotonyl-CoA-carboxylase-mangel 2 og kombinationer heraf.

7. Fremgangsmåde til at teste for risikoen for en genetisk abnormitet i en DNA-prøve, der overvejende omfatter føtalt eller embryonalt DNA og omfatter genomiske sekvenser af interesse, hvilken fremgangsmåde omfatter:

(a) at forberede et sekventeringsbibliotek fra DNA-prøven der omfatter overvejende føtalt eller embryonalt DNA;

(b) at hybridisere sekventeringsbiblioteket til en pulje af dobbeltstrengede targetcaptursekvenser (TACS), hvor puljen af TACS omfatter et antal TACS-familier, der er rettet mod forskellige genomiske sekvenser af interesse, hvor hver TACS-familie omfatter et antal medlemssekvenser, hvor hver medlemssekvens binder til den samme genomiske sekvens af interesse, men har forskellige start- og/eller stoppositioner i forhold til et referencekoordinatsystem for den genomiske sekvens af interesse, og yderligere hvor:

(i) hver medlemssekvens inden for hver TACS-familie er mellem 150-260 basepar i længden, hvor hver medlemssekvens har en 5'-ende og en 3'-ende;

(ii) hver medlemssekvens binder til den samme genomiske sekvens af interesse mindst 50 basepar væk, på både 5'-enden og 3'-enden, fra regioner, der huser kopinummervariationer (CNV'er), segmentduplikationer eller gentagne DNA-elementer; og

(iii) GC-indholdet i puljen af TACS er mellem 19 % og 80 %, som bestemt ved at beregne GC-indholdet for hvert medlem inden for hver familie af TACS;

(c) at isolere medlemmer af sekventeringsbiblioteket, der binder til puljen af TACS for at opnå et beriget bibliotek;

(d) at amplificere og sekventere det berigede bibliotek; og

e) at udføre statistisk analyse af de berigede biblioteksekvenser for derved at bestemme risikoen for en genetisk abnormitet i DNA-prøven,

hvor start- og/eller stoppositionerne for medlemssekvenserne inden for en TACS-familie, med hensyn til et referencekoordinatsystem for den genomiske sekvens af interesse, er forskudt med 5 til 10 basepar.

8. Fremgangsmåden ifølge krav 7, hvor DNA-prøven er fra et præimplantationsembryo eller fra intakte trofoblaster der er opsamlet fra en maternel Papanicolaou-smear, eller fra føtale celler der er fundet i maternel plasma, hvor DNA-prøven er opnået direkte fra føtalt væv, eller fostervand eller villi chorii eller undfangelsesprodukter.

9. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor antallet af TACS-familier omfatter medlemmer, der binder til kromosomerne 1-22, X og Y i det humane genom.

10. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor puljen af TACS omfatter mindst 5 forskellige TACS-familier, eventuelt hvor hver TACS-familie omfatter mindst 3 medlemssekvenser.

11. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den genetiske abnormitet er en kromosomal aneuploidi, eller hvor den genetiske abnormitet er en strukturel abnormitet, herunder omfatter men er ikke begrænset til kopiantalsændringer, herunder mikrodeletioner og mikroduplikationer, insertioner, deletioner, translokationer, inversioner og mutationer af mindre størrelse inklusive punktmutationer og mutationssignaturer.

12. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor sekventering af det berigede bibliotek tilvejebringer en sekventeringsdybde for de genomiske sekvenser af interesse og sekventeringsdybder for referenceloci, og den statistiske analyse omfatter anvendelse af en algoritme, der sekventielt tester sekventeringsdybden af loci fra de genomiske sekvenser af interesse mod sekventeringsdybden af referenceloci, hvilken algoritme omfatter trin til: (a) fjernelsen af utilstrækkeligt sekventerede loci; (b) forringelse af GC-indholdsforvrængning; og (c) bestemmelse af ploiditetsstatus, eventuelt, hvor GC-indholdsforvrængningen afhjælpes ved at gruppere loci af matchende GC-indhold sammen.

13. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor sekventering af det berigede bibliotek tilvejebringer antallet og størrelsen af sekventerede fragmenter for TACS-specifikke koordinater, og den statistiske analyse omfatter anvendelse af en algoritme, der sekventielt tester fragmentstørrelsesandelen for den genomiske sekvens af interesse i forhold til fragmentstørrelsesandelen af referenceloci, hvilken algoritme omfatter trin til: (a) fjernelse af fragmentstørrelsesoutliers; b) beregning af andel af fragmentstørrelse; og (c) bestemmelse af ploiditetsstatus.

14. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor puljen af TACS er fikseret til en fast bærer, eventuelt, hvor TACS er biotinyleret og er bundet til streptavidin-belagte magnetiske perler.

15. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor den statistiske analyse omfatter en segmenteringsalgoritme, hvilken segmenteringsalgoritme eventuelt er valgt fra gruppen, der består af: likelihood-baseret segmentering, segmentering ved brug af små overlappende vinduer, segmentering ved hjælp af parallel parvis testning, og kombinationer deraf.

DRAWINGS

FIG 1

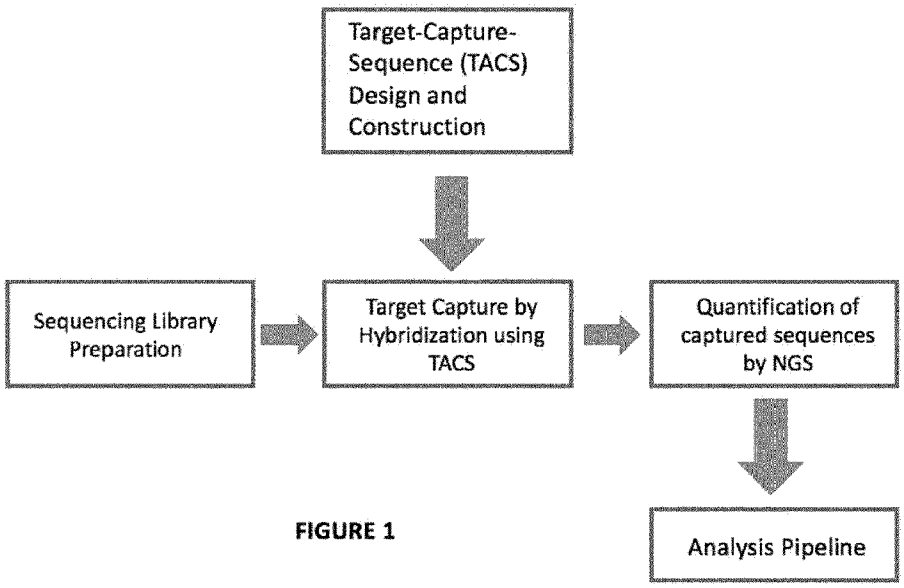


FIGURE 1

chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	91049752	91050001	TCT TCT CGT TCA AGA TGC CG	AAG GGT GGA AGC ACA TTG AC	1, 2
chr13	92177139	92177388	AAA TTA CAT GCA AGG CTA CCA C	ATA GAG GAA CAA GCT GCA CA	3, 4
chr13	93071049	93071298	ACA GGA AGA AAG GGG AGT TAC	CTC GTC CCT TGC ACA TCT TA	5, 6
chr13	94326973	94327222	TTC AGG TTG TGT GAT GTG TC	AGG TGG GGA AGA ACA AAA CA	7, 8
chr13	94415507	94415756	TAA GCA GAG GTT TTG TTG CC	GAG AGT AGG TGC AGG GAA AC	9, 10
chr13	95600034	95600283	GTG AAG TAT TTG CTG CCA CC	ATC CCG CAT TCT TAA CCA CA	11, 12
chr13	112910529	112910778	ATG GTC ATC TCA ACA GCA CA	GAC TAA GCA AAA GCA TCT CCC	13, 14
chr13	53328405	53328654	TCC ACT GAC GTT GAG ATT CG	TTC TTA CAG GCT CAG GGT AT	15, 16
chr13	20533985	20534234	CGA ACC TCC TTG ACC TCT TA	CCC ATA GCT TAA CCC CTA CAA	17, 18
chr13	20535728	20535977	GTT ATG TTG ATA GGG GAA GCT T	TCT TCA TCT TAC TGT CTA GCA C	19, 20
chr13	20544227	20544476	AAG CCC TCT ACT CCA TCT GT	TCA TAC CCT ATC CCT GTG ATC	21, 22
chr13	20567359	20567607	TCA GAA CTC GTC AGT GGA AG	TCT TGC CCT TGA TTT GTT TCC	23, 24
chr13	20577159	20577407	GAA GGG CCA GAC AGC TTA T	GCC TTT TAT CCA TAT GCC ACC	25, 26
chr13	20598772	20599021	ATT TGC TTT GTT TTT GTC CCT	GCC CTC AAC TTT GCT TTT CA	27, 28
chr13	20600556	20600804	GAT CAT TGC TTT GTT TGG ACC	AAA AAC CTG CAC TGT GTT CG	29, 30
chr13	20610779	20611027	AGG AGA GGA AAA ATC TTG ACC	TTT TTA CAG CAA TCT TCA CTG C	31, 32

FIGURE 2 (Page 1 of 80)

Fig. 3


sequence of interest
sequence of interest
single TACS
family of TACS

Fig. 4

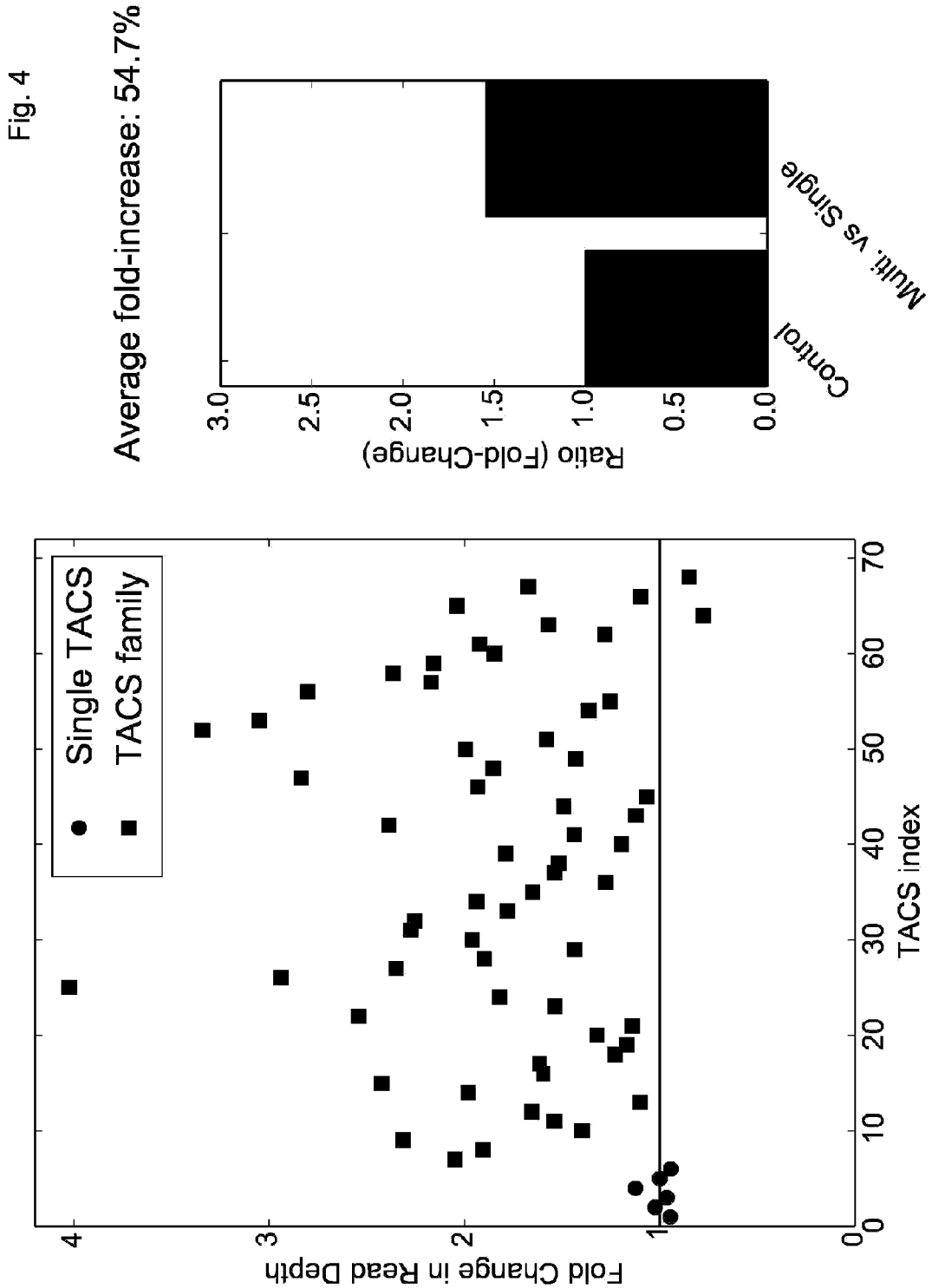

Average fold-increase: 54.7%
Ratio (Fold-Change)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
Multi. vs Single
Single TACS
TACS family
Fold Change in Read Depth
TACS index
0
10
20
30
40
50
60
70
1
2
3
4

Fig. 5

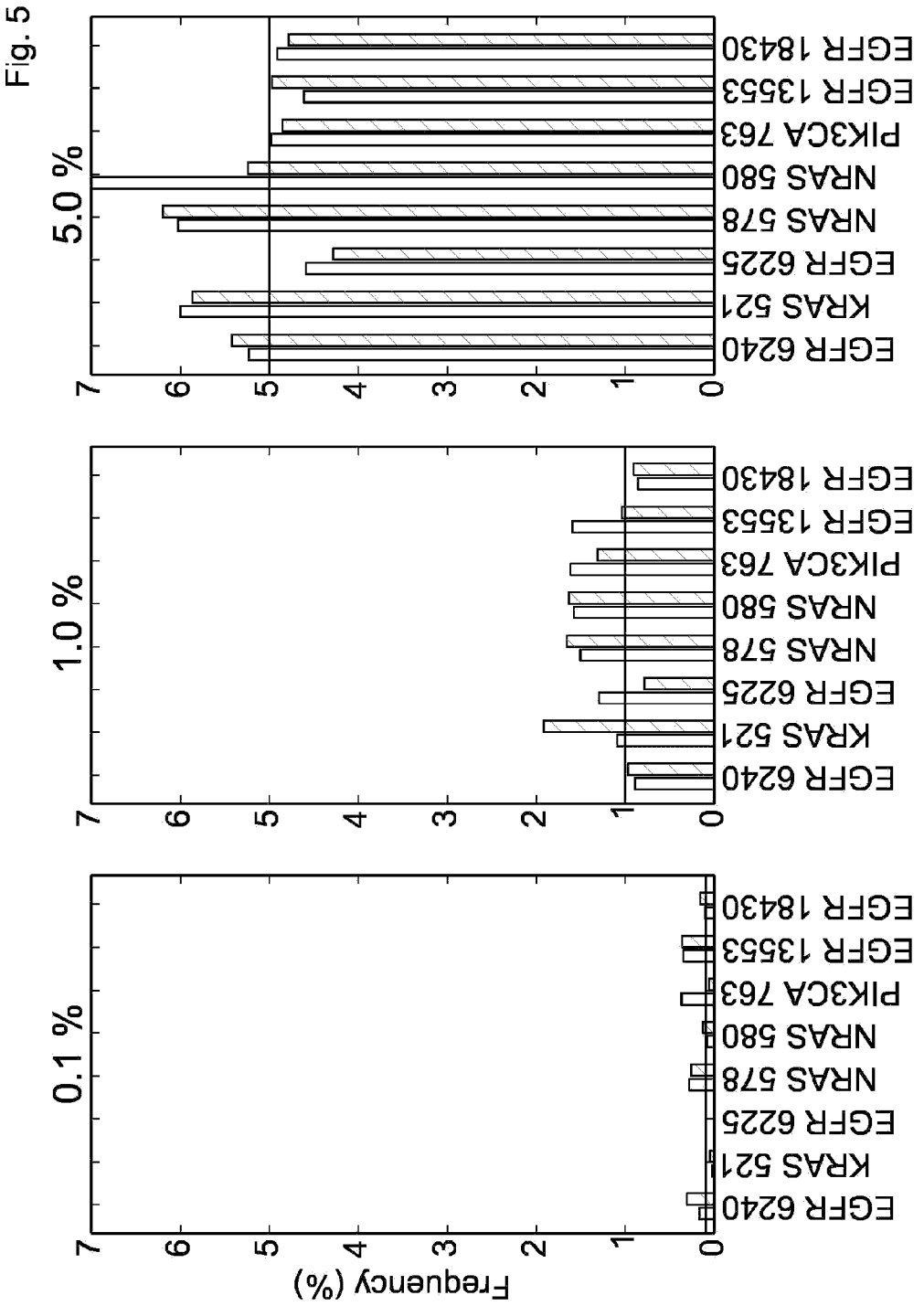
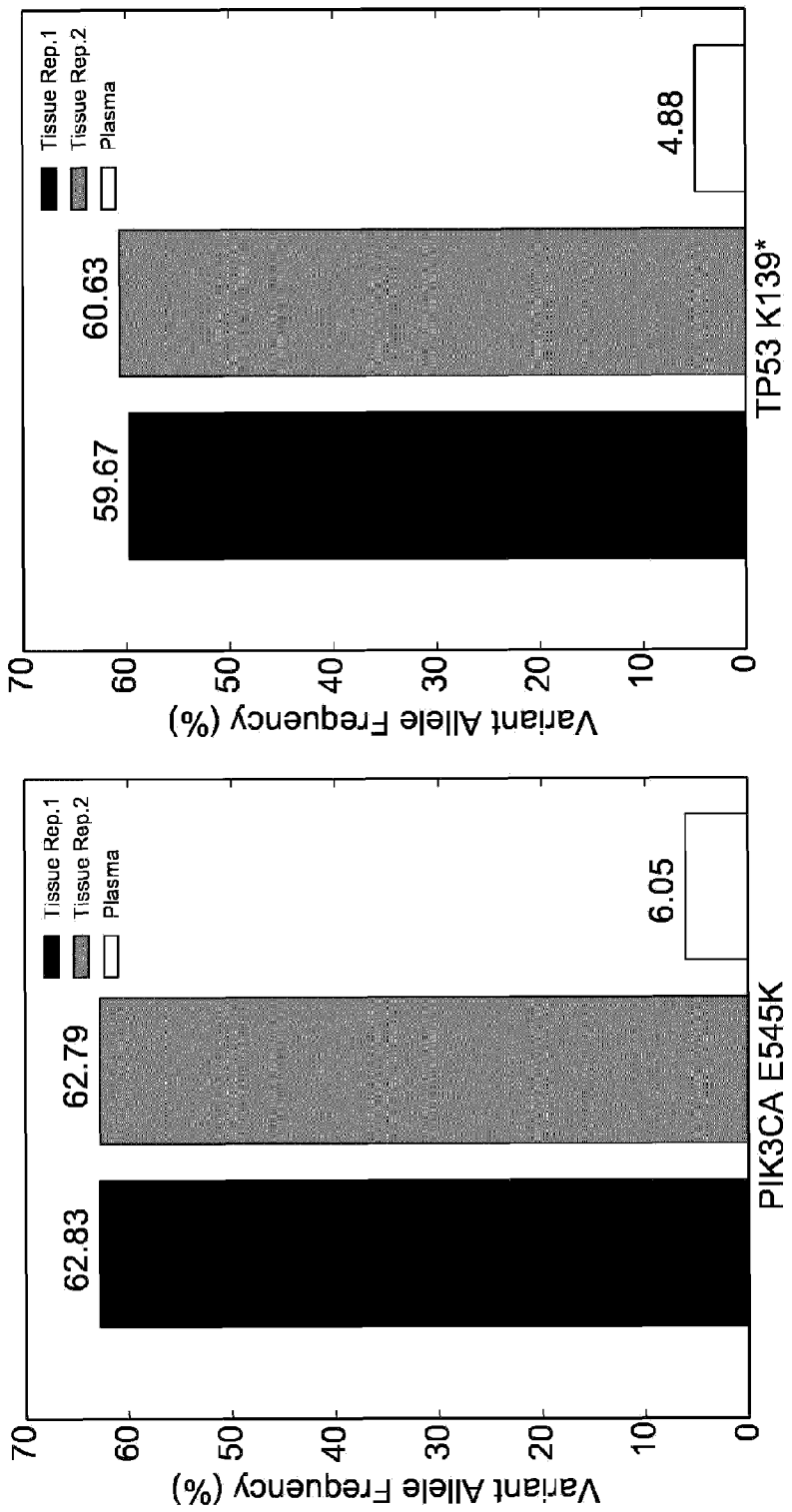

0.1 %
1.0 %
5.0 %
Frequency (%)
0
1
2
3
4
5
6
7
EGFR 6240
KRAS 521
EGFR 6225
NRAS 578
NRAS 580
PIK3CA 763
EGFR 13553
EGFR 18430

Fig. 6


Tissue Rep.1
Tissue Rep.2
Plasma
Variant Allele Frequency (%)
0
10
20
30
40
50
60
70
59.67
60.63
4.88
TP53 K139*
62.83
62.79
6.05
PIK3CA E545K

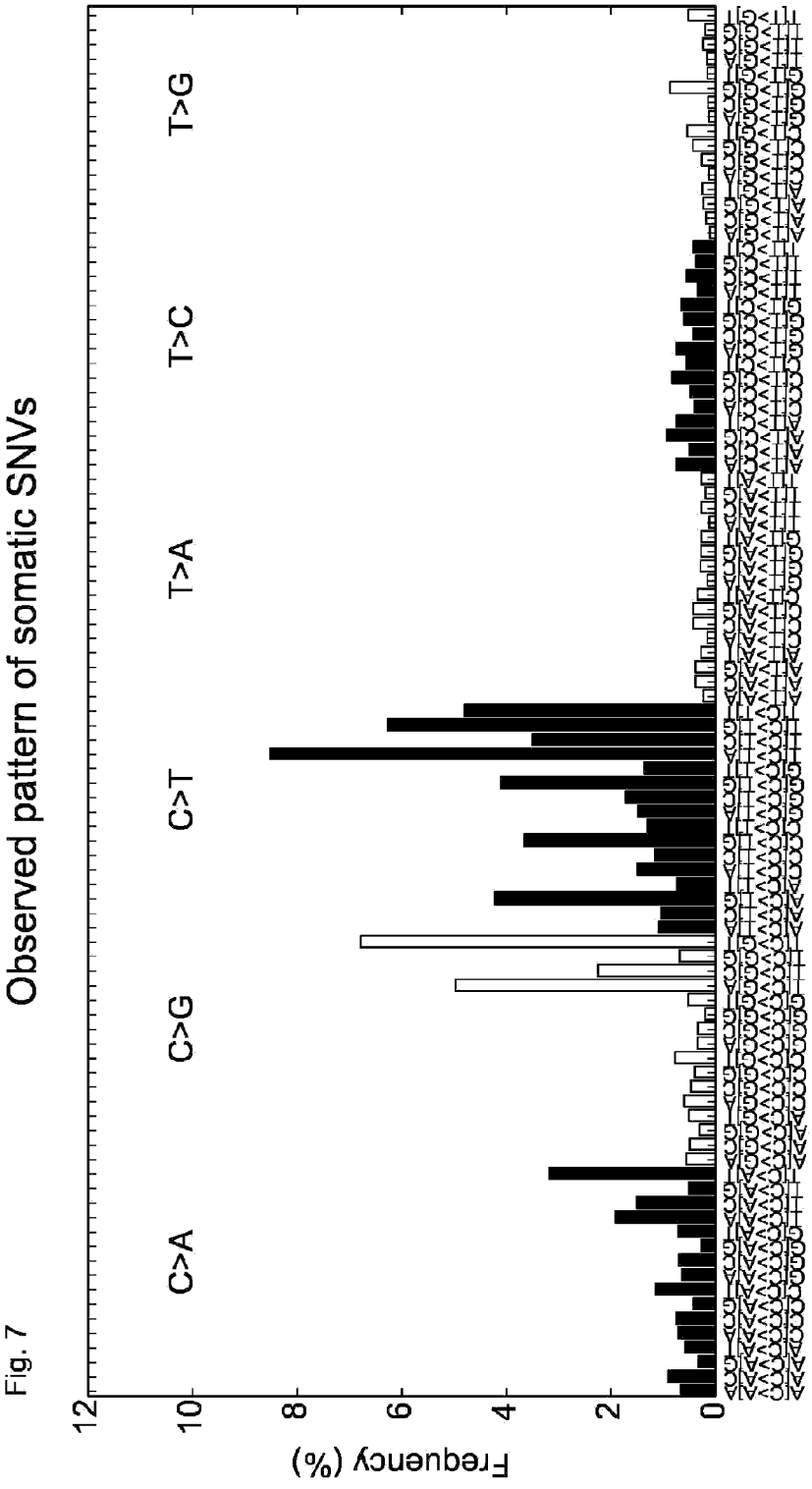

Observed pattern of somatic SNVs
C>A
C>G
C>T
T>A
T>C
T>G
Frequency (%)
0
2
4
6
8
10
12

Fig. 7

Fig. 8

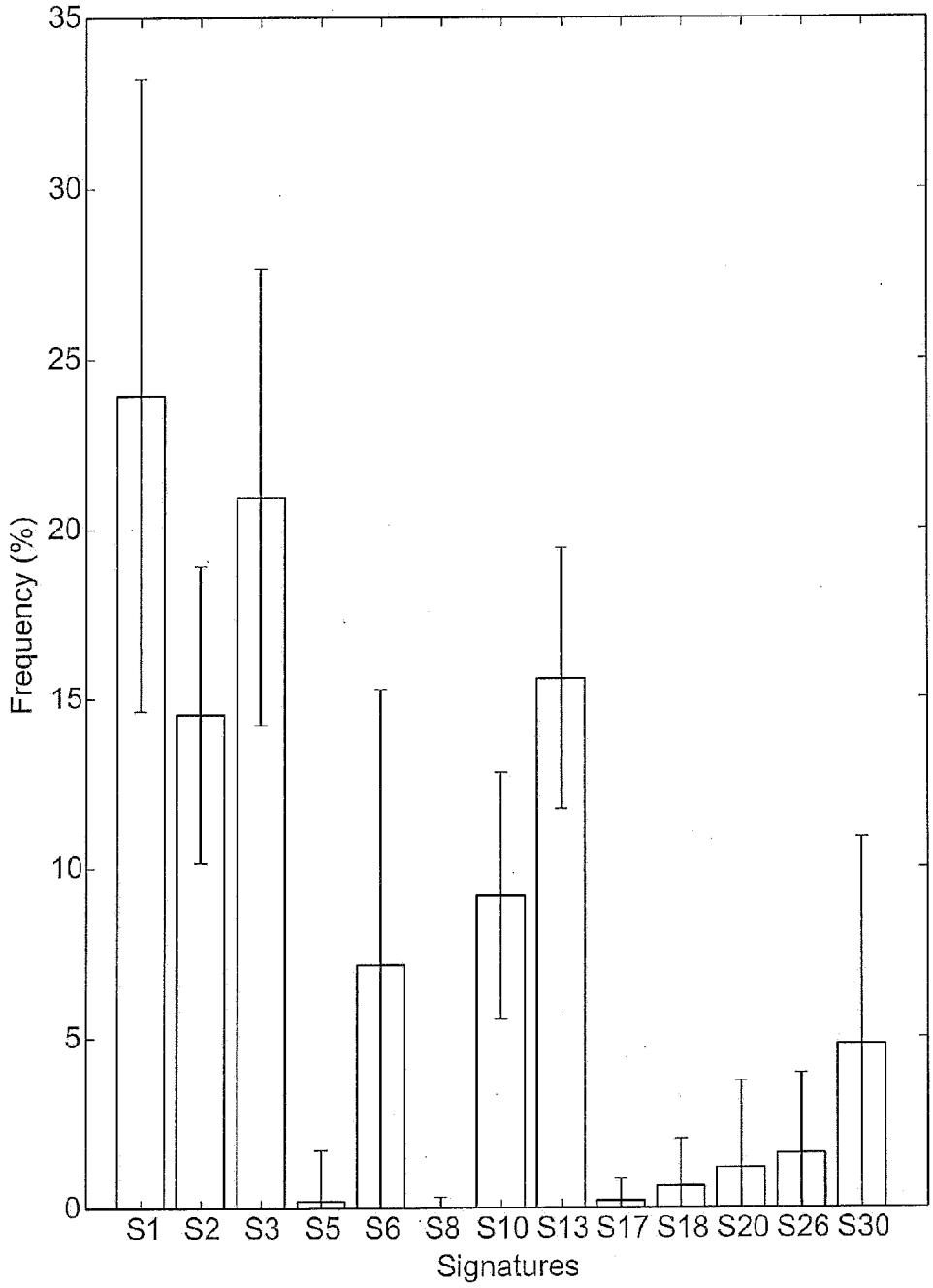

35
30
25
20
15
10
5
0
Frequency (%)
S1
S2
S3
S5
S6
S8
S10
S13
S17
S18
S20
S26
S30
Signatures

Fig. 9

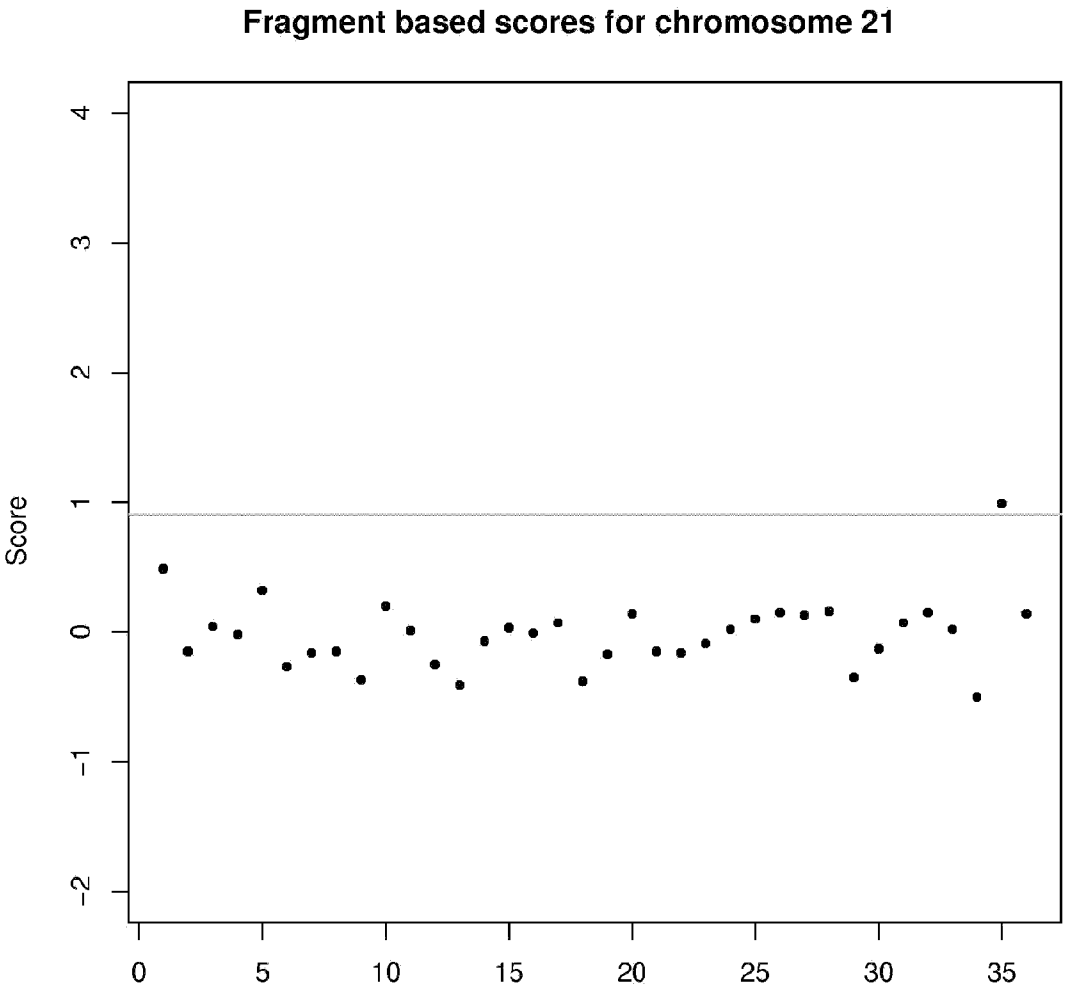

Fragment based scores for chromosome 21
Score
4
3
2
1
0
−1
−2
0
5
10
15
20
25
30
35

Fig. 10

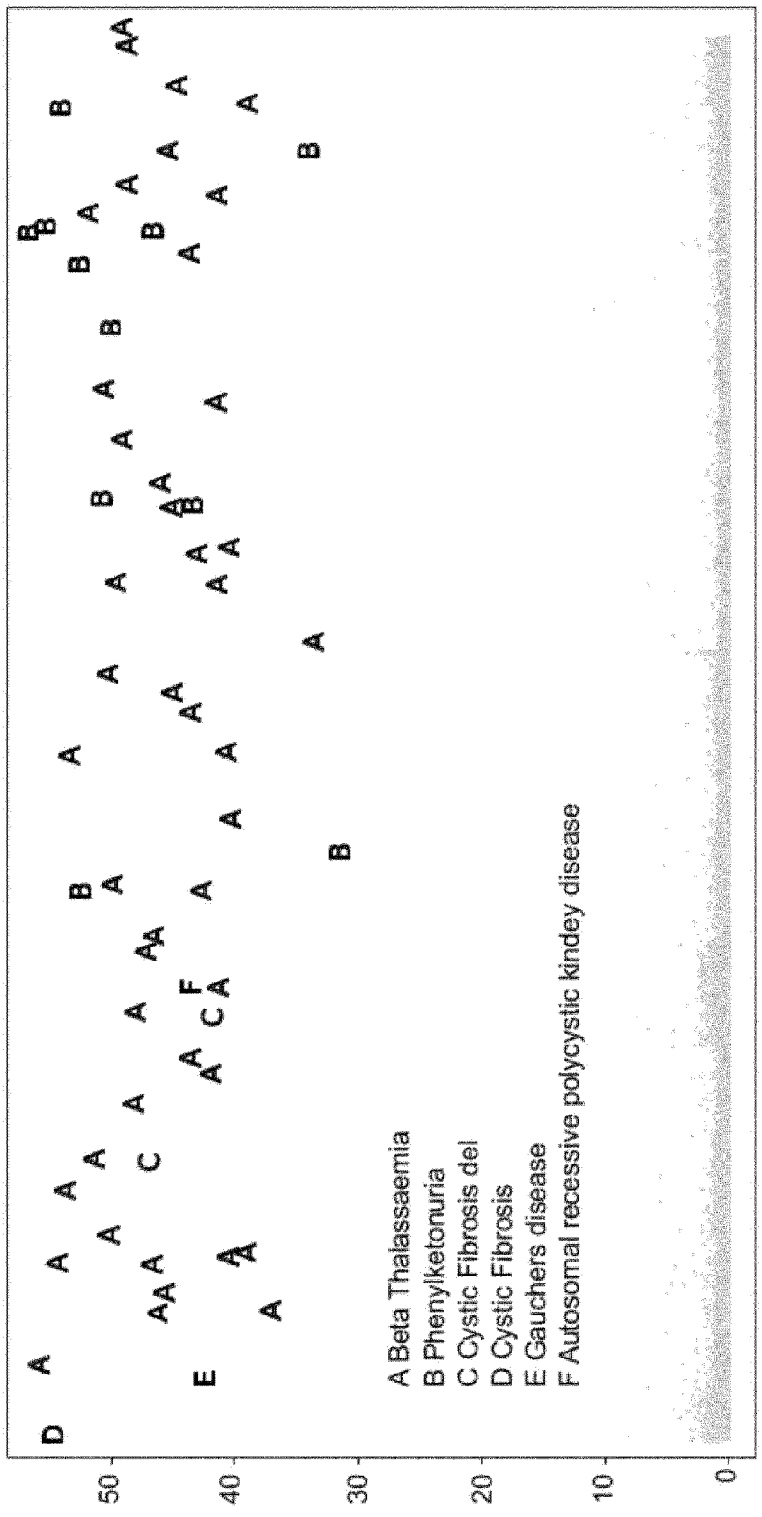

A Beta Thalassaemia
B Phenylketonuria
C Cystic Fibrosis del
D Cystic Fibrosis
E Gauchers disease
F Autosomal recessive polycystic kindey disease
50
40
30
20
10
0

Fig. 11A

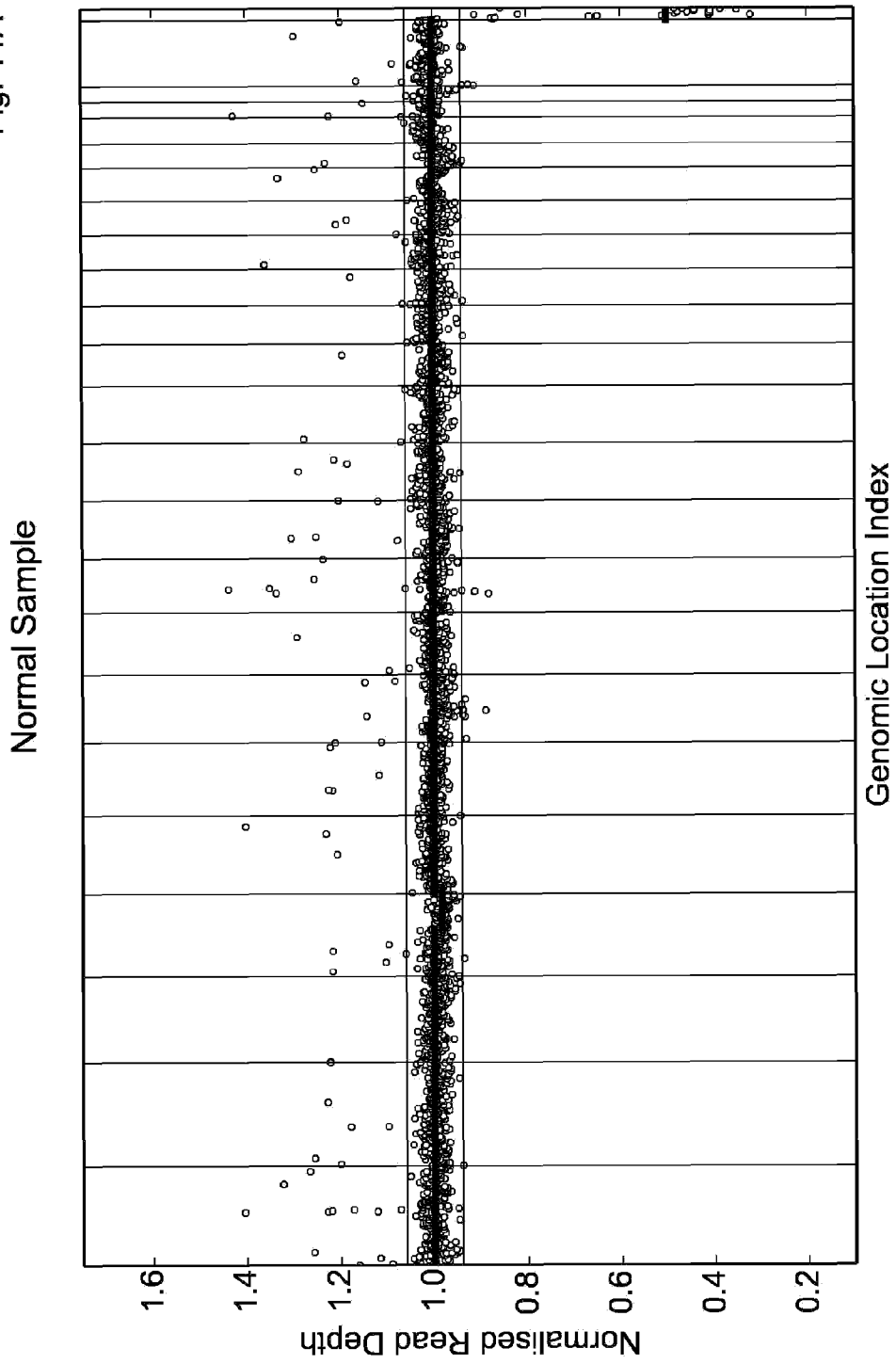

Normal Sample
Normalised Read Depth
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Genomic Location Index

Fig. 11B

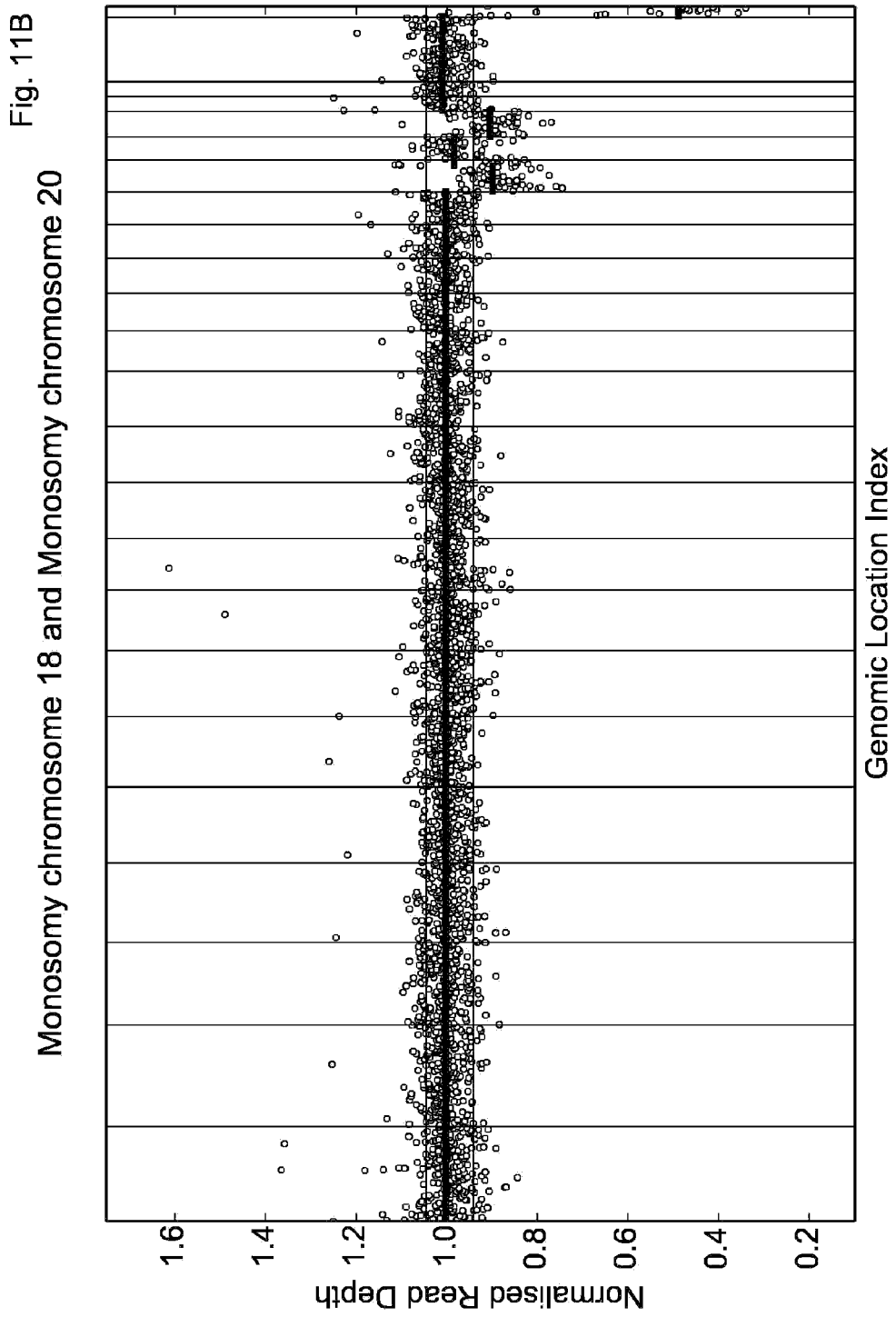

Monosomy chromosome 18 and Monosomy chromosome 20
Normalised Read Depth
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Genomic Location Index

Fig. 12

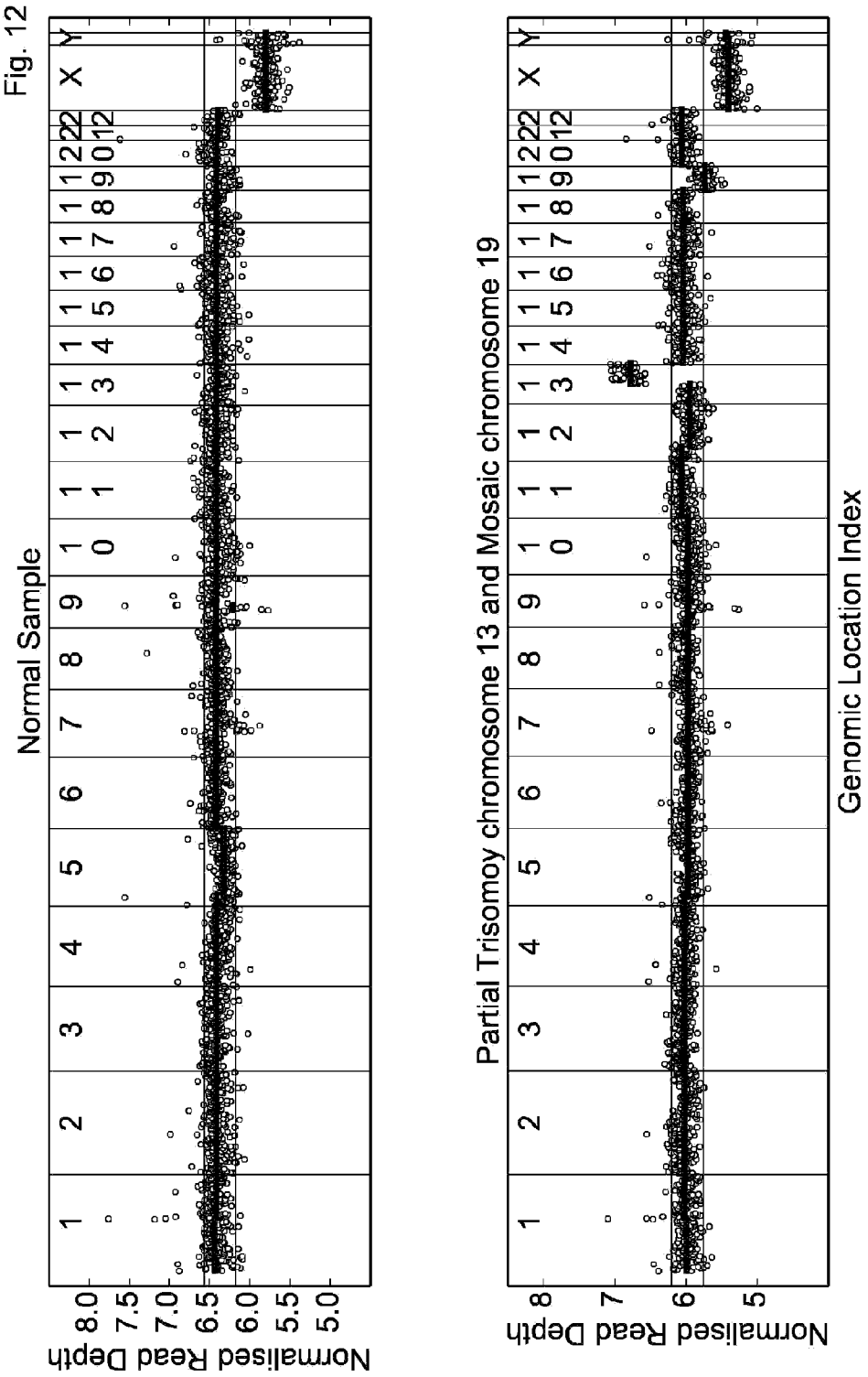

Normal Sample
Normalised Read Depth
8.0
7.5
7.0
6.5
6.0
5.5
5.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Partial Trisomoy chromosome 13 and Mosaic chromosome 19
Normalised Read Depth
8
7
6
5
Genomic Location Index

Fig 13

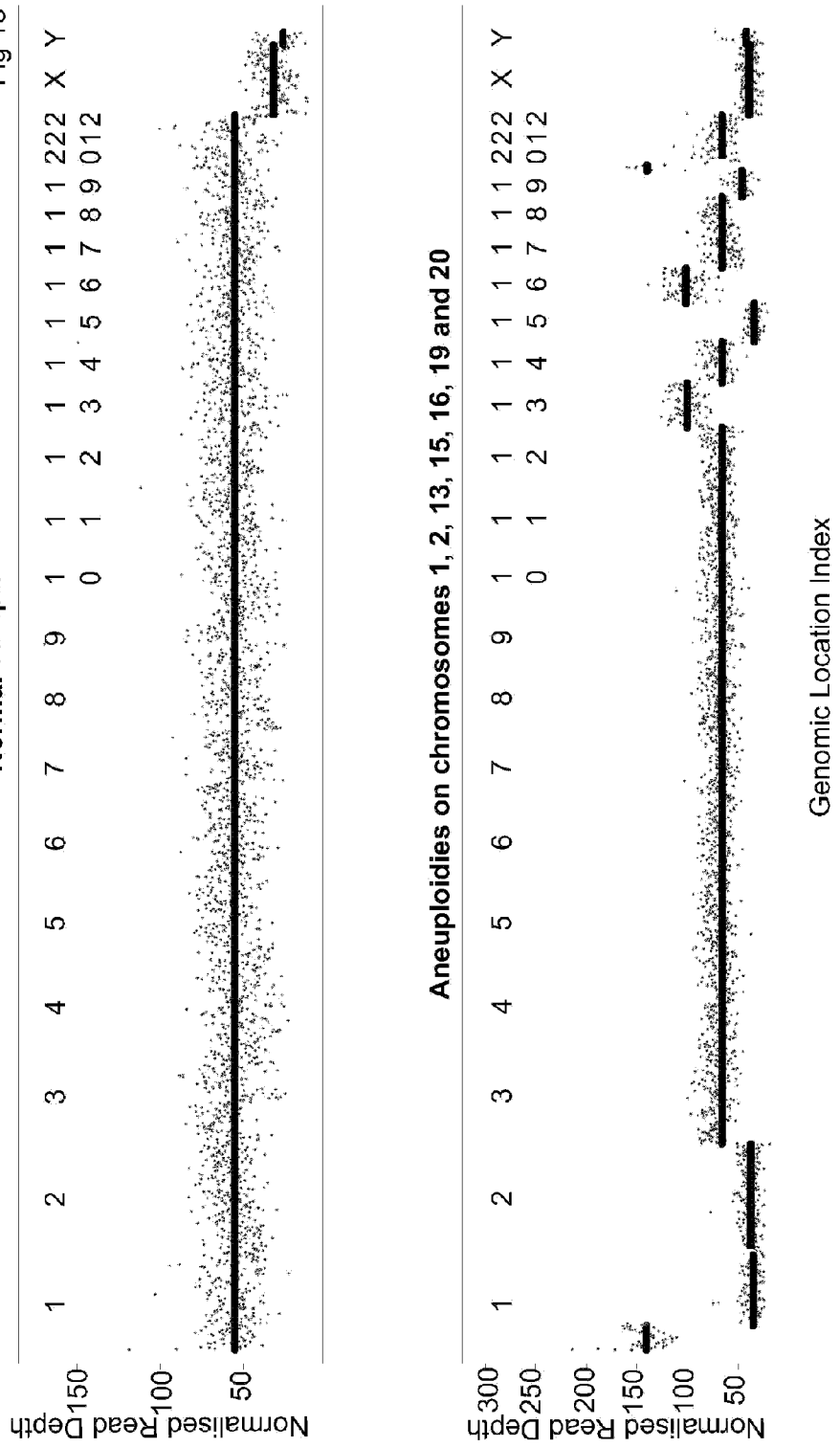
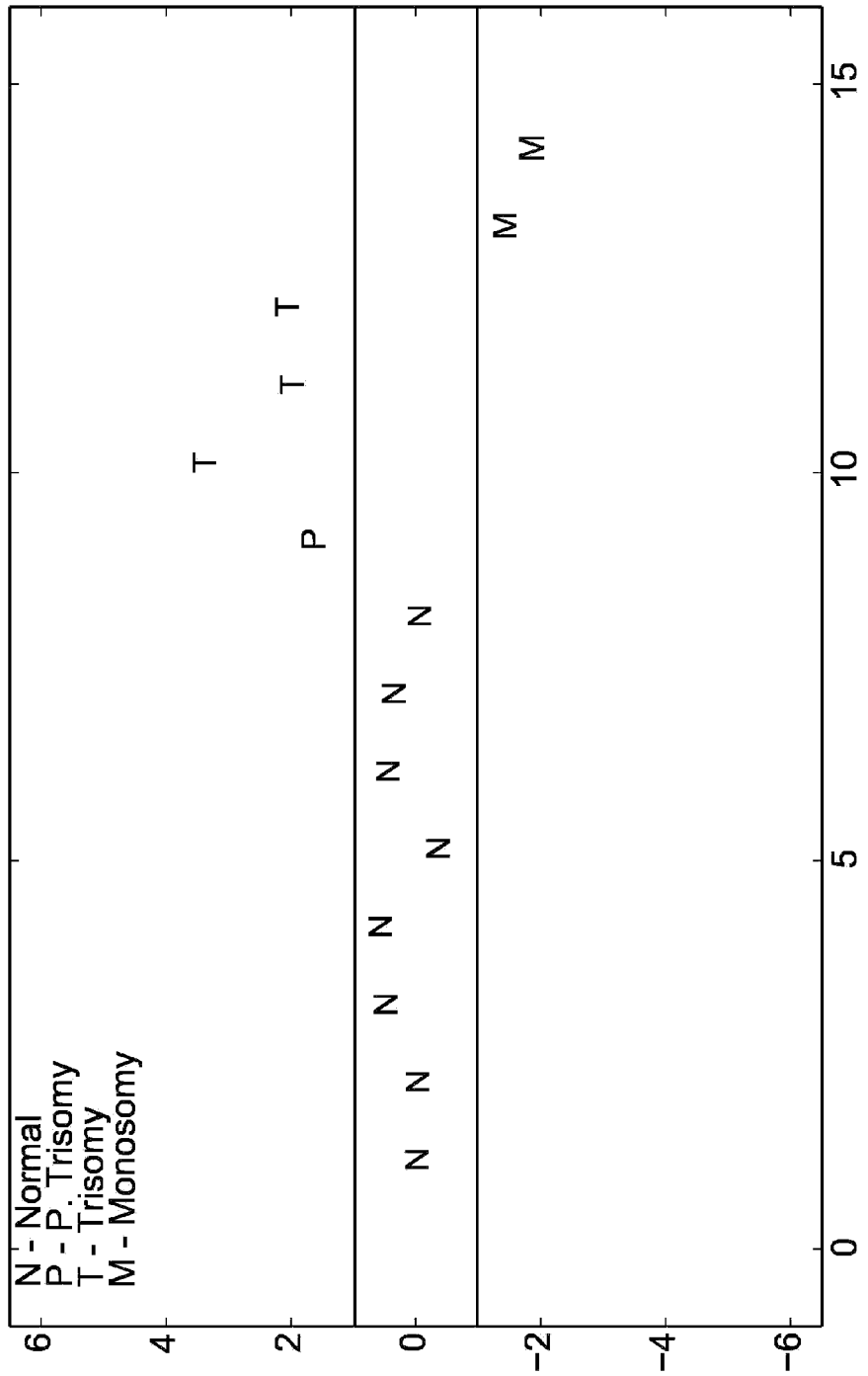

Normal Sample
Normalised Read Depth
150
100
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Aneuploidies on chromosomes 1, 2, 13, 15, 16, 19 and 20
Normalised Read Depth
300
250
200
150
100
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Genomic Location Index

Fig. 14


N - Normal
P - P. Trisomy
T - Trisomy
M - Monosomy
6
4
2
0
−2
−4
−6
0
5
10
15
N
N
N
N
N
N
N
N
N
P
T
T
T
M
M

chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	20621336	20621585	CTG CCT GCA GCC ATT ATT GT	TAA GCC GGA ATG ATT TGT AAG G	33, 34
chr13	20624392	20624641	CCT TGG GGT ATG TTT GTT ATG T	TAC CCT TTG GCT TAA CAG CT	35, 36
chr13	20625461	20625710	ACT TTT TCT GTA TCA GTC ACG G	AGT TGT CAT GTT GGG CTC AT	37, 38
chr13	20632609	20632857	CTG CTT TTG TGT GTT CCC TT	TGT CCT AAG TTA CCT GTC TGA C	39, 40
chr13	20635196	20635444	CCA GTT CCT GTT TTT CTG CC	GCC CTG GAA AGT ACT GTA ACA	41, 42
chr13	21127405	21127653	GCC TTC ACT GAT CCT ACT TTC	TGT TAC AGC CAG GCT TTC AT	43, 44
chr13	21163998	21164247	GGA TAT GGG GTA GGT TTT TGT	CAA CGA ACA CAG GGT TTA CA	45, 46
chr13	21170950	21171199	ACT CAT AGA ACT GGG GCT TT	CCA GGA CTC TCT CTT TTC TTC	47, 48
chr13	21172515	21172764	CTT TGT AGG TCC TCC AGA GA	GAC ACA TAA GAC CAC TTT AGG C	49, 50
chr13	21173774	21174022	CCT GTT TTC AGT GGG TTG AA	GAT ATG TTC TGG AGG ACT GCT	51, 52
chr13	21182580	21182828	CCA CGT TGT ACC TTT CCA TG	TGA TTC TCA CAG GCT CCT TG	53, 54
chr13	21203385	21203634	GAG ATT CAA AAC AGT GGT GGC	TGA TGG AAG TTT CTA GGT CAG T	55, 56
chr13	21217173	21217422	ACA ACA CTG TCC TTG GGT T	AAC ACT CTT GCT CCC TAT GT	57, 58
chr13	21228403	21228652	TAA ATG TCC TGT GTG CTC GG	TTT CTC TCC CAG CTT GAT CTT	59, 60
chr13	21237418	21237666	GAC AGG ACA CAT GGA GAG AG	CTG TGC AGA GAC GAA CTA AG	61, 62
chr13	21239719	21239968	AAC TGA TGG TGA TTT GCA TGT	CTC TCG GAG CAA AGA CCT T	63, 64

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	21245232	21245481	GGC TTC TGG AAA CAT CTT GC	ACC TCA TAA GTA CGC CCA TC	65, 66
chr13	21251423	21251672	CAA TAG AAG TAG GGG GTG AGG	TGA TCT TCC TTT GCT CCT GT	67, 68
chr13	21276601	21276850	AGC TGG GAT GGG TTG TTT AT	ATG ACG ACG ATG TTG GAG AG	69, 70
chr13	21296989	21297237	GCC AGT TGT CAG AAG AAT CC	TCC TTC AGC AAG CCT CTT TT	71, 72
chr13	21321012	21321261	CAA CTG CAT TCC AAA ACA GC	TGA GGG TGA TAA CCT GTG AG	73, 74
chr13	21330138	21330386	GAA GGG AAA CAG TGA GAA AGA	GGC TTG AAG TTT GTC TGT GA	75, 76
chr13	21348854	21349102	TCA AGC GCC GTA AGT ATG TA	GGA TTT TCA CAT TGC TCA GC	77, 78
chr13	21351692	21351941	TAA CCA TAA CAT CCA GGG CA	TAG CAT GAG AGT GAA CTG AGG	79, 80
chr13	21356527	21356775	TTG TAA GCT GGC ACA CTG AA	CAG CCA CAT AGC CCA TAT CT	81, 82
chr13	21361621	21361869	TTA AGA AAG TGC CGT GTT GC	TGC CCA TGA GTC TAC TTG TG	83, 84
chr13	21396343	21396591	ATG GGT CCA TGA AGA GAA GC	AAG TGG ACT GAG GGA CAA TT	85, 86
chr13	21399744	21399992	AGT TGT TTC CAG TAC TGC CA	TCA GAC TGA GCA TTA AAT CAC C	87, 88
chr13	21417352	21417601	GTA TGC TTT CAA GTG ACG CC	TAG GTC TGG AAG AAT GCC AG	89, 90
chr13	21422981	21423229	GAA GAC AAT GCA ATG AGG TGT	AGT ATC TTG GGC TTG TGA CA	91, 92
chr13	21607075	21607324	AGA CCC AAT GAG AAC AGG AA	GCT CCA CTT CCA GTC TTT CT	93, 94
chr13	21631007	21631256	TGA TGG AGG AAG TGT GAA GC	TTG GAA TAG TGA GCC TCC CT	95, 96

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	22114658	22114907	TGG CTT TTC TGT AGT TTG GG	TCT GGG GCT CTT TGT CTT TG	97, 98
chr13	22130956	22131205	AGC AGC AAG AGT TGA GAA GA	ATT TTT CCT CCC CTG TAG CT	99, 100
chr13	22189915	22190164	GGC TTT GAA AAA TCA CCA TGG	CTG GGC ACA CTG TAT TAC CA	101, 102
chr13	22270443	22270692	AGA GGT TTC CAT CGT TGC TA	CAC CAC GTT TCT AAT GCA GA	103, 104
chr13	22396225	22396474	AAC AGC CCC AAA CTT CCT AC	GAG TTG AAA AAG GTC CAC GC	105, 106
chr13	22609278	22609527	ATC ACT GCC AAC AAG CCA TT	CAG AGG AAA GAC ACA GTG CT	107, 108
chr13	22691882	22692131	TGA GTT GTG GGG GAT AAA GG	TCT TGG TTT TGA GGC TGT CA	109, 110
chr13	22765144	22765393	TCT GGG CAC TTT CCT TAT GA	ATC AGC CTA ATT CTC CCC AC	111, 112
chr13	27175379	27175628	GCA GTT TTG AGG GGA GAA GA	ATC CCC CAT CAT CCA TAC TC	113, 114
chr13	27405837	27406086	TTC ATG ATG CCA CCT CCT C	CAT AGC TAG GCC TGT GAG TG	115, 116
chr13	27646720	27646969	GCC CAA ATA CCC CTT CAG TA	TCA GCT TGC TCC TTC TCT G	117, 118
chr13	27674747	27674996	GCA CCT CAA TCC CGT ACA AT	CTA GGT CCT CAG CAG TGT TT	119, 120
chr13	27969360	27969609	GCG TTT TAA GCA GCT GTG TA	TCC CAG AGT TAA CAA TAC CCC	121, 122
chr13	27978231	27978480	TCT CCA GAT CGA AAC AGC AT	CCA TTT GCA CTG CCG ATT TC	123, 124
chr13	28003481	28003730	TCA TTC AAA GCC AAG ATG CC	ACT AGT CCC AAA AGC CTA CAC	125, 126
chr13	28073180	28073429	ACA CGC TAC ATA GAC ACT GG	AAC AGC AGC GTC AGA ATA AC	127, 128

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	28077809	28078058	CAA TCG AGG TCA CAT TCA CC	AGG TCT TTA CGG GAA GGA AA	129, 130
chr13	28212786	28213035	TGA CAC GCT GAA GAA AAT AGC	TGA GGG AGA AGT TTG GTA GG	131, 132
chr13	28302961	28303210	GGA GGG AAT GGC AGA AGT AA	CCC TGT CTA AAG AGC CAT GT	133, 134
chr13	28325874	28326123	GGA TTT TCA GAG CAG AGG TTG	GTT TGG AGT TTC GAT GCC TT	135, 136
chr13	28395893	28396142	AAG AGT TGC CTG TAC CCT TC	ATG TTT TGG TCC TGG GAG AA	137, 138
chr13	28549591	28549840	GTC TCT TTA CTG GGA GCG T	GGA AGT GGT TAG GGC AGA TT	139, 140
chr13	28592602	28592851	ACA TTG CCC CTG ACA ACA TA	AGC CCA GTA AAG ATA AGA GGC	141, 142
chr13	28608293	28608542	TCA TTA TCT GAG GAG CCG G	CCC TTC CCT TTC ATC CAA GA	143, 144
chr13	28617043	28617292	GGC TCC ATA ATC TTC TGC AAT	CTG AAA CCT TCT CCT TAG CC	145, 146
chr13	28622471	28622720	CGT ACA TCT GAT TTG TGG GT	ATT ATC CAA CCT GAC CTG CA	147, 148
chr13	28623594	28623843	TGC CAC TGA TGA TAC AAA AGC	AGG TGC AAA GCT GTT CAT G	149, 150
chr13	28941450	28941699	GAA ACG TGT GGT GTC CTC TA	AAT GCC AAG ATT GTC CTT CA	151, 152
chr13	28958898	28959147	AGT GTT TGG GGC TCT ATC AG	TGC GAA ACC TCA GTG ATC A	153, 154
chr13	28960375	28960624	CTT TCC CTT TTG AGT CCT GC	CAC TTC CTT CAG CAC ACT TT	155, 156
chr13	29003985	29004234	TCT CGC TTA CCT TGC TAC AT	CCG GCT CTC TAT GAA AGT GA	157, 158
chr13	29052686	29052935	AAA GCG GGA ATT GGA ACT TT	ACT TGT CTG TCT GCC TGT TT	159, 160

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	52293092	52293341	GGG GAA CAT TGG GAA GAG AT	CTG ATG CGC TGA AAA CCA A	161, 162
chr13	52302656	52302905	TCT ACC AGC TAC AAA CCC AT	TTC CAT GTG TTC TTC CTC CC	163, 164
chr13	53286404	53286653	TCA GCC AAT ACC CAT AGC AG	AGC AGT AGG GTT AAC AGG AG	165, 166
chr13	53306303	53306552	AAT CAG AGG AAG ATG GGT CG	GCA GCA ATG TTT CGG TGT A	167, 168
chr13	53497224	53497473	GAG AAC TCC ACC CTG TCT TT	TAC TAA TGG CTG GGG GTA AC	169, 170
chr13	53548644	53548893	GGG AGT GTG TGA ATG TGT CT	CTG ATG AGG CTA AAG GAC CA	171, 172
chr13	53652715	53652964	AAG CTT TAC ATC ATG GCA CTG	CAG AGT TCT CCA TCC CAG AC	173, 174
chr13	53861872	53862121	AAA GGT CCA TAG GCT CAC AT	CTG AGT TCC TCC TTT TGC CT	175, 176
chr13	53897429	53897678	TTG ACC AAT GCC ATT AAG CC	GCA AAA GGT GGT GTT AGC TG	177, 178
chr13	53938205	53938454	ACC AGG GAA ATG TTA GCT TCT	ATC CAC ATC CCA TGC CTA AG	179, 180
chr13	54626806	54627055	TGC TCT GTT ATG GTT GGA GTT	TCT ATT CCT TTG GCA CCT CC	181, 182
chr13	54740174	54740423	GAA GCG GCA GTA ATT CAG GA	AGG AAA GGA GAG CTT TGT CC	183, 184
chr13	54753834	54754083	AAC TGT GTC CTA AGC AGT GA	CCT TTC AGC TTC CAA GTC CT	185, 186
chr13	54768238	54768487	GTC ACC TCC AGA GCT TTC AT	GCT CTC TTC CTC CCA CTA AAA	187, 188
chr13	54773333	54773582	GAA CAA TGC AAC CTG AGA ACT	ACT GCC TGT GTT TTC TTC CT	189, 190
chr13	54910969	54911218	TGA TTG TCC TCT ACC ATG CAT	AAA GCA ATT TCT TCC CCA GC	191, 192

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	54917347	54917596	AGG CTT GAA ACA CCA CCT TTA	TGT CTG TTG CCA TTC CTT CT	193, 194
chr13	54926251	54926500	TTG ATT TGT TGG GTG GTT GG	TGA CTG TGA CTT GTG CTT TC	195, 196
chr13	55009732	55009981	TGC CAA TCT GAG GTT TTT CC	GAA TTA CAT TTC CCT GGG CG	197, 198
chr13	59785342	59785591	ACT CTG CTT TAG GGC TTC TG	TGA AAC CGT CTT CCT TGT CT	199, 200
chr13	60086302	60086551	GCA GAA AAG CTC CCA AAC AA	TTT AAA GCA GAG CAG GAC CT	201, 202
chr13	60668020	60668269	GAA AAG AGG TGG AGA GGG AG	TTT ATG ACA CAC AGA GCA GC	203, 204
chr13	60707747	60707996	AGG ACC CTT TTG CTG ATT TC	ATG TGT TTG ACC CTT TCC CT	205, 206
chr13	60975949	60976198	CTG GCC CAA GTG CAT ACA TA	ATC CTG AAG TTG TTC CAC ATC	207, 208
chr13	61177164	61177413	AAC CAG AGA GAC ACC TTG AC	GAC CCT GCT TTG TTA CTA GGA	209, 210
chr13	61495655	61495904	TCC TCC CTA TCT CCT GTG AC	TGG ACA TGG ACA TTT CAA CG	211, 212
chr13	67720914	67721163	ACT CTG CCA GAA AAG CCT AC	AAA AAT GCT TCC ACT TGC CT	213, 214
chr13	67799557	67799806	CCT TTG TCT TGA AGC CTC CT	GCA CCT CCA ACA ACA TTC AA	215, 216
chr13	70101783	70102032	TTG ACT GAG CAG AGT AGA GC	TTG GAA ATG GGG CTG GAG	217, 218
chr13	71512697	71512946	TTC TAC CTA CAA GCA AAG AGA G	ACT GGT CTA TTG GGG GAA AAT	219, 220
chr13	71821654	71821903	TCA CCA ACA GAG GAT CAA ACT	AGT GTT AGG AAA GCA GAG TG	221, 222
chr13	72405844	72406093	CCG AGG GAT AAC ATA CAG CT	TGG ACA GGG TTT CAC AAG AT	223, 224

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	74087455	74087704	CAC TAG TCA CAG AAG CAG GT	CTC CTC TCC ATC TTT CCA GG	225, 226
chr13	74192477	74192726	GTA GAA ACC CCG AGA CAA CT	ATA GGC TGA CTT CCA CAT CTC	227, 228
chr13	74260893	74261142	CAA TTT GCT GTT CAG AGG CT	TGT GGG GGT CAA TTC TAA CG	229, 230
chr13	78218064	78218313	TCA TGA TGT GGC TTA GTG GG	CCA ACG GGT AGT GGT AGA TT	231, 232
chr13	88033439	88033688	TAC ACC CAC ATG CAT ACA CA	CTT ACC CCA CTT CTT CCT GA	233, 234
chr13	90954378	90954627	GAA GTG TGC ATG GGA GAG T	CCT GTC ACA ACT GCC TTT G	235, 236
chr13	91290109	91290358	TTT GGG TGG CTC TAT GTT AGG	TTT GCC ATT TTG TGA TGC CA	237, 238
chr13	91874767	91875016	CTC CTT GAC TCA TTT CCC GT	GGA GTT TCA GGT TGG CAG AA	239, 240
chr13	92125914	92126163	AGA GTA CCA CTG CCA AGA AA	ACA GCT TGC TTC AAA CTA CA	241, 242
chr13	92308079	92308328	CTG CTA GTC TGT CAG GAG AG	TTG AAG GGG CAA AAT ACA GC	243, 244
chr13	92971372	92971621	GGG CAG CAG TAT AAA CAT CC	GCC CCA AAT TGT AAC AAA GC	245, 246
chr13	94063232	94063481	GAC ATT CCC TTC CAT TGA GC	TGA CGA AGA CTC CAA CAC AA	247, 248
chr13	94453836	94454085	TTC CTG GTA AAT GTG CTG GT	AAG TCA GGG AAA TGA AGC TG	249, 250
chr13	94713643	94713892	GGC CAG ATT TGC AGT GAT TT	GTA ACA CAG TGC TCC TTC TC	251, 252
chr13	95766126	95766375	AGA ATC AAC AAC AAT GGC AGG	GGC CCC AAT TAG CTG ATT TC	253, 254
chr13	95814599	95814848	CTC TGA GGA AAG CTT GTA GGA	CTG AGC AGG GAA AAA TCC AG	255, 256

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	111956688	111956937	AGG TTT TCG TTC TGC TTC AG	ACA AAG GAT TCA GGT GCA GT	257, 258
chr13	112517966	112518215	CGG GTC AGT GAT TCT AGC T	ACA GGT TTT GCT CTT CAG GA	259, 260
chr13	112531519	112531768	GTC ACT ATG GAA TGG GGG TT	GGC AAG TTT GTC TGG TTC ATT	261, 262
chr13	112986709	112986958	GCT TCT TCC CCG CAA TAT GA	CCA TCT GCA TCT GTC TCC TT	263, 264
chr13	113000054	113000303	CGG TTC AGA GTC AAT GCC TA	GCT CCT CTC CTT CTC CCT T	265, 266
chr13	21391429	21391678	TAC CCA ACA AGC CAG AGA AAT	TGT ATA AGG GCA ATC GTG GT	267, 268
chr13	21394616	21394865	AGA GGG AAA GTG CAA GGA AT	AAG GAA CCA GGT CAG ACA AG	269, 270
chr13	21404268	21404516	GTT TTT CAG CAC ACT GTC CC	GTT AGA AGG CAA ACA TCA TGC	271, 272
chr13	21618233	21618482	ACC ACA TTA CTC ACA ACC CT	TGC AGT CAT AGG AAA AGG CT	273, 274
chr13	21619521	21619769	AAG CCC TTT TCA TCT CCA CA	GCA GTC AGA ATG GTT TGG C	275, 276
chr13	21629602	21629851	CCA GAG CTG AGA CAA CTA CT	AGA CAT TGG TTT GGT TGG TTC	277, 278
chr13	21654127	21654376	GAA GCA ATT CCT CAC ACC AC	ACA CAA ATG AAA GCC CGT AC	279, 280
chr13	22215795	22216044	TTC AGT CAG AAT GAG GAG CC	GGT CTG CTG TTT CTC TTT GC	281, 282
chr13	22227261	22227510	CTC CTC TCC CCT CTG ATT TT	AGA GCC TTA CCA AGC TGA AG	283, 284
chr13	22243011	22243260	TTT TAA AGC GAC AGT CAC ACG	GGG ATG GTT ACT TAG TGG GG	285, 286
chr13	22255153	22255402	TCC TCT GCC TTC TAC CCT TT	TTA CAC TCG CCT TCC AAA CA	287, 288

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	22639387	22639636	TGT TGT GCC TTT TGT TCT GG	GAG AGA GGA GGA GTT GGA AG	289, 290
chr13	27183101	27183350	TTA GGA GGT AAG GCT GGA AAA A	ATC CAC GAC ATC CAA AAT CA	291, 292
chr13	27387690	27387939	CTG TCA GCA ATT TCA GGT CAG	TTG TGC AAG AAG AAA CCT GC	293, 294
chr13	30466189	30466438	AGA CAA AGG CTT CAC GGA AC	GGC CTT GCA TAA ACC ACA TT	295, 296
chr13	30977198	30977447	TTC CTC TGT GTC TTG AAG GT	TTT GTA ATT GGT CCT CGC CT	297, 298
chr13	31061235	31061484	ATC AAT GCA GGT GAG TGT GA	CAA GTA TTT CAT GGC GCT CC	299, 300
chr13	31205037	31205286	CAC TCC ACA TAA GCC TCA GA	GTC AAC AGT ATC AGC TTC CAA	301, 302
chr13	32625308	32625557	AGG ATG TAG TTG GGT GAG GA	TGG GCT TCT TTT TCA TTC CG	303, 304
chr13	34428102	34428351	CAG TCA TCA CGG GGA GAT AC	TCA ACA AGC TCT CTG TTC AC	305, 306
chr13	36054899	36055148	GAC AGA TAT TTG TGC AGG GT	GTG CAA ACA GTG ACC TCA AT	307, 308
chr13	36105686	36105935	TCC TAG CCC TTA CCT TTC CT	GCT GGG CTG CTT TAA TTT CT	309, 310
chr13	40676702	40676951	AGC CTG AAT GTC ACT GAT CA	GGA ATT GTG GGG TCA AAT GG	311, 312
chr13	40745383	40745632	ACT CAT CAC TTC TGG CTG C	CTA GTG CTT CTA CCT CCA GAC	313, 314
chr13	49281723	49281972	TCT TGT GTT TCC TGC CCT AT	AAC CAC ACA CTA ACA GGG AA	315, 316
chr13	53624527	53624776	TTG CTG TGG ATG AGA ATG GA	TCT AGT TTG CCC TCT TTC CC	317, 318
chr13	60069277	60069526	TGA GGG CAG AAA GAA ACA GA	CGA ATT GCT TCC TTG CTC TG	319, 320

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	60654975	60655224	GGG GTC ACA CAT CAC TTT TC	TCT ATC ACA GCA GGA AAT CAC T	321, 322
chr13	61151386	61151635	ATA GGG TCA CAA TCC ACT GC	TCT TCG TGT TTC TCT AGC CC	323, 324
chr13	62064603	62064852	ATA TTG AGC CCC GCA TGT TA	TTG AAG AGC TAA AGG GGG AG	325, 326
chr13	71479359	71479608	GGT TGC AGG AGA AAG AAC AT	TGT CAC CGT ACT ACC TAA GC	327, 328
chr13	39233534	39233783	TGC CAT GTA ATT GCC AAG AT	AGT ACG CTC CTT TGC AGA G	329, 330
chr13	39281667	39281916	CCT GTT CTC CAT CCC TCT G	TTA CGG GGA CAC AAA ATG GT	331, 332
chr13	39343524	39343773	TCA CAA ACT ACC CAA CAC CTA	CTG TGC TTT GCC CTT GAA G	333, 334
chr13	39444897	39445146	CAG AAT TAG TTG GGG AGC TGT	AAG TCA ACC CAT ATG CCA CT	335, 336
chr13	39469458	39469707	GCC ATC TCC TGA AAT AGT GC	ACA TTC AGG CTG TCA CAC AT	337, 338
chr13	39486237	39486486	GCA AGT GTT CCC ATC TAG AA	TC ATG TCA CTA GTT TTA TAA GGC	339, 340
chr13	39541262	39541511	ATT GAT ACC CCT CTC CCC AG	AGT AGT GAG GCT CCA AAG TG	341, 342
chr13	39597307	39597556	AAG TAA GCT GTC TCC TGG C	TGG GAG GAG TTT GCT GTT TA	343, 344
chr13	39662550	39662799	AGG TTG GTT GGC ATG AAG AA	TTC TAA GCC TGT GAC TGA CA	345, 346
chr13	39681445	39681694	AGC AGA GTT TCA AGA CAA GC	TGA ACC TGA CTT TCC TTG GG	347, 348
chr13	39707053	39707302	TTT TAC ACA GCA GGC CTC TT	GCC ATT CTA TCA TCT CGG GA	349, 350
chr13	39791099	39791348	GCA ACT CCA AAT TAT CAG GGC	AGT CTC TCC CTG AAA CCC A	351, 352

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	39882804	39883053	CAG CAC CTT CCC TTA GCA AA	AAG AGT TGG CTT GGA GTT GA	353, 354
chr13	39887397	39887646	CTT GTT GTC TTG TAG CCC TG	CCC ACA ATT ATG AAA GGA GGT	355, 356
chr13	40023278	40023527	CAC AGA TAC AGA CGT CCA CA	TTG ACC AGG ACA AAT GAG GA	357, 358
chr13	40038277	40038526	AGC TCA GCA ATT AAA CAG TCC	CAC TTT GTT GGT CTG GGT CA	359, 360
chr13	40071926	40072175	TGT TGA GAG TGC CAG AGA TG	GGG ACT CTA GGT GGG GTT AA	361, 362
chr13	40138914	40139163	CTT CCA CCT TCT GCC AAT GA	GTT TAT GCC TTG GGA TTG CC	363, 364
chr13	40171414	40171663	TTC TGA CAT TTG CAA GCA CC	GTG TGG TAA GGA TGC TAG GA	365, 366
chr13	40405751	40406000	CTG TTG CTA GTT TCT TGG GC	GAA AGT GAC TCC TCC CTG AC	367, 368
chr13	40483291	40483540	TTT TCC AGT CCC AGC ACA T	CCA GGT TCT GTT CTC TGT CA	369, 370
chr13	40562330	40562579	TCT CTC TCT TCC TGA AAC AGC	GTG AGA CAT GGT TGC TGT TC	371, 372
chr13	40590631	40590880	AAC CTC TGC TTT GTG TAG TGA	CTT TCT CCT GCT CCA CCT AT	373, 374
chr13	40633944	40634193	GAG AGA ATG CAA GGT TCA GC	CTA TGT GTG TTC CAA CCC GA	375, 376
chr13	40644042	40644291	TAC AGC ATC AAA GAG GAA GC	GGG ACC TTC TAA CCA TGT GT	377, 378
chr13	40661582	40661831	GAG GGG ATG AGG GGA AAA AG	CGG GAT TTT GAA AAG GCA GA	379, 380
chr13	40667939	40668188	CAT GAC CTC TGA CGG ATC TG	GTG TTG TCT CTC AGC TCC TC	381, 382
chr13	40684306	40684555	AGG CAA TGA GGT CAA GGA C	GTT CTC TGG TTA AGG CCC TT	383, 384

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	40747946	40748195	ATG ATG GCC CCA ACT TCT TC	CCT CTT CAC CTA TAA GCC CC	385, 386
chr13	40767281	40767530	TTC TAG ACA CTG AGG GAG CA	TAG GGG ACA GTA AGC CAG AT	387, 388
chr13	41165017	41165266	AAC GCT ACA CTT TAC GAG CT	ACG ATG GAC CTC TGT TGA AC	389, 390
chr13	41176869	41177118	TCA ACA CTA CCT GCC AAT CA	ATT CCC ATC CAT CCA TCA CTC	391, 392
chr13	41573543	41573792	TGC CGA CAC AAA AGA ATG C	TCG TTT TTG GAT GGT GGT TG	393, 394
chr13	41581284	41581533	AAA GTG TTC CTC CCT GCT G	GAA GTT CCT CCA GTA GAC TCA	395, 396
chr13	41588844	41589093	AGG AGC AAA ATA GTC TGG CT	TTG TTT GAG TCT GGG AGG AA	397, 398
chr13	41628316	41628565	ACC ACT CTT GAA TCA TTG CAG	AAT ACT GTG AGA CTG CCA CC	399, 400
chr13	41633944	41634193	CCA GCC AAT TTT CTC TTT CCC	CAG TCA CGG AAA GTA CCC TC	401, 402
chr13	41827812	41828061	ACT GTC CCT ACT GCC AAT TT	ACC ATG TTT CCC TCT GTC AC	403, 404
chr13	42280338	42280587	ATC TGG TTT GAA CTT GCC AAC	TTA CCA AGG GAC AGG ATG GA	405, 406
chr13	42349979	42350228	CAC TCT GAA TAG CTC TCC CC	CCC TAG AGG TCA AGG TAT GG	407, 408
chr13	42589616	42589865	TTA TCC GGG ACA GTT TCA GG	ATA GGC CCT GTG TGT TAG TT	409, 410
chr13	42773088	42773337	GAA TCT TTT GGC CCA CAC TG	AGA AAG TCC CCT CCA TTT CT	411, 412
chr13	42811933	42812182	GTC AGA CAC ACT TAG CTG GT	GCC AAT GCC AAA GTC AGT TA	413, 414
chr13	44211957	44212206	ACA CAT TTC ACC TTC ACC CT	GAG GAC GAG TTG AAC AAA GC	415, 416

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	44239246	44239495	GCA CTA ATC CAG GGG CTT AA	ATA CTG GTC TCA AGG TAG CAC	417, 418
chr13	44247433	44247682	CCC CTT ACC ACC ACT TCT AC	ACC AAG TGA AGC TGA GTT AAT G	419, 420
chr13	44359516	44359765	TTC AGA TCC CTT AAG CAC GC	CCC AGA TAC ACT CCT GCT TC	421, 422
chr13	44388992	44389241	ACC TAA GGC CTC AAA TTC CA	CAA ACA TGA GAG GGG GAG AA	423, 424
chr13	44493443	44493692	CAG ACC ACG GGC ATA AGA AA	AAA CAC AGC AAT GAG GAA GG	425, 426
chr13	44557886	44558135	TGG ATG TGT GGA TTT GGA GA	GAT ACT CCC CTG TGT TGC TT	427, 428
chr13	44959162	44959411	CTG GCT GTC TTC TGG GAA A	GCT CTT ACT AGG ATG GCA GG	429, 430
chr13	45043950	45044199	CAA GCA GAA CTG AGA AGA GTC	GTT TCC AGC AGC AAT CCT TT	431, 432
chr13	45115235	45115484	AGT GGA ACG AGG ATT GTG TT	CTG TGC AGA AGG GTT AGC T	433, 434
chr13	45147386	45147635	CTC CCA TCT GAA ACT GCT GA	AAA CCC CTG CTA CCC AAA AT	435, 436
chr13	47067063	47067312	ACA TCA CAA CCA CCC TGA C	AAT GCC CAG ATG CTG TTT TC	437, 438
chr13	47120595	47120844	GTG TTG ACC TGA TTT GCC AA	GAG TGG TTG TTC TCT CCA GAT	439, 440
chr13	47154937	47155186	GAA CAA AGA GGA ACA GAG CC	CAG TCT AGA AGC TCA CCC AG	441, 442
chr13	47258834	47259083	TTC ATG ATT CCA GGG TCC TC	TCT CCT CTA CCC CTA CAC TG	443, 444
chr13	47261849	47262098	GGA CGG ATT TAG TGT ACA TTG G	GGT GTA AAT GTG GCC TCT CC	445, 446
chr13	47286641	47286890	TTT CCT TCC AAC ACC ACA GA	ACA AAG CTA CAA ACT CTG GC	447, 448

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	47314093	47314342	TCC TTA GGG TTC TGC GAA AT	TTT CAC TGG GAG ACT GAT GC	449, 450
chr13	47623498	47623747	GGA AAC TCC CTG CCT TCT AC	CTG TTC TGT TCC TGA GGC TA	451, 452
chr13	48562058	48562307	TCT TCC AAA CAC CAG GTC TA	TGT GTA TCC ATT GCC TCA TCT	453, 454
chr13	48698166	48698415	ACT CAA TGG AAG GAA GGG C	GAA GAG GGT GTG TGT AGG AC	455, 456
chr13	48762184	48762433	AAG GAC TTG TGC TGT ATT GC	TGG TTG CTC TTC CTA GTT CC	457, 458
chr13	48806515	48806764	GAC GGG AGC CAG TAT TCT AC	CAA TGT GGA GGA AGC TCT TG	459, 460
chr13	48811339	48811588	GGG ATT GAG AGC TTG GTT CT	TTG CAC ACC CAA TAT GCT AC	461, 462
chr13	49119202	49119451	CTC CCC ACC AAG ATG TTC AA	TCC AAG GTT TCT CTA GCG AC	463, 464
chr13	49479756	49480005	GTT TTG GGT CAT GCA GTG TT	TCG CTA TTC TCC TTG CCA TA	465, 466
chr13	49505070	49505319	GAC AAA AAC ACT TGC CAG AC	AAC AGC CTC TTT CCT TAG CA	467, 468
chr13	49530727	49530976	AAC CAT GGC TTT GCA AGT AC	TTT TTG GCT CAG TGG GAT GT	469, 470
chr13	50387096	50387317	GGA ACC CTC TGC TAT TTT GC	CTG TTC ATT CTT CTT CAG GGC	471, 472
chr13	50960526	50960775	TAC TCC TTG TGT GAA CCC CT	TTC CCG AGC CCA TAA ACT AC	473, 474
chr13	51783208	51783457	ACC TTT ACC CCA TAC CAT CC	TGC TCA GAT TTC AGC TTC CT	475, 476
chr13	51816719	51816968	CAT TCC TTT GGT TGG TGT CC	GTC AGC GAT GTG GAT GTC TA	477, 478
chr13	51825241	51825490	CTA ATG GGC CTG TTG TTC CT	AAC TGA CTC CAT GAC CTG TG	479, 480

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	52025153	52025402	CAA CCT ACC TGC CCA TAG TT	GAA GCT GCT ACT TGG TGA AC	481, 482
chr13	52249504	52249753	CGT AGC AAA TTA TGG CGA GG	TTT TCC TCC TGT TCT GTT GC	483, 484
chr13	29111011	29111260	GGT CAG AAG GGA AAG GGT TC	TTC TCA AAT GCA ACC ACT CC	485, 486
chr13	29192994	29193243	CCA GAT TAA AAC GTG GTG CC	CTG GCC CTT CAA TTT CAT GC	487, 488
chr13	29233772	29234021	CCC ACA ACT ATA GGT CGC AT	CCA GCA GTA CCG ATA TCA GAG	489, 490
chr13	29310139	29310388	AGT TGT TCC ATT TGT ACC AGC	CAG AAG GCA GGA GAT GGA TT	491, 492
chr13	29563140	29563389	TTG GCT GCA CTT TGA GTC A	AAG CAA CCA TTT TCC TGA GC	493, 494
chr13	29580073	29580322	CAG ATG GCC CAT TGT AAC AA	TCA CCC TTC ATC TAC CCA CT	495, 496
chr13	29929175	29929424	TAT TGA GGT TCC CGT GCT G	AAG CTG GTG ACC TTC TAC AG	497, 498
chr13	29934475	29934724	AGA ATG TGA AGT GGC TCC AT	AAA ATT CTG GTT GGG GAG GA	499, 500
chr13	29939640	29939889	TTT GGG TTT GTG TGT GTG TG	CCA TAC CTC ATC TGC TCT GT	501, 502
chr13	29957853	29958102	AGG AAT CTC TCT CTG CCA AG	GGC TGT CCC TGA ACT ACT TT	503, 504
chr13	29995732	29995981	TCT TCA AGG CAG GTC ATA GG	CTT GGC TTA AAC TCT GCT CC	505, 506
chr13	30003375	30003624	GAA ACC TAA GAC GTT CCA CTG	GAC TTG AAC ACA CCC TCA GA	507, 508
chr13	30014954	30015203	GAG TGA AGG GAT TGG AGC AA	AGG AGA AGA GAC CAT TGC AG	509, 510
chr13	30114022	30114271	ATG TCT CAG GCT AGG TGT TC	TTA GCT AAG TCT GTG CGG AG	511, 512

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	30145894	30146143	ACA CTC ACA AAG CCC AGT TA	GAA GCA ACA CTG TAC ACG C	513, 514
chr13	30187253	30187502	GTG CAG ACT CAT GTT ATG GC	TCT GAT AAA GGC TGG CTC AT	515, 516
chr13	30421518	30421767	AGG ATC TCA AAG CAC CAC AG	TAA AAC AGT GCC GCT ACT TC	517, 518
chr13	30531947	30532196	AAC GGG AAG AGG GAA ACT TT	AGT GCT ATG AGT CTT GGT CC	519, 520
chr13	30778718	30778967	ATG TTC AAC AGA GTC AGG CT	GGA AAA CAT GCG GTG GTC TA	521, 522
chr13	30905760	30906009	CAG TAA CAG TCC AGG GTC TT	GCA GAG AAA TGG GTT AAG GG	523, 524
chr13	31011560	31011809	AGT CTG GGA GCC TAG AAT CA	GGT AGA GGT GGG TTA TCT GT	525, 526
chr13	31089936	31090185	CAT TGT AGT TTC AGG ACA CCA A	CTG TAA ATC TCC GGG GGT G	527, 528
chr13	31227324	31227573	ACC ACA GAA TGA CTT GCA GC	GGC GAG AAT GGA GAG AGA AA	529, 530
chr13	31430437	31430686	TTT CAC GTG TAA CAG GAG CA	TCC CAA GCC AGG ATT CTT TT	531, 532
chr13	31575060	31575309	TGC TAC AGG GAA AAT GGT CT	GAA CAA GTA CAA CCG TGC AG	533, 534
chr13	31641685	31641934	TCC ACT GCT TAG TTT GCC TT	ACA AAT GCC CCA TAT CAA CC	535, 536
chr13	31718167	31718416	ACA GGT GGG GAG AAA AGG TA	GGA AAG AGG CCT GGA GTA AT	537, 538
chr13	31723797	31724044	CTG CCA CTA CTA CAC AGC TA	TTT CCA CTG GAT GTC GTC AT	539, 540
chr13	32249897	32250146	ATG GGT CTC TGG AAT GCA TG	AGG ACA AAG TTT CAG CCT CT	541, 542
chr13	32296888	32297137	AGT TCT CCA CAG CAC ATC AT	ACT CAG GAC ACG ACT TCA TAC	543, 544

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	32311823	32312072	TCT TTC ATC TCA GCT CTG CA	ACA TCT TTG GCT CAC TGG TT	545, 546
chr13	32316395	32316644	CCG AAC AGT ATT TTG AGG GG	TCA CAG TGG GCT TCA TTC AG	547, 548
chr13	32321163	32321412	TTT GGC TGT TTC CTG TTT CC	AGC TGG AAT CTA TGT AGG ATG G	549, 550
chr13	32348262	32348511	ACC TAA CTT GCC TTG TCC TT	CTG CGG AAG GAT CTA GTC TT	551, 552
chr13	32565108	32565357	ACA GGA GAA CAA GCA GCA TA	TGA GAA GTA TTC AGC ATT TCC C	553, 554
chr13	32578964	32579213	CAG CCT AGT ATA TGG GAA CGT	CAC TCA CGG ACT TTT AGG C	555, 556
chr13	32584041	32584290	ACC CAT ATG TAG TAT CGC TCT TG		557, 558
chr13	32596887	32597136	TAT GGG TTT TTC TGC TCC ACT	TCA CAC GCC AGG TTA TTA CA	559, 560
chr13	32651705	32651954	AAA TGT GAG GGA GAG TCG TC	AAG TGG GTT TGC AGT TTG GA	561, 562
chr13	32760468	32760717	GCA GGA CCC TTC AGC ATT A	ATG TAC GTG TGT GTC CAT GT	563, 564
chr13	32829312	32829561	GAC TGG ATG ATG CAA AGG TG	AGG CGG GTT GGT CAA TAA TA	565, 566
chr13	32852622	32852871	AAC ATT TGC AGG GGG ATC AA	CCA CAA ATC CCA TCA ACA CA	567, 568
chr13	32872003	32872252	AAA GAT GCC TCC TTG TGT CT	TTT CAC AGT AAC ATC GGC AC	569, 570
chr13	33535420	33535669	AAG TTA TCT GCC CAG GGA AA	CTG ACA GCC TGC ATT TGA TT	571, 572
chr13	33634729	33634978	TCC TGG CTA GTT TTG CTG AA	TTT CCT GGA GTA AAG CGA TCT	573, 574
chr13	33660366	33660615	CTC CTT GCT TGC CTT TAC AC	CCC ACA ATC ACC CAT CTC TA	575, 576

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	33742309	33742558	AGC CTT AAT TCC CCA TGC AT	TAT CTC ACT CCA CAG CTT CC	577, 578
chr13	33760763	33761012	TTT TTC TGT GGA GTG TGG CT	ACA GGT AGT TTG GTG GTG TC	579, 580
chr13	33787480	33787729	AAA GAG TCA ACC ATG CAC TG	GCT GTT GAA TGC CAG AAC TT	581, 582
chr13	33803219	33803468	GGT GAA GCA GCC TGA ATA AA	ATA GGG TCG GTT TTG GTC TG	583, 584
chr13	33925858	33926107	TCT TTG TAC CAA GCT GCC A	CAT CAT CCC TGT CAT TCC CA	585, 586
chr13	34286418	34286667	GCT TCT ACT TTC CCC TCC AG	GTG GGC TAA GAA AAC ACC TC	587, 588
chr13	34301780	34302029	AGA AAA GCC AAC CTC CTC TT	ATT GTG GTT TGT GGC ATG TG	589, 590
chr13	34380783	34381032	CCA GGG TAC TAA AAG GGG AC	CCA GAT TCA GCC TGT ATT CC	591, 592
chr13	35504924	35505173	TTG TGG GTC AAT GTC AAC AC	GCC CAT GGA AGT AAA CAG TC	593, 594
chr13	35532835	35533084	AGA AAA GGT GGA GGA AGG GA	AGG TTT GAC ATA ATA GTG CTG C	595, 596
chr13	35538772	35539021	GGA GTT GTT TAC AGG TGG ACT	AAT CCT TTC CCC ACT CAC TG	597, 598
chr13	36233165	36233414	TAG CTT CCA ATT CAC AGG TCA	CAC AAA GCA GTT CCA TGT CC	599, 600
chr13	36343466	36343715	TTA AAT GCG CCA AGT CCC TA	GGA CAA TTT CTC ACT TGC CA	601, 602
chr13	36348003	36348252	ATT TCC TGG GTC AAG CTC TT	AAG GGG TGT TGT TAG ATG CT	603, 604
chr13	36380590	36380839	CTT GGA CCA GGA ATG CTC TA	GCA TCA CAC ACA GCA GAT AC	605, 606
chr13	36386774	36387023	AGG CAG TCA GAT CCA CCT AT	AAA ATG TCC GTC CCA GAT GA	607, 608

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr13	36410707	36410956	AAA ATT GCC TGC TGT TTG GA	GGG GGA AAA TGT GTT GTG TT	609, 610
chr13	36427833	36428082	GCA CCA TCA TGA AAC CTC CT	AAC TGT TAG CTT CTC CAC CC	611, 612
chr13	36445018	36445267	ATA GGC CAG TCT CAG GTA GA	CGA GTG TAG GTT CCG GTT TA	613, 614
chr13	36451093	36451342	AGA TTG CAG CCT ACC CAA AG	AGA ATG CCC ATT TCA GGA GT	615, 616
chr13	36568336	36568585	TTG ACT GAA GTG TTC CAG GT	ATA TGT GGT TTG AGG TCA GCT	617, 618
chr13	36576882	36577131	GCT TCT TTC AAC CAT CCA CC	GGC TTT GGT CAC ATG GAG A	619, 620
chr13	36663599	36663848	GCA GCA CTC AAC TAT TCC AC	TGA GGC AAG ATT CAG TGA CT	621, 622
chr13	37595672	37595921	CAG GAG TTA TGG CAC CAG TG	ACT TCA TCT TGA CAG CAG CT	623, 624
chr13	38283224	38283473	GAG AGT GTG GAG GCA GAA AA	CAC TTC CTC ATG ATG TTT TGG A	625, 626
chr13	38320894	38321143	GCC CAA CTT ATT TTC CAG CT	GGC CAG CCC ACT TAT TTT TG	627, 628
chr13	38361974	38362223	TCA AGC CCC TTA GAT TGA ACA	CTC AGG GTG GAG TTT CAA AC	629, 630
chr13	38380696	38380945	GCT GGG CAT GTA GAA CTC AA	GAT CTT TCT TCC CCT CCT CC	631, 632
chr13	38789453	38789702	GGG AAA TTG TCA AGG GCT TT	TGT ACA TCC ACC ACT TGT TTG	633, 634
chr13	39009512	39009761	CTT AGT TGC TGT TGT GCT TCT	AGC GGT AGT AAG AAG GCA AA	635, 636
chr13	39096011	39096260	AGT TGT AGC TGT ATC TGG GT	AGA CAG GTG ACC ATT TTC CC	637, 638
chr13	39202882	39203131	AGC TGA GTC ATG TTT AAG GC	TGT GGA ACT TTT GAG CCA GA	639, 640

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	44004660	44004909	CAA TTC AGA CTT TGC CCA AAC	CAT AAC GAA ATA GGG CCT TCC	641, 642
chrX	44005572	44005821	CAA TCA TTC CCA CAG TTC CAA	ATT TCT GCC TCT TCT CTT CCC	643, 644
chrX	80622102	80622351	CTG TGA TGG TCC ATT CAA GG	TCA TCA AGT CAC CTC TCC AC	645, 646
chrX	81576088	81576337	TAG CTG GAA ATT GCA AGG AG	TGG AAA ACT AGA CAG CAG CC	647, 648
chrX	83458870	83459119	TCT GTT CAC CTG AGC CTT T	GGA AAG GGG AAA AGG TGA CA	649, 650
chrX	83461989	83462238	TAA CTT GGA CTG TGA ACC CA	CTG TCC TCT GTC CCA CAT AA	651, 652
chrX	83972195	83972444	CGC AAC AGG ATG AAG GAA AT	TTT CTG AGT CCA TTC CCC AT	653, 654
chrX	83973091	83973340	GAC TCA CAC TCT GAA AGC CT	GGC CAT CCT GAT ATC TTC CA	655, 656
chrX	84308155	84308404	AAT TTA GGT AGC ACT GAC CCC	CAG AGA GAT GCA GAG GTT CA	657, 658
chrX	84412933	84413182	CTG GGG AAT TAG GAA GCA GA	TGT GTG TGA GCT AGC TGA AT	659, 660
chrX	84530826	84531075	ATG ACA AGG CTG GCT CAT C	GGT TTC CCA TCC TAC CAC AT	661, 662
chrX	84531356	84531605	TTA GTT TTG GCA TGT GGT GG	GGT GCT TTT GTT GCC TTA CT	663, 664
chrX	84561384	84561633	TGG TGA GGG AGT GTT CTT TT	ACT AGA AAG CAG GGT ACA GT	665, 666
chrX	85583338	85583587	TCA TTG GGG GAG TCA TTC AC	GCT TTT TCC AAC TTC TGC TG	667, 668
chrX	85584098	85584347	GAA GTG GTG TGA TGA GGG TG	CAA CAG ACA AGT CAC CTC CT	669, 670
chrX	85643367	85643616	AAG TTA GGC CCT GTT AAG CA	GGT TCT TCC TGG ACT TCA AA	671, 672

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	85643847	85644096	TGT TGG TCG GAG TCA GAA AT	GAA AGC AGT AGT TTC AGG TGT	673, 674
chrX	85644765	85645014	GTA AAA GAG GTT GGG ATG CC	TGA AAG ACT CTG TTG CCA TG	675, 676
chrX	85682212	85682461	CGT TGG ACA TGG ATC ATA CC	CAC TAC ACG CTC AGA ACA AA	677, 678
chrX	85715710	85715959	TCA CAA CAA GGG AAA TAG CCT A	AAG GCA AGC AAT AAT GAG GC	679, 680
chrX	85764768	85765017	TAG TCA GGT AAA CAA CGC CT	GGA CAG TCT GTG AAA ATT GCT	681, 682
chrX	85988749	85988998	TCT GTT TCT TGT TTG GCT GAG	ACA AAC AAC CCT TAA TGC CC	683, 684
chrX	85989214	85989463	ACA TAG GTC ACA CAA AGG GT	TGA AAC AGT GAA TCC GCA AT	685, 686
chrX	85997574	85997823	GTG TGA CAC TTT TCT GCC TT	AAG GGA AAT GTG GAT GCA GTA	687, 688
chrX	87225841	87226090	CTG GAA ATA GAA GGC CTT TGC	TTG AAG GGA AGC GGA AAG T	689, 690
chrX	91517109	91517358	TCT TGG TCT GGG AAT AAG CC	ATT TCC AGC TAA TGA TGC TCC	691, 692
chrX	91517578	91517827	TGC CCC TAT GAA CAA CAG AA	GCT CAC TTA CGC ATT AAC CA	693, 694
chrX	94601042	94601291	TGA ACG TCT TGC TTA CCC AC	ACA GGC AAA ATT CAG TTG GA	695, 696
chrX	95282210	95282459	GAA GGA AGG CAG AGG TCA A	AGG CTG AAT CAC GTC AAA AC	697, 698
chrX	95700693	95700942	CTT CCA CAA AGT CCT GCA AC	CCT CGT TCA CAT TTG ACG C	699, 700
chrX	96016317	96016566	ATG TGA ACC ATT GAG AGG CA	TGT CTG GGT TCA ACT GTT TG	701, 702
chrX	96474722	96474971	AAG AGA AAC TAC CCT GGC AA	ATT TTG CAT GCC TGT TGA GA	703, 704

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	96475190	96475439	GCA TGT AGT TCA GTT CAG GC	GCT CTC CTC AAA ACC CAA GT	705, 706
chrX	96522258	96522507	ATG AAA TGT AAT GGG GTG CG	CTT AAT GAG GGG GCA CAA AG	707, 708
chrX	96685785	96686034	TTT TGG CAG TGA TGA CCT TG	TTT TCG TCC AGT CTT CCA CC	709, 710
chrX	98226881	98227130	TTC TTG GCT TTT CTG ACC CT	TAC AGG ACC GTC AGT GAG AG	711, 712
chrX	98227467	98227716	TTG AGA AAG ACC CCA ACA GAA	TTA GCT ACT GAC GCT TCA CC	713, 714
chrX	98314697	98314946	GAA AAT AAC ACA GTA GGG ATG C	GCA CAG ACA ACA TGC TAG TT	715, 716
chrX	98331718	98331967	GAA TGG AGA GGC AGT TTT CA	TTA GTC TGT TCA CTG GCA CA	717, 718
chrX	102986351	102986600	CAC CCT TTT CCT GTT TTG CA	CTT ATC AGC AGG GCA CAG T	719, 720
chrX	112117885	112118134	AGA AGA AAC TTG CAG TGT TGG	TTG CAT CAA ACA AAG CCA CA	721, 722
chrX	116461543	116461792	TGC AGC ATT ATT CTT TCT GGG	GAG AGA CAA GTC ACC CCT TC	723, 724
chrX	116746667	116746916	ACA CAC ATA TTA GGG AAC AGC	AGT CAA CTA CAA ATG GGG GA	725, 726
chrX	117121287	117121536	GAG TGT AGG TGC TTG GGT AT	GTG CCA AAA TCA ACG AAA GC	727, 728
chrX	117494627	117494876	CCT TAG AAT CCT AGC GCC TT	AAG GAG GGA GTA CAA AGT GAG	729, 730
chrX	118400460	118400709	TCA TGA GGT TGC CAG TGT TT	ATC ACA TTT TCA GCA CGA GG	731, 732
chrX	119760145	119760394	CCC CAT ACA TCA TCA CAT GC	CAG GGA GGG ATG ATT TGG AA	733, 734
chrX	120436725	120436974	GGG TAA TGC TTT CTT GGG GA	AGA ACT GAG AGG GGA GCA TA	735, 736

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	120437307	120437556	AGT TGA GAA GGG AAG GCA AG	CAG TCA CCA ACA AAG GCT TT	737, 738
chrX	121854317	121854566	CTC CTG GTG GCT TAT TTT TGA	TCC CCA TCT CCC TAA CTC AT	739, 740
chrX	121855104	121855353	TCT GGA TTT TGG CTA CTC ATG A	TTT TTC TGC TGC ATC CAA GG	741, 742
chrX	122863038	122863287	GTA GTC CTC CTT TGC CCT TC	ACT GTA CGC CAT GAA AAA CA	743, 744
chrX	123203756	123204005	GAC ATG CAC AGA TCG AAA CC	GGC AAA TCA AGT GAG CTG AC	745, 746
chrX	123989030	123989279	CGC ATT TGA CAA CAG GGA TC	CGT GGG TGG AGA ATT TCA CA	747, 748
chrX	124394967	124395216	AAG CCA CCT GTT CTC TCT CA	ACT TAG GTC AGT TGC TTG GT	749, 750
chrX	125455385	125455634	ATT CCA ACC ATT CCG ACA CC	GAC TTC ATC AGC ACG TAC TT	751, 752
chrX	125500101	125500350	AAA GAA AAT GGT GAA CGT GC	TGC AGG CAA AAT TAG CAT GG	753, 754
chrX	125500582	125500831	TGA TCA GGG CTT TAG AGG TC	GAC TTA TCT GCT TTC ACC CC	755, 756
chrX	127973758	127974007	ACT CCC TAT TGT TCT CCC CT	CCC CAT GAA CCT AAG ACC AT	757, 758
chrX	128274367	128274616	CTC CTT GAC AGA TGT GAC CC	TCT GGA CAT GTC TTT GCG TA	759, 760
chrX	128274689	128274938	TTT TGG AGT CTG AGC CAC AA	CTC CAG GAC ATC TCA GCA AT	761, 762
chrX	128275238	128275487	TTG AAG TCC CGT TGC TGA T	CCT CTC GTG TGG GAA ATG TA	763, 764
chrX	128694864	128695113	TTG GGG TCA GTT CTA ACA GT	TAT CTC TGG CTA CCT CCT GT	765, 766
chrX	131201343	131201592	TTT TCA CCA CCT CTT CCC TC	CCC CAT CCC TGT ACC AAA G	767, 768

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	132160888	132161137	ACC TGA CCA CAA GCT TTA CA	CTG CAG AGA TAT TCC ATG GC	769, 770
chrX	133560531	133560780	CAC ATA TTG GCG CAC AGT AC	AAA GCT GGG TTC TTA GGC TT	771, 772
chrX	133847304	133847553	GCC ATG CAC CGA TGA AAA AT	GCT GTT TTA GGG GCA CAT TT	773, 774
chrX	133886077	133886326	TTG TGA GGA GAT TTC TGG GC	AAG AGG CAA TGT GGA GGT TA	775, 776
chrX	133886778	133887027	CAC ACT AAG AGC ACT GGG AA	GCC CAA ACA ATC TGC CTT TTA	777, 778
chrX	135279738	135279987	ATG ACC TAG CAC ATC TTC CC	TGG CTT CAA ATA ACT GGG CT	779, 780
chrX	135581031	135581280	ACA TTT TCC CCA TTC CAT GC	AGA GCA CAC AGA ACA GAA CT	781, 782
chrX	136941543	136941792	GTC TGT CAA CCA CAC TTT GC	CTG GTG GAT TTC TCG TCA GA	783, 784
chrX	138913313	138913562	CTG TCT CTC CTT TTG CCA AA	TAG GTG TTT GTG TGA GGC TT	785, 786
chrX	138913920	138914169	AGT AAC CTG CGA CTC TCA GT	GCC TCA CTG CTC CTA TCT TT	787, 788
chrX	139857034	139857283	TAG CAT TTA AGG AGT GGG CT	CTC TAG CAG CTG TTC CTC C	789, 790
chrX	139857318	139857567	GGC CTC CTC AGT GAT TTG AA	CGT CGT ATC TCT GGC TTT GT	791, 792
chrX	141400062	141400311	CTT TCT TTG CCT CCC CTG TA	GTC CCC AAC CTC ATC TTT CA	793, 794
chrX	141408144	141408393	ACT TCC ATT TGT GTC AAC GG	TCT TCA AAG ATG GCT GCA AA	795, 796
chrX	141408548	141408797	TCT TGC TTT GGG TTA GAG GG	AGT ATA ACC AGA TAG CCG TGC	797, 798
chrX	142517923	142518172	AAC CAC ACC TCC ACA AGA AA	TGT CCT CAG GGC AAT AAA GT	799, 800

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	143466384	143466633	CAA GAT ATG AGG GAG GGG AA	TTT GCT GCT TGA AGT GGA AC	801, 802
chrX	147766073	147766322	TCT AAC CTG GGC CCT TTC TT	GCT GCA ATG ACC TGA TTT CT	803, 804
chrX	147869477	147869726	TGC CCT TCC AGA ACT GTA AA	TCC CTC TCT CCT CCA AAT GA	805, 806
chrX	147880766	147881015	GCC AGG TCA CTT AAC AAA GC	TGC CAC AGT AGG TAT AGG TTG	807, 808
chrX	147881811	147882060	CAA TAA GGC GCC AAG TTC G	CCC TCG CCC TAA AGA AAC TA	809, 810
chrX	151796091	151796340	GTC ATC AGG GGA GCA AAT GT	CCC CTG AAT CCC TAC CTC AT	811, 812
chrX	104102171	104102420	AAC ATG CAA TCC CTG GAA TTC	TGT TGT ACA AGT GAG CCA TTC	813, 814
chrX	104124449	104124698	AGT TGT TTC AGG ACA GGA TCT	ACC AAG CAA TCA ACT CAC TCT	815, 816
chrX	104234605	104234854	TCC TCC TGC CTT TAA TAA GCT	GCC CAA TTT GTC TAG CCA ATA	817, 818
chrX	104235195	104235444	TTA CAA GGC ATC TGA CAG GAA	CTA CTG ATC CCA AAG AAG GCA	819, 820
chrX	104291380	104291629	TTA CTG GTA GGT TTG AGC ACA	GTG AAA GGT TCT ATC TGC CAA	821, 822
chrX	104291786	104292035	GAG CTA CGT TCT TTC TCA TCA C	GGC ATA CCG AGC ATA CAT AGA	823, 824
chrX	104647680	104647929	CAC CAA TTA AAG TGT GCT GCA	CAC CTG TTT CAC CAA ATC ACT	825, 826
chrX	104648691	104648940	TCC CTT CCA AAG TGC CTT ATA	TCT CAT GCT CTG ACA GAC AAG	827, 828
chrX	106886942	106887191	TGG TTG GTT TGG GAT ACT TGA	TTG TCC TGT TTC TCT TGT GAC	829, 830
chrX	3343487	3343736	GAA CCC AAA TCG ATC ATG CAT	CAC AGG ACT GCA TGC CTA TTA	831, 832

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	4013011	4013260	AGA GTT AAA CGT GCA ATG TGG	GAC TCT CTC AGC ATC GAG TTT	833, 834
chrX	5008870	5009119	GTT CGT TGG TCA TAG TTG TTG T	CTG ATG GAA GGG CAT TAT CCA	835, 836
chrX	5687782	5688031	AGT TTA GCC AAA GGA TTC AGC	TTC TGG TTC CAT AAA TCC ATG C	837, 838
chrX	5717003	5717252	TCA ACC ATT TAG AAC CAC CTT G	GGG ATT ATT GTT GGC TAC TGA G	839, 840
chrX	5808998	5809247	AAT GTC CAC TTT AGC GGA GAG	AAT TGG TGA CCT AGG GAT CAG	841, 842
chrX	5814187	5814436	GGA GTA TTC TGT TCA TGT TGG G	GGG TTT GGT AAG GGA GAA TGA	843, 844
chrX	5836574	5836823	TTT CTA GAA TTG AGG AAG GGC A	AA GAC ATC CCA GTT ATG CAT TGT	845, 846
chrX	5849776	5850025	AGG ATA AGA CGA GGC ATC AAT	AGA TGG GAG GGA GAT TAG ACA	847, 848
chrX	6895474	6895723	TTC TGT GTT GAC ATG TAC CTC T	GAG TAG CAA CAA CAC ATG GAG	849, 850
chrX	6965226	6965475	TCC AAC ATT TCT CTC TGT CCC	TTC TCC TTC ATT AGC CAC ACA	851, 852
chrX	6965573	6965822	ATA ACG TGT ACT CCT CAG CC	AGT CTG CAC TGT ACT CTT CTG	853, 854
chrX	7152743	7152992	GCA CTT GGA GGA TGT AAA GAC	ACA AAT GGT TCA TGA TGG TGG	855, 856
chrX	9529739	9529988	TGT ATG CTT TAG GAC CCA GTT	GGT ACT CAC GTT TCA GTT TCC	857, 858
chrX	9605323	9605572	AAC TCC ACA GGA ATC TTT CTG A	GTT CAT TTC TAC AGT CCA GGC	859, 860
chrX	9615158	9615407	CAT TTC TCC TGG GAC CGA AT	CTC TCA CTG TGC TGC TTA AAG	861, 862
chrX	9766329	9766578	TTC TGA AGC TGA CGA AAT TCC	CAA AGA ATT CCA CAG AGA TGG G	863, 864

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	9793372	9793621	GTT GCT TAG TCC TTG CTT CAC	TGA GCT ACA GAC AAG ATT GCA	865, 866
chrX	9849298	9849547	AGG TCA TTG GTC TGC AGT TAT	TCA CAT GGG ATC GAC ATA TGC	867, 868
chrX	9850182	9850431	AGC ATT TAG AGA ACA GCA GTC	CCA CAC AAT TTC CTG GCT ATG	869, 870
chrX	10002581	10002830	GTG TAA GAA GTG GTT GGG TTT	TCT TGC TTC TGG AGA GTT CTT	871, 872
chrX	10301512	10301761	AAA GGC AGA GCA GTG TAT TTA G	AGG TTA TGC AGA CTT CAG GAA	873, 874
chrX	10354762	10355011	AGA CAA GAG AAC AAT CAG GTG A	AAG CCA ATT CTG CCT CTC TAG	875, 876
chrX	10375361	10375610	CAA AGA AGC TCT AGG ACA GGA	TCC CTT CCT TAT TCT GGC AAC	877, 878
chrX	10384260	10384509	TGG TGG AGG AAA TCA ATG TTG	GCT TCA AAC ACT CTA AAG GGC	879, 880
chrX	10398645	10398894	CAC AAG GGA GGA AAC GTT CT	TGC CAT TAA TGA GAA GTG CTG	881, 882
chrX	10572719	10572968	TGA GAT TTA GTG CCA GCT AGA	TGG CAA TCC TGT TAA ACA ACT C	883, 884
chrX	10579800	10580049	AGG ATT AGT TTG GCT CCT CAG	CCC GAA CAT TGA TAA CAG AAG A	885, 886
chrX	10580482	10580731	CCT GCA CTA TTT CCT CAA AGC	GCA ACT CAG GAA AGA CTA CAT C	887, 888
chrX	10627502	10627751	CAA TTT CCT TCT CAC TGA GCC	TTA AGA AAG TAC CCA TCC TCC C	889, 890
chrX	10645064	10645313	ACG CTT CCC AAA TCT ATC TGG	GTC ATG CCT TAC AAC TTA GCA	891, 892
chrX	10669664	10669913	CTT ATT TGT GTG CCC AAT ACC A	AAT CTT TGC CAA GGT ATG AGC	893, 894
chrX	10709674	10709923	TTG CAG CAG GAA CAC CAT AAA	GCC ACT TAT ACC TCC AGA CAT	895, 896

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	10710087	10710336	CTG TTC ATG TTG CTA CCA CAG	CCA CAA TCC TGA ATG CCA TG	897, 898
chrX	11139863	11140112	CTG CCT TAG ATT CAC TTT CGG	CTG CAA GGT ACA ACA CAA GTC	899, 900
chrX	11658912	11659161	CCA TCT GTG AGG TCT TCT TTG	ATC TCT GTG CCA GCA AGT ATT	901, 902
chrX	11681358	11681607	TCC TTG GTT GTG TAT TTA GCC	ATT GGG AAA CTG TCA CTG ATG	903, 904
chrX	11686196	11686445	AGA CTC AAC TCA CAT TGG CC	GCC CTA ATA GAG AAG CAA AGC	905, 906
chrX	11686913	11687162	GGT CTG ACT CTG TGG TTT GG	GGG AGC CAA TCA GAT AGA AGT	907, 908
chrX	11687182	11687431	TTT CAT TTC ATC CTG CCC ATG	GGG AAG TTG GGC TAT TTA ATG C	909, 910
chrX	11917847	11918096	TTC TGT TAT TCG CCA TCA GTC	AAC TGT GTA GAG CGA CCA AAT	911, 912
chrX	12016474	12016723	GAA TTG GGA ACT TGG GAA GC	CAG TAA GGC CAT GGT CTA GAT	913, 914
chrX	12158815	12159064	GAA CTT TGG AGA GGA CAG TGT	CTC AGA ACA TTT GCA CCT TCT	915, 916
chrX	12485152	12485401	AAC TGT CAT GTG TGT CTG CTA	ACC TGA TAC AAT GGA GCA TGT	917, 918
chrX	12608017	12608266	TGA TTC CTT CCA CCT ACC AAA	GGG ACC TAA ACT CCT TTG GAA	919, 920
chrX	12724960	12725209	GCC AAG GTC CAT TAT CTC AAG	TCT GCA GTG GTG TTA TCT AGT	921, 922
chrX	12841791	12842040	GAA CCT GCA TTG TCA TTC TCT	CAC TTA AGT TTC CAC GCC AG	923, 924
chrX	12904558	12904807	AAG AAC ATC AAC AAA CTC CAG G	GTA ATG GCG AGA GGT TAA AGC	925, 926
chrX	12937788	12938037	TCA ATT CTC TTT CAC ACG TGC	TTT AGG TAT CGA AGT TGG GTC A	927, 928

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	12938100	12938349	AGG TGC TGG ATC TTG AAT TCA	GAT AAG TTT GGA AGC TGC ATC A	929, 930
chrX	12992266	12992515	TCC CTC TAC CCG AAT CTC TTA	AAG CTC TGC CAT TGA CTT TAC	931, 932
chrX	12995926	12996175	TGG GTT TAA AGG ACA CTA GCA	CCC TAC AGA ACC GAG GAA TC	933, 934
chrX	12997273	12997522	CGA AGG TCA CAC AGT TTA GTC	TCA ATC TTT GCA TAC ACA GCC	935, 936
chrX	13293562	13293811	ATG TGC CTT GTT GAT TGA TGG	GTA GGT TTA CAT GGA CAG ATG C	937, 938
chrX	13338207	13338456	AGA TGG TAT GTC ACA AAG CAC	TCT TCT GTT TAG TGC TGT GGT	939, 940
chrX	13340422	13340671	ACA CTT TGG AGA GCT TCA GAT	CAA GTT CAT TTC TTC CCT GCA	941, 942
chrX	13351532	13351781	GTG CCC AGA ATT ATT TGT GTC T	TGC AGG AAT ACA TGG TAG ACA	943, 944
chrX	13624857	13625106	GCT CTC TTG TGG AAA CGA TTA	CAT AGG CCT TCA TGT CTC TCA	945, 946
chrX	15525635	15525884	AGT TTG TTT CTC TGG CCT ACT	TAT GAG GGT GCA CTA ACA GAT	947, 948
chrX	15543469	15543718	ACC ACC TCA AAG ATT TCA TGG	GGT GCT ACT ACT GGT GTA TGT	949, 950
chrX	15543853	15544102	GTC TTC ATC TAT TTC GTG AGC C	GCC ATG CAA TAT CAA ATC CCA	951, 952
chrX	15844810	15845059	TCA TCC CAG ATT CAG AAT GCC	GAA ACC AAA GAC TAG TGC AGC	953, 954
chrX	16860721	16860970	CGT TCA ATG AAG TCC CTT GTC	ATG ACT AAC ACT CTG CCA AGT	955, 956
chrX	16861729	16861978	TTC ACA AGA ACT CTG CTG GAT	AAC AAA TGC ATC CCA GAC AGA	957, 958
chrX	18307677	18307926	GAA GCC TTC TAG TGG GAC TAA	GCA GAG AGG AGT ATG TGG TAT	959, 960

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	18600682	18600931	GTT AGG GTC ATG GGT CAC TTT	ATG GGT CAT TCT ACG AAG CAA	961, 962
chrX	19376384	19376633	GCC TTT GTA GAG TGG ACT TCT	TGG TGT GTG TGG TGA TAA TTA G	963, 964
chrX	20026004	20026253	GGC TTC TTG ATA CTG CTT TCC	AGT GAC CCT CTG AAT AAC CTG	965, 966
chrX	20627108	20627357	AGG TGT GCA ATA CTC AAG GAA	TCA GCA AGT AAA CCT GAG ACC	967, 968
chrX	21537202	21537451	AAG GTC TTA GGA GTG AGG ACA	GTA CCT TCA CCC TCC AGA TC	969, 970
chrX	21629967	21630216	TCG TGC TAT TTC AGT CAG ATC T	TCA TCA AAT TGC CCA CTC CTA	971, 972
chrX	21887062	21887311	TGT CCA GCC GTA ACA TTT CAT	CAT CAG ACT GTC TTG CCT TTC	973, 974
chrX	22202443	22202692	AAT GGA CAT CTT TCA GGT CTG	CAT TCT TGC TGA CAT TTC CCA	975, 976
chrX	23018625	23018874	TCA AAT TGG GAT CGC ATT AGG	GAA GAT CAG GGT ATT GCT GAA A	977, 978
chrX	23019006	23019255	ATG CCT GGG TTT ATT CAT CTT G	TTT GTA GGT CAT TCA GCC TCC	979, 980
chrX	24521525	24521774	GGA GAA GTT TGG GTT TGA TCC	TGG CTA GGA TTC ACT TAG GAA A	981, 982
chrX	24522047	24522296	TGA TAG GAG CCA TCA GTT CTT	GCA AAC AGG GTG AAT TAT GCT	983, 984
chrX	25401534	25401783	AGC AGA TGT TGT TAG CTT TCC	AGA AGT CTG GGA AAC GAA GAG	985, 986
chrX	25403300	25403549	TTC TCT GTC ACT TCC ATG AGG	CAG ATG CTC CAT TAC TAG GTG	987, 988
chrX	28689766	28690015	CCA GTA ACT TAT TCT GCC AGA G	CAC ATG GAG AAA GGT GAA TCA	989, 990
chrX	28693600	28693849	TGA GAG ACA AGC TGC ATT ACA	TCC CAT CCA ATA CTG CCT TC	991, 992

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	28717026	28717275	GCA CAG AAA TTA CAG TTC ATG G	TCT GGC TCA AAG GAT CAC ATG	993, 994
chrX	28720131	28720380	GCT CTC GTA TCT GAC AGT GAA	AAG ATC CCA TTG ACC CTG AAT	995, 996
chrX	28808128	28808377	CAG GCA TCT TGG TTT GTA GTG	CCA CAG GCT CTC TAG AAC TAA	997, 998
chrX	28824813	28825062	CGT GAT GAA CAG TGA TGA CTT	TTG AGA GGG TTT ACA AGG TCC	999, 1000
chrX	28825844	28826093	CGC CAT TTG TTC TCC TAT TCA	CTT CTC CTA CTC TGC ATT CTC A	1001, 1002
chrX	29487025	29487274	TTC GTT AGC TAC TGG GTA CTC	GAC ATT AGT GGA TTC AGG CCA	1003, 1004
chrX	30847194	30847443	CCA CCC TTT ACA CCT ATC CAA	GCT GAA GTG GAG GCA ATT AAC	1005, 1006
chrX	32660941	32661190	AGA GTG CAC AAA GGA GAA GAC	TAT AAC TGT TGA GTC TGC CCA	1007, 1008
chrX	32949975	32950224	TCT ACT GTG TCA AAG CAG ATT G	ACT GAG CTT ACA TTC ATG CAC	1009, 1010
chrX	35971182	35971431	TTC TTC CTA GCC TTC CTT TCC	GCC ACT TTC TCT GCA AAG AAT	1011, 1012
chrX	35971544	35971793	TCT CTG GCT GTG CAG TAA ATT	ACT TCC TAC GGA CTC AAA TCT	1013, 1014
chrX	43599991	43600240	AAA CTC CCA GCT TTA ATC CCT	GGC TCT CAT TAC AAT TGG CTG	1015, 1016
chrX	43600262	43600511	AAG AAT GGG TGA GTT GGG TTC	ATT GCT TTC AGT GGT GGA TTG	1017, 1018
chrX	43807492	43807741	GGA AAC TGA ATT GCC AAG TCT	GGA ATG AAA CAG AGG AGT CCC	1019, 1020
chrX	63137429	63137678	CTC CCA ACT TTT ATG CAG CC	AAG ATG CTA GAA ACC CAC AAG	1021, 1022
chrX	63137928	63138177	GCT CAG GGA ATA TCT TGG GAA	AAT GGG TGG GTT ACA GAG AG	1023, 1024

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	74269728	74269977	ACA TTC AGC AAG TAG GAA GGA	GCA TTT GGA CAT GAA CAA GC	1025, 1026
chrX	74270926	74271175	CCC AGA AGA GCA GTA ACA AC	CAT TGG TGG GTG GAT AGC TG	1027, 1028
chrX	74282022	74282271	GAA AAA GGG GGA TAG GCA TT	ACC AGG AGG AGA AAA GCA AA	1029, 1030
chrX	74329664	74329913	CCC AAC AAC TGC AAT AAA AGG	CGG AAA ACA AAC CCT GAA GT	1031, 1032
chrX	74344769	74345018	ACC GAA ATT GCT TGC TCT TA	ACC TGC ATA TTG AGC CAT AC	1033, 1034
chrX	74741441	74741690	CCT TAG TGT GAC AGG ACA GG	TGT TCT TTC ACT TTT AGC CCC	1035, 1036
chrX	76192017	76192266	CCA AGA CAA CTA GGC CAA TG	TTT CTA GAA CCC TCA GCA ACT	1037, 1038
chrX	76194203	76194452	GAA GAG ATG ATG CAA AAG AGC	CCC CCA ACA GTT TTT AGT GGT	1039, 1040
chrX	76221949	76222198	TCC AAG CAA GGG ATC TCT TC	AAG GCA AAA CAC TCC CTT TT	1041, 1042
chrX	76226569	76226818	GAA TGG TCA GGG AAG GGT TT	CAG TGA TTG CCT CTA GAA AAG G	1043, 1044
chrX	76234682	76234931	AGT CTT CAG CCA TCT TCC TG	CTA AGC AGA TTG AAG CAG CT	1045, 1046
chrX	76308591	76308840	GCA TTT CCA GGC TTT ACA AGT	TGT CCC CAG GCT TAA GAA TC	1047, 1048
chrX	76647025	76647274	CTC TCT CTC CCT GGT CAG AT	AGC CAG AAT AAG CAA CTG TC	1049, 1050
chrX	76711476	76711725	CAG CAA TTC TCA GGC TCA GA	TTT CTC CTA TCC CAG CTT GC	1051, 1052
chrX	76759387	76759636	TCT GAA ACA AAG CCT CCT TAG	AGT ACA GAG GAT AAC AAG GGT	1053, 1054
chrX	76778190	76778439	AGG ATA AGG TTT CCC ATG CTC	CTC CCA TCA GTA CCC TCT CT	1055, 1056

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	76778847	76779096	ATG TAT CTG AAG GAG CTC TGC	GGA ATG CCT AAA CCA TAC TGT	1057, 1058
chrX	76875971	76876220	GGC CTT CAT CAC AAA CAA CA	AGT GGT ATT TCA ATG CTC TAC C	1059, 1060
chrX	76923678	76923927	TGT GTC CCA TCT ACA AAG CC	GCA GTA CAT CGT CCT GGA A	1061, 1062
chrX	76937441	76937690	TCC TTG AAC TCT TTC CAA GC	CCT GAG CGA GAA GAA ATT TGT	1063, 1064
chrX	76938848	76939097	TGT TTT CCT GTC CAA GTC CA	GGA ACT GAA CAA GAA GTG GAG	1065, 1066
chrX	76939481	76939730	CAG CAT CCA TCG CTC GAA A	ACC TAC TCT TAT TCC GCA CT	1067, 1068
chrX	76949897	76950146	GGT ATT GGT GGG GGA AAT GA	TGA CAG CCT CTC TCT TCA AT	1069, 1070
chrX	76951314	76951563	GCA CTG CTG TAA AAG ATC TAT GAG		1071, 1072
chrX	77040328	77040577	ACT CAA AGG CAC ATT TCG C	GCT GTT TTT CTG TGT GCT TC	1073, 1074
chrX	77168344	77168593	ATT CTA TTC CGA TCA CAG CCT T	ATG TAT TTC CTT TAG CGC CC	1075, 1076
chrX	77634270	77634519	CTA CCT GAC AAA TGG AGC TT	AAT TTT GCA AGA CTT CCG GT	1077, 1078
chrX	78176076	78176325	GAA ATG GCC ATG TGT ACT GAG	TCC CAG TTG TGA ACA TTT GC	1079, 1080
chrX	78176555	78176804	GAA GCC TCT CAA GCT ACA AG	ATG GGT TTT TGC ACA GAT GAC	1081, 1082
chrX	78178901	78179150	GAA TGA GAT TAG GGA GCA AAG T	GGG AGT CAG AAG GAG GTC A	1083, 1084
chrX	78208245	78208494	GGA AGT AAG AAG AGT GCT GC	CCT CTT TTT GCA TGA ACC TGA	1085, 1086
chrX	78290291	78290540	GCT CCT GAT TGA AGA AGT GT	CCT TAC CCT TTC CAC TCA GA	1087, 1088

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	78401753	78402002	CTG CTC CTT TGT CTC CTG T	GAC TGG TAT AAT CTT GCC GTG	1089, 1090
chrX	78403248	78403497	TAA GGT GAG AGT GTG AGG AAG	TGT CTG CAT CTT GAT CTC TGG	1091, 1092
chrX	78405536	78405785	CCA GGG GAA CAT TTA CTC AGA	GGT TCC ACA GCA TTT GAG C	1093, 1094
chrX	78411791	78412040	AGG GTA CAT GTA AGG CAG CT	AGG AGC CCT TAA CTA TGG TG	1095, 1096
chrX	78427326	78427575	TTC TGT GGC ATT GTG TCT TG	TGG CTT GGG TAT TGC AGA TA	1097, 1098
chrX	78430991	78431240	CCC CAA ATT TAC CCC ACT CT	GGG GAC AGT AGA AGA TGA GT	1099, 1100
chrX	78431372	78431621	ATC CCT AGC ACT TTC AGG AC	AGT TGT TTC TGG ACG GAC TT	1101, 1102
chrX	78437851	78438100	TTA CCA ATC CAT CCA GCC TG	AGA ATT TCC CTG TCC ATA CCA	1103, 1104
chrX	78526539	78526788	AGA AGC TAA ACA GGT TGC CC	AGT GAG TGA ACT TGC CAT CA	1105, 1106
chrX	78531686	78531935	CAT AAG AGC ACA GCC AAG AT	GGC TTT TCT CTG CAC TGA TT	1107, 1108
chrX	78996675	78996924	TGT CCT GCC ACT TTT ACA TC	GCA AAG GAA ACA GGC TAA CTA	1109, 1110
chrX	107682036	107682285	GAG CTA AGC AGA ACC TAA GGA	ATG GCA GCT GAA TCG ATA TCT	1111, 1112
chrX	107683930	107684179	TTA AGC AAC AGG AAC CTA CCC	TAG GTG ATG GGC AAA TTC TCA	1113, 1114
chrX	110366745	110366994	GCC AAG GGT AAT CAT AGC AAC	GAA ATG CCT TCC CAC TTA CAA T	1115, 1116
chrX	110367927	110368176	TCA TAA TGA AAC CCT TGC TGC	ATT GGC AAA TGT ACC TGA AGC	1117, 1118
chrX	110368330	110368579	AAT CCT AGT GCA TGA GAC TCC	CAA TGA TTG CTC TTG TGC CAA	1119, 1120

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	110443307	110443556	TAC TGC CTG CAT CAT TAC CAC	AGC TGT CCT CCT CTC CAT ATA	1121, 1122
chrX	110466084	110466333	ATT AAC AAT GAG GAG CCA GGT	GGC GTT CAA GTT ACT CGA TTG	1123, 1124
chrX	110537791	110538040	GAG AGA TGG TTG AGA AAT GCC	TAG TGA CTA GCT TTG GAG AGT	1125, 1126
chrX	110538060	110538309	ACT ACA ACC ACC AAT TAC AGC	AGC CAA TGA TCC CTT ATG ACT T	1127, 1128
chrX	110538444	110538693	AGT GAC ATT TCC AAG GGC TTT	AAC TAA ACT GGT AGG CAA TCG	1129, 1130
chrX	110575740	110575989	GGA GAT GGG AAG ACG ATT AGA	CAA TCA GAC CAC AGG AAG GAA	1131, 1132
chrX	110576099	110576348	AGT CTT TCA GTC TTA CAT GGG T	GAT GTG TTT ATT GCC TGT GGT	1133, 1134
chrX	110950649	110950898	TAG TTT CTG TGA TCC TGG CAG	GCT CCT TTC ATA GTT TCA GGG	1135, 1136
chrX	110971144	110971393	TTT CTC CCA GCT GTT CCT AG	ACA CAC TGC AGT TCT CAC TAT A	1137, 1138
chrX	121597454	121597703	TAC GGA ACT TCG AAT CAA CTC	TGA ATC AAG TGA CAT GAC AGC	1139, 1140
chrX	135558463	135558712	TCA AGT CAC CCT CAT TGT AGG	TTC TTA CAG TCC TCA GCA CTT	1141, 1142
chrX	135570815	135571064	TGT AAC GTG GAT GTG AGA TTG	CCA CTA GGC TGC ACT AAT GTA	1143, 1144
chrX	135760826	135761075	TTG AGC TAA GTC TGC ATC ACT	CAC TAG CTT CTG TAA CTG TGT G	1145, 1146
chrX	135831981	135832230	CTA GAG AAA GCA ACG CCT AAG	GTC TCA CAC TGC TCA TTT CCA	1147, 1148
chrX	136044394	136044643	AGC ACC TTC CAT AGC TTC TTT	CAC TAG GGT TCA TCA GCT GTT	1149, 1150
chrX	136318161	136318410	GTG TCT TCT GAT GGC CAA ATG	CAA CCA ACA TTA GAG TGA CCC	1151, 1152

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	136320360	136320609	AAT CAG GTG AAA GGT ACC TCC	GCT GGT TGA GAG AGA GAG AGA	1153, 1154
chrX	139380500	139380749	CCT TCA CAA TTC AGG GAA CAA	ACA CCA GCC GAA TAC AGA TTT	1155, 1156
chrX	139381074	139381323	TGG ACC CAG TTC TAT GCA ATT	TGC TTC ATA CCT TTC TGC TTC	1157, 1158
chrX	144897923	144898172	AAT GAC CCT TAC AAC TCC GAA	ATT TCC CAT GCC TTT CAA CTC	1159, 1160
chrX	144908837	144909086	TTT GGG AAG TGA TTG TGA AGG	TCT CTC AGT AGG CGT CTT TAA	1161, 1162
chrX	144958353	144958602	TCA GCT GCA TAG ACC TTG TTT	TGA CTG CTA CGC TAG ACT TG	1163, 1164
chrX	144959377	144959626	TCA TTT GTT CTC ATT ACG GGC	GGA TAG AGG AAA CCC AGG TG	1165, 1166
chrX	145799354	145799603	TTC TAC CTG GGT TCT CTT GG	GGC TCT TTC AAA GTA TCC AGG	1167, 1168
chrX	145922717	145922966	TAA CTT CCT GAG CAC ACA TCA	AGA CTT TCT TTG TTG CCT TCA G	1169, 1170
chrX	146994477	146994726	GGG TAA ATT CAG GAA TGC ACA	GCC TCA ACA ATT CAG TCC AC	1171, 1172
chrX	146995311	146995560	ATT TCT TCT GGT GAG TTT GCG	CAT AAG TTG CTG GAA GAG AAC A	1173, 1174
chrX	147540977	147541226	CCT CTT CCT GAC ATG TTG TTG	TCG ACT GCT TTA AGT GAA GGA	1175, 1176
chrX	149764787	149765036	GTT GGT GCC AGA TTG TAA CTT	ACC AGT GAT TAG TGT TTC TCC T	1177, 1178
chrX	150808513	150808762	GCC TGG CTC AAT AAT AGT CCT	CAA GAC ACA CAC AAA CAC ACA	1179, 1180
chrX	150809248	150809497	ATG TTG CCT TCT CTA CGT TTG	GGT AAG AGT TGC CCT AAT GTC	1181, 1182
chrX	150942801	150943050	GAG TGG AGC GCT ACC TTT AT	GCT TGG CTT CTC ACA AAT GT	1183, 1184

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chrX	79712826	79713075	AGA ATG GGA AAT GGG AAG GA	GTT TGC CAG TAG AAA TGG GA	1185, 1186
chrX	80374192	80374441	CAC AAT ACA TGG GCT GCT TT	GTA AAG TGT TCA GAG GAC GG	1187, 1188
chrX	80491243	80491492	CCA CTC CAA CTC TGC TTT TAC	ACT AGG GAG AAG AAT TGG CAG	1189, 1190
chrX	80514786	80515035	GAT GTG GAT TGT CTT TGT TGC	CCA GTT CAT TCC AGC TTC CA	1191, 1192
chrX	80553311	80553560	AGC CTT TCA TTG CAC ATT TCA G	GAT CGC CAA CCT GTT TTA TAA G	1193, 1194
chrX	104028154	104028403	TCC CAC CAC AAG ACC AAT TT	GCT GTC AAA GTG GAG ATA ACC	1195, 1196
chrX	104039199	104039448	GAA GAT AGG TGG TGG AGT TCA	AAC GCT TCC ATC CAC CTA ATT	1197, 1198
chrX	104101675	104101924	CTG TTT GAA TGA AGT TGG CTG	ACC TGC CTG TCT TAC CAT TAA	1199, 1200
chr21	17021878	17022127	TAT ACA TGG GTG GGA TTT GTC	GAT ACT TTC CTT TCT CCA GAT CT	1201, 1202
chr21	17046718	17046967	TTT GTG ATG GAC CAT CTA ACC	GGC CGC AGT TTT TGA TTT AG	1203, 1204
chr21	17088576	17088825	GAA GAT CTG GTG TCC CAC TA	TGG GAA GGA CGG TTT GTT A	1205, 1206
chr21	17134784	17135033	ATC TGA AGA TCT CCG TGG TA	TGA ATC ATG CTG TGG AGA AC	1207, 1208
chr21	17161709	17161958	ATG TAT GAA CCA TTT CCT GCT	ATA GAA GAG GTA CCC AGC AA	1209, 1210
chr21	17183683	17183932	AGC ACA GAA TTG AAT GAA GGA A	CCC TGA CTA TGC TAA GTT GC	1211, 1212
chr21	17301983	17302232	GCA TGA GTA AGG CTG AAG TG	TGC AAG CAA AAT GAA ACC AG	1213, 1214
chr21	17302814	17303063	AAA GGC TTA TAT TGC TTT TGA ATC A		1215, 1216

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	17336649	17336898	TTG CTG TTG TTG GGA TCA AG	CAG CTG CTC CTT CTT TAG C	1217, 1218
chr21	17346228	17346477	GCT TCT GCA TCC ACC TAT CT	TAT ACT GCC AAA GGT GAC CT	1219, 1220
chr21	17348050	17348299	AAA AGC TGC CTA AAA TGC CA	GAA GCA TAA TGA GAA CCT CCA	1221, 1222
chr21	17351309	17351558	AGT CAG CAA GTT AGC AGA AA	AAC CCA ACT TGC AGA CAA TC	1223, 1224
chr21	17362239	17362488	CCC ACA TTT ATC CCT TGT CC	ATC TGT TTG CTG TGT CAG AA	1225, 1226
chr21	17367713	17367962	TGT CTG ATT CCA TCT TTC CC	TCC AGA TAT ATT CAA AAG GGA GA	1227, 1228
chr21	17506670	17506919	CAG CCA CCA AAA CAC AAT G	AGG TTT TTA TCA AAG CCC CA	1229, 1230
chr21	17548792	17549041	AAG GCC TGT TTT GTG TGT AG	GGG ACT TGA TGT TCT AAG CAA	1231, 1232
chr21	17591325	17591574	ACA GCT CAC AGA TCT TTA AGC	CCT ACA TGA TAC GCA CAG TC	1233, 1234
chr21	17695933	17696182	GGC ATT ATA AAG AGA TAG CTC CA		1235, 1236
chr21	19087043	19087292	TGT TTT TCC TTG CCC TGT AA	GTT TTT CTC TAC CCA GCA CA	1237, 1238
chr21	19178365	19178614	TAA CCA GAT GAA TGA GGG CA	CTC TAA TTT GCC ACC CTC TTT	1239, 1240
chr21	22579319	22579568	CAA CAA GCC AAA ACC ACA TC	GAA CGC TAA AGC TTT TCC CA	1241, 1242
chr21	22605212	22605461	GTT GGT TCT TGA AGA CCT GA	TCT TTC TCC TGC CAA GTA GA	1243, 1244
chr21	22964667	22964916	TGT GGT AAG TAG TCT CTA AAG A	ATA ACA CTG TCC TTC TGG GC	1245, 1246
chr21	22984832	22985081	ACC AAA TTT CCA GAT CAC GG	GAG CCT ACT CTC TGA TAC GA	1247, 1248

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	22985386	22985635	AAT GAA TGG TCA TGG CTC AC	CCA GTT GTT CAC TTC TCT GAT	1249, 1250
chr21	36076596	36076845	GCC CTG AAG CAC ATT AAA GT	GCC TTA AAA CCA AAC TGT GT	1251, 1252
chr21	36169368	36169617	GCC TCC AGG TTT ATG ACA AC	CTG GAC TTC AAT CAC CCA AG	1253, 1254
chr21	36789088	36789337	CAG CTC TAT TCC CCT TCT GA	TGA ACA AAT TGC TGT GCT GA	1255, 1256
chr21	37939587	37939836	CCT TGG AAG GGA AAG TTG AT	CCC CCT AAA TGA AAG TGG TC	1257, 1258
chr21	37959766	37960015	GTC CCA CCC TGC TCT TAG		1259, 1260
chr21	37983316	37983565	CAG AGT AAG ACA GTG GGA CA	TGA GTG CTT GGA ATT TTG CA	1261, 1262
chr21	38559539	38559788	AAG TGT GGT GCA TAA AGG AT	GGT TGG CTA CTT CAT GGT AC	1263, 1264
chr21	38751525	38751774	CTG TAA GAA GGA GGG TTT GG	TCA CTG CAT TCC TAG AAC CT	1265, 1266
chr21	39173262	39173511	GAG GTT GAT GAG AGG TAG GG	ATA ACT CAG GCA AAA TGG GG	1267, 1268
chr21	39285267	39285516	AGC CAG GGA TAT TGT TGA AG	CCT TCT GCT CTC ACT TTA CG	1269, 1270
chr21	39330340	39330589	CAG GTA AGT GTG TGT TCC AG	GGA TAC TAG CAG AGG TGG AG	1271, 1272
chr21	39442392	39442641	TTG ATT TCC ATG CAG AAG GG	TGT ACA CAA TAT GCC AGG AAC	1273, 1274
chr21	39499219	39499468	TAA TTT GGC CTT AGG GGT TG	GCA CTT GAG TTA TGG GAC TT	1275, 1276
chr21	39541298	39541547	TCC ATA AAA GGT GCT TAA AGC	TGT GAT ATG TAG TGT GTA TCA GT	1277, 1278
chr21	39563720	39563969	TTG TAT CAC ACC ATC GTG GA	GAA TGT GTT CAA AGG AGG GT	1279, 1280

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	39584873	39585122	ACT GCT GAG AAC AAT CAT GC	GAT GAA GGG GAT TAC GGG AA	1281, 1282
chr21	46530377	46530731	GGT GTG GAG AAT TTG TTT GC	TTT AGG AAG CAG CAC AAG AA	1283, 1284
chr21	46534043	46534292	TGA TAT TAG GCG GTG GCT TA	GAG GGT GCT GGG GTT ATC	1285, 1286
chr21	46535763	46536012	TCT CAC CTA AAA TCT GGG GC	CCC GAA AGC ACT TAC CTT TT	1287, 1288
chr21	46537454	46537703	AGG ACT GTG ACA CTT TAT CTT T	TTC ATG AGC TGC AAT GTG TT	1289, 1290
chr21	46542089	46542338	ATG CGA GGT AGA AAA TGA GAG	TGC TAT GAA AAG AGG GAC CA	1291, 1292
chr21	46556856	46557105	TGT GGC TTT AAG GTT CTG AAG	GTC CTG GAT CTA CAC GTG AA	1293, 1294
chr21	46564465	46564714	TCG TTG CTA TTC TGC TTT GA	GTA AAT CTG TGT GCC AGC AA	1295, 1296
chr21	46567255	46567504	TCC CTT TTG TGG TTT CTT GG	TAA AAG AGG CGT GTG GAA AA	1297, 1298
chr21	46572969	46573218	TTG CCG CAC TCT TCA TTA AT	CCA GTA GCT TGT GAT GTG TA	1299, 1300
chr21	46605329	46605578	ACT GGC TTC TCC TCA TTA GT	TAC AGG CCT CTG AAA GAT GA	1301, 1302
chr21	32134809	32135058	ACT GAT TTG CCA TGT AGA GC	AGA GCA TGC TAG ACG TCT TT	1303, 1304
chr21	32148136	32148385	GAA TGT CAT ATT GCC TGC CA	CTG GGC TGT AAT TAA GGC TC	1305, 1306
chr21	32165994	32166243	TCA CTC GCT TAG AAT GTT GC	AAC ACC TGC ACA CTT TGA AA	1307, 1308
chr21	32174173	32174422	ACC ATT AAC TTC CTG CAA ACT	AAT GAA CTT TGT GGG CTG AA	1309, 1310
chr21	32179882	32180131	TAA AGC CCC ATA CCA GGA TT	CCA CAC TCT CAC TGG TTC TA	1311, 1312

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	32181384	32181633	CCA AAA ATC ACC CAT ATG CG	CAC AGT GGA TAC CTC AGG AA	1313, 1314
chr21	32184463	32184712	ATA ACC CAG GTG CTT CAA AG	CCA ACA CAG GAG GAA CTT TT	1315, 1316
chr21	32201994	32202243	TCC ACA GCA GAA GTA ACG A	GAG AAA AGC CAA CAA AAA TGT G	1317, 1318
chr21	32208609	32208858	AGG AAA GCT ATG AAG AAA GGG	TGT CAT TAC TAG AAG CAC CTT T	1319, 1320
chr21	32319600	32319849	AGC TCC AGA GTG TCA GTA TT	TGG TGT GAG TTC AGG AGG GTT TA	1321, 1322
chr21	35034581	35034830	TTG ATT TCC AGC ACT GAA CTT T	ACC ATT TCT GAC AGA ACA GA	1323, 1324
chr21	35060177	35060426	TGT TTG ATT TTT CAG GCT GA	TGA TGC TTA AAC ACA TGC CT	1325, 1326
chr21	35070605	35070854	CTG GTC ATT CCT GAG TGT CT	AAG GGA TAT GCA GCT TGT TC	1327, 1328
chr21	35093305	35093554	TCC TGT TTT ACA CTT TTC TAA CTT T		1329, 1330
chr21	35094930	35095179	ACC TCA ACC TGT TTT AGC AC	GTT GTC ATT CAA ATG TCA CCA C	1331, 1332
chr21	35099418	35099667	CAG TGT GTG TCA TGC CAA AT	CTC AGA TTC CAG AGC CCT C	1333, 1334
chr21	35138457	35138706	GGT CTA TGT TAA TCT TGG GCC	ACA TTG CTA GCA GCT TTT GT	1335, 1336
chr21	35152877	35153126	AGC CAG TCT GTA TCT AAA GGT	ACT TGC CAA GAA CAG TAT CTG	1337, 1338
chr21	35157173	35157422	ACT CTT GGG GTT TCT TCA GT	TTC CTT CTT CCA GGT GAA CA	1339, 1340
chr21	35161795	35162044	CGA ACC AAA AGC AAA ATC CTT	TGT TTC TCC TTC ATC TGG TC	1341, 1342
chr21	35296082	35296331	AAT CTA TGG AGG TCA CTG GG	TGT CAC CTT GCA GAT ACA GG	1343, 1344

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	35450331	35450580	AGG TCC TTG TAG TTT GCT TG	ATT GTT CAC AGG GTC AAG TC	1345, 1346
chr21	35473022	35473271	GTG AGA GCC AAT AGA GTG TG	ATG AGC CAG GAG AAT CAT CA	1347, 1348
chr21	35473971	35474220	GCA TGG TGT GTG AAA GTG AT	CAT CAT TTC AAA GGG GCT CA	1349, 1350
chr21	35498164	35498413	ATG CTG CTT TTG ACT GAT GT	TCT TCT CTA ACA CCC ACT CC	1351, 1352
chr21	35540943	35541192	CGA AGC TGT ATT CCT GTC TC	ACC CTT ACC AAA GTA GCA TC	1353, 1354
chr21	35702965	35703214	ATG GAC TAA CTG GAG AGC G	ATC ACG ACG CCT TGT TTA TT	1355, 1356
chr21	28574819	28575068	ATG TCC TGC CAG TAA ACA CA	ACT TTC CTG CCA ACA ATC TC	1357, 1358
chr21	28586377	28586626	TGC CAC TAA ACA CCT AAG GA	TTG ATA GTT GCA TCT GGG GA	1359, 1360
chr21	28597009	28597258	TTG GAA ATT TTG GGG TCA GG	TTT GTT AAC TAA CGT GAT TCC A	1361, 1362
chr21	28616121	28616370	TTC TGA CCT CCC TTA CTG AG	CCC ACT CTC CAT GTG TTC TT	1363, 1364
chr21	28631408	28631657	TAT GTG CTT GCG ATG TGT T	TCC TGC TTC AAC TCA ATA CG	1365, 1366
chr21	28891355	28891604	GAA CCT TAG GGC CAG TCT AT	ACC CCT CAT TTT CGT ATG TC	1367, 1368
chr21	28892223	28892472	AGA ACC TGA TGT GTT TTC CTC	ACA GTT ATG GAG GAA TTG CG	1369, 1370
chr21	28919446	28919695	ATG TGA GAG AAG CAA AAC CC	TCA AAC CTC TTT TAT CTG TCC C	1371, 1372
chr21	29012106	29012355	GTA GTT GTC TTG AGG GCT TT	ATT TCA CGT AAC ACT CTG GT	1373, 1374
chr21	29021007	29021256	CCT GGT CAA CAA CAT ATG GG	CTC TGG CAA AAC TTT CTG GAT	1375, 1376

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	29753618	29753867	AGA ATA TTG CAT TTG GCC AGA	GCT TCT CAT GCT CAC ACT G	1377, 1378
chr21	29796479	29796728	AAA GCT ATG CAA ATA GTG GCA	AAT ATG ACT TGC CCT TTT GAA	1379, 1380
chr21	29812165	29812414	AGT TCC CAA CAG AGG CTA AT	TCA CAG CCA GTT ACA CAG A	1381, 1382
chr21	29813966	29814215	AGT TAT AGG TGA GGA AGG GC	CTA CAG TGC AGA AGA GTC CC	1383, 1384
chr21	29814951	29815200	CAC TGC AAA AGA AGG AGG TT	TTG GTT TCA TGT GGC TTT GA	1385, 1386
chr21	29820028	29820277	TCT CAA CAT CGC TGA TCT AGT	TCC TTC TCC CCA ACT TTC TT	1387, 1388
chr21	29825992	29826241	AGG TAG CTG GAA AAG GAG AA	CTT TAG ATT CCA GGG CTC TTG	1389, 1390
chr21	29827616	29827865	TGA AAT TGC CCA GAA TTG AGT	TGA AAG CGT GAA AAT CAG CT	1391, 1392
chr21	29841662	29841911	ATT TTC CTG GAC TTC TGA CA	ACT CGA TCC CTA GGT AAT GT	1393, 1394
chr21	29884231	29884480	TGT TAT TCC TCT TCC TGT CCA	ACA AGT GGG TAG GGA TGT TC	1395, 1396
chr21	29898255	29898504	TAA CCA CAC AAC TAC AGC TT	GCA CAA GTT TTC AGG GAA TG	1397, 1398
chr21	30368230	30368479	ATT CAA AAT GGG GAC GAG AG	GTG GAA AGT CTC GTC AGA AT	1399, 1400
chr21	30413906	30414155	TCC CAG TTT GCT ACT CTG G	TCC ACC TCT GAG CAT AAC AT	1401, 1402
chr21	30415555	30415804	TCT CAT TAT GTG AAG ATT GCT TTC		1403, 1404
chr21	30428953	30429202	TTA AGA TTA AGC AGT CTT CTT GG		1405, 1406
chr21	30477650	30477899	CAA CAG ATC TGA TTC TGC CC	CCA TCC CAC TTC TCC AGA TA	1407, 1408

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	30528161	30528410	CAT GTG TTT CAA AGT TGG CT	CCA CCT TCC TGC TTA AAG AA	1409, 1410
chr21	30550132	30550381	ACT ATA GCA TTA GGG TGA GGG	CTC CTT ACT TGC ACT GAG TT	1411, 1412
chr21	30580738	30580987	GAG ATT TGG ACA TGC TTT CA	GGG AAA ACT TAC GGG AAC TT	1413, 1414
chr21	30600446	30600695	CCC ACC ACC AGT TGT CAT C	TAG AAA TGT TTA GGG TGC ATG A	1415, 1416
chr21	30628668	30628917	GCC ACT CAC TTC CTA GAT AAT	TAC CCC TCT TTT CCA TCT GC	1417, 1418
chr21	30660609	30660858	ACA GTA TCT CAG GGC CTT AT	GCA TTT TGA CAG GAA AGT GG	1419, 1420
chr21	30680391	30680640	TCA GTA GTT CCT CAG ATG CTA	TAG GGC CAC AGT TTC TCA AT	1421, 1422
chr21	30720214	30720463	AAT GCA TGA AAG TCC AGG AA	GAA TGA GAA ATC TGG CAG GA	1423, 1424
chr21	30782462	30782711	ACA TGC ACT CTT GTC TTA TGC	TCA GAT GCC TGA TGA CCA TA	1425, 1426
chr21	30810199	30810448	CAC ATA TAC TGG CTT TCT GGT C	AAT ATG CAG TGG GTA GGA GC	1427, 1428
chr21	30849402	30849651	GCA CAT GTC ATT AGC AGG G	GAC AAC AGC TGA CTT CCA TT	1429, 1430
chr21	30868525	30868774	ACA TGC TCA ATT ATG GAG CC	CAC TCT CTG GAA CAA ACA CA	1431, 1432
chr21	30988776	30989025	ACA AAG ACA GGA ATA GGG CT	GTT TTG AGG CGG TTT CAT GA	1433, 1434
chr21	31012406	31012655	CAA TCT GCT GAC TTG CTT CTT TTC A		1435, 1436
chr21	31032720	31032969	CTT TGG CTC AGA ATC TTC CAA	GAA CAC CTC AAA GTT GCT CA	1437, 1438
chr21	31085356	31085605	TGT ATG AGG ACC AGC AGT AAA	AAG AAG CAA GGA CAA GGA TG	1439, 1440

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	31494755	31495004	TCT TTT CCC CTT GTG CAT AG	GAA ACA ACC ACC ACA ACA AA	1441, 1442
chr21	31560667	31560916	GCT TAG TGT GTG TGA TCC GT	CCA CCA TGT TTA CAC CGT TT	1443, 1444
chr21	31605973	31606222	ACT CAC CTT TCC CAA GAA GA	TTT ACC CAT GAA TTG CTG CA	1445, 1446
chr21	31621826	31622075	TGG TAG TGG GAA GAG GTT GA	AGT TTG CAT TTG TTC AGG GA	1447, 1448
chr21	31653822	31654071	GCA TGG AGA ACA AAA GCT GA	TTC GGA TGG CTT TGA TTG TC	1449, 1450
chr21	31670830	31671079	TGT GAG CAA GAA ACT GAA GG	CAT TGT AAA TTA AAC GGC CTC	1451, 1452
chr21	31691347	31691596	AGT ATC ACT TGT CCA GCT CA	CTA ATG CAA GCT GCT TCT CT	1453, 1454
chr21	31708936	31709185	CAC AGG ACT AGG TAG GCT TT	TGT CTA GTG GTA ATC TGG GG	1455, 1456
chr21	31733443	31733692	GTG TTA ACA GCT TTC CCT TCA	TCT GGA CAG TGG AGT TGA AA	1457, 1458
chr21	31768996	31769245	TCA TGT ACA GAA AGA ATT AGC CT		1459, 1460
chr21	31886189	31886438	AGA GGC CAA GTG ACC AAA TA	AGG AGA GAC ATA ACT GGT CT	1461, 1462
chr21	31886808	31887057	AAG CCA GTA ATT CAT CTT CCC	ATT GCA ATG TCT GTG GAT GT	1463, 1464
chr21	32119833	32120082	CCA TGA CAT AAC ACA TCA CCA	AAG AAG TTG AGG TAG CAC G	1465, 1466
chr21	32125263	32125512	ATA GAT TTC CTC CTG GGC TG	AGT GTC CTT TCC TCC AGT TC	1467, 1468
chr21	25795207	25795456	CCA CCT CTG TAC CCA CTA TC	TTG AAC CTG AAA GGA ACT GTG	1469, 1470
chr21	25802273	25802522	TTC CCT GGA AGA TAG CCA AT	GCT CAA TCA CCT GTT CCC TT	1471, 1472

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	26355452	26355702	TGG ACA CGT AAA AGA AGG TG	CAT TGA AGC TCA CTC TAA GGG	1473, 1474
chr21	26365770	26366019	CTG AGA ACC TTG TCC AAC TG	CTA AAG TTT GGG TTA GGG GT	1475, 1476
chr21	26373440	26373689	TTT CGC TAG TCT TTG CAC TT	TGC CTT ACA TTT TCT GTG GG	1477, 1478
chr21	26374774	26375023	GCG GCA ATA ATT GTC ACA AA	GCC ATA AGA TTT CCC CAC TC	1479, 1480
chr21	26390279	26390528	CAG AAA TGT GTC AGG CTA CA	ACA AAG CAA GAG GAT GAA ACA	1481, 1482
chr21	26390951	26391200	AAC TCT TCA TTT TGA CGG GG	CAA AGC ACC ATC AAC ACT TA	1483, 1484
chr21	26402802	26403051	TGG GTT GTG CTG TTG TTT AG	CAC TGC AAC TCC TAG AAT GA	1485, 1486
chr21	26496769	26497018	GCT CAG CTC CTT TCA TCT G	TGT GGG GAA ATT GCT GTT TA	1487, 1488
chr21	26515400	26515649	AGG ACA TTC AGC CTA TTT GC	GTA TGG GCA GCT GTA ACT TG	1489, 1490
chr21	26571662	26571911	ACA CAG TAT CAA GGT CAA CA	TCA CAA GCC ACT GAA AAT GT	1491, 1492
chr21	26589786	26590035	GCA GAA CCA CAG TCT ATG AG	TGT ATG TTA AGC TAG CCA ACA	1493, 1494
chr21	26604824	26605073	AAT GCT CCA AGT TAT TCC AGA	TGT GTT ATT GAA CTT TGC CA	1495, 1496
chr21	26933670	26933919	AAG AGA GAA GCG ACA AAA CC	TTC ATC CCT ACC TCA TCA CC	1497, 1498
chr21	26950703	26950952	AAT GAG AAG GAA TTG GGT GC	AGA TCA CTT TTG GCT GTA ACC	1499, 1500
chr21	27045134	27045383	AAT GAA GTG TTA GGG CCA TC	CAA GTG ACA ATC TCA GCC AA	1501, 1502
chr21	27398390	27398639	GCT CAT CAC AGT TTA AGG AGT	GCA TTT TCA GAT GGT TCC CT	1503, 1504

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	27441346	27441595	ATG TGG AAG CAA GAG AAA GG	ATG GAG AGT TTG AGT GGA GT	1505, 1506
chr21	27465490	27465739	TGA AAG GTG AAG TGG CTT TT	CTG CCA CTG GGT TTA TAG AAA A	1507, 1508
chr21	27485182	27485431	GTA AGT AAG GGG TCC TAG CT	ACA CCT TTA CTC CTG TGG AT	1509, 1510
chr21	27486006	27486255	TGT GAC TTC CAT GAA ACT GG	GAT GTA GGG CCT TAT CCA CA	1511, 1512
chr21	27502429	27502678	GCT GAC AAA CTA ACC TTC CA	AGC TTG GTG ATC TTC AAA CA	1513, 1514
chr21	27520192	27520441	AAA ACC TCC CAA AAC AGA CT	TGA AAT GGT GGG TAA TGC TC	1515, 1516
chr21	27539803	27540052	AGT TTA GTG GCC ACG TGA AA	CAT GCA AGT TCA CGA GGT TA	1517, 1518
chr21	27623130	27623379	GGC AGA AGT TTC AAT TCC CT	AGT CTG AGG AAG AAG CAA CT	1519, 1520
chr21	27623692	27623941	AAA GCC TCT GTT TGC ACT TT	GAG TCC AAT CTT TTC CCA CA	1521, 1522
chr21	27763679	27763928	TCA GTC AGC TTC TTG AGT CA	TCC ACT GCG TTC TTA TCC TT	1523, 1524
chr21	27894000	27894249	TGT TTC ATT TGG GTC ATG GA	GGA CAG AAC AGC TAC AAA GG	1525, 1526
chr21	27926498	27926747	TAC AGC ACT AGG ATC ACT CTG	CTG GCT TGT GAA TTA GAG GG	1527, 1528
chr21	28077177	28077426	ATG CAT GTT TCT TGC AAA GG	GTA AGA TGG TGG GCA GGA T	1529, 1530
chr21	28122462	28122711	GTA GGA TTC AGG GCA TTT CA	AGA AGG GCT CAA AAC ACA TC	1531, 1532
chr21	28161337	28161586	AAC TGG AAC TGA GCG TGA G	TCA TGT AGG CTT TCT GAT TTT	1533, 1534
chr21	28180925	28181174	TGT ATG CAG TTA CCT CCA GA	TGT AGC TCT TGA CCT AGC AA	1535, 1536

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	28275754	28276003	GGA TGT ATA CCA GAC CCC TT	TAA GTT CAC GGT GAA GTC AAC	1537, 1538
chr21	28291449	28291698	CGA AAG ATG TTA GCA CCT CA	GTG GCT TCA ACT AAC TGG AC	1539, 1540
chr21	28294820	28295069	AAT TGG CTT TGC AGT GTT TC	ACC CGT AAG TGT TTG AGT GA	1541, 1542
chr21	28295351	28295600	GCT TGC TGG TAG GAG GTA TA	TGT AGA AGT AGG GTT TGC GT	1543, 1544
chr21	28295857	28296106	CTG GAA ACG GAA GGA AGT TG	CAG GGG ACA TTT GAA GAT GG	1545, 1546
chr21	28311866	28312115	CTG TGT TCA GTA AGT GGC TG	GAA ATT GTG CAG TGA AAG CA	1547, 1548
chr21	28334784	28335033	GAC AGT GAA GTG TGA TCG TT	ATG TAA GAA GTG CGT TGC TTA	1549, 1550
chr21	28354814	28355063	TGG TGA TGC TGT TTT GGA AA	AGA TCT GGA ATC TGA GAC TCC	1551, 1552
chr21	28415341	28415590	TTC TTT CAG GCA GAA GAA ATG A	TAT AGG ATA AGG TCA AGC AGG T	1553, 1554
chr21	28433186	28433435	AAG TTC CAT TAC TGT ATT GAA AAT T		1555, 1556
chr21	28450226	28450475	GGA CGT ACG TGC TTA TTT CA	TCA GTG AAT GAG GAA TCA TGC	1557, 1558
chr21	28474083	28474332	CAG CCT GAT ATT CCC ATT GAG	TTC TCA GGA GTA CCA CAA GC	1559, 1560
chr21	28491062	28491311	TCA TGA GGC TGA GTG AGT AT	GGA TGA AAC ACA GAA CCA TG	1561, 1562
chr21	28509439	28509688	AAA GCT CTC CTA TCT CCA GT	CAG ATC CCT TTC ATT TTG CA	1563, 1564
chr21	28534176	28534425	ACA AAG AGT CTG GAC TAT CCT	GGA AAA GTG CTC AAT TAG GC	1565, 1566
chr21	40042441	40042690	TGC CTA AAA ATA CCC AAA GCT	CTG GCA GTT ATA GTC ACC AA	1567, 1568

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	40427338	40427587	CCA AGA CAC TCA CTC CAA AG	AGG AAG TCC TTA TGA TGC CA	1569, 1570
chr21	40509976	40510225	CCA GGA AAA GGA GCA GTT TT	GCT GGT GAA GTT GGA GTT TT	1571, 1572
chr21	40531291	40531540	GTT CAA CTC TTT CTG CGA AC	GCT GGG TTT AAG CCA CAT AT	1573, 1574
chr21	40578323	40578572	CCA CTT CTT CTG TTT CCA AC	TGT AGC CTA AAT AGC AGC CT	1575, 1576
chr21	40601158	40601407	GGG AAG GGC ATG CTA ATC A	AGG AAT TGC TTT TAT TTT AAC CA	1577, 1578
chr21	40630577	40630826	TGT TCA ATC ACC TCT CCA TC	GCT GCT TTC AGG TTT TTG TG	1579, 1580
chr21	41280723	41280972	AGA GGA GAG GCT AAG CTT TG	ATG GTG CAG AAA AGA GCA AT	1581, 1582
chr21	41607273	41607522	ACA AGA GAG GAA GCT GTC AG	GTG TGG TGC CAT TTT CTT TC	1583, 1584
chr21	41629820	41630069	AGC CCC TTT CTC CTT ATT CT	GAA CAA TAC TTT CTC CCC GG	1585, 1586
chr21	41703462	41703711	AGG GAA GCA GGA TTT TAA CG	GAA ATG GCC ATG CTA GGA AT	1587, 1588
chr21	41729089	41729338	AAG TTT CAC AAT ACC CAG GT	ACT TCC AGT AAC ATG GAT GC	1589, 1590
chr21	41761636	41761885	TCG CAG AAT GGA CAA GTA CT	GAA ACA CCC AGA CTT GTA GC	1591, 1592
chr21	41839021	41839270	GGC TCC CAG ATT TTG ATC AT	TTA AAT CTT TGT GTG CGT GT	1593, 1594
chr21	41908902	41909151	CCT AGA ACT GCA AAA CAC CT	CCT CCA GGA ACT TTG TTC AG	1595, 1596
chr21	41933321	41933570	TTC CAT TAT TTT CTC ACC GGC	AAA AAC CTT CAC AAA CCC CA	1597, 1598
chr21	41981868	41982117	CAG TCC AAC AAA GAG GTC AC	GAG TGG ATA TTG TCT CGC TG	1599, 1600

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	42029142	42029391	TTG TGT GTG TTG AAG CCT AG	ACT AGC CCA CTA ATG TTG CT	1601, 1602
chr21	42030642	42030891	AGT GGG GGT AGG AAG AAA AA	CGA TTA TCA GAA CAG ATG AGG T	1603, 1604
chr21	42080190	42080439	GTT CCA ACA ATG TAA GGC AC	ATT GCA CAG CTG AAA ATC CT	1605, 1606
chr21	42251219	42251468	GGA ACC CTC TAT GGT CAA AG	GGC ACA CTG ACC GTA TTT AT	1607, 1608
chr21	42559559	42559808	ATT GCT GTG TAG TTC CTT GA	TGG TAG TGG GTC AGG AAT TT	1609, 1610
chr21	42601828	42602077	TTT CTT GCC ACC ATT CTG AC	TCT CTT GAA AAG AAA GGC GG	1611, 1612
chr21	42851340	42851589	CAC ACG TTC TAA CCA AGT GC	TGA ATT ACA CAG CAA AGC CC	1613, 1614
chr21	42872713	42872962	TAG CCA CAT CTT AAC AGA CCT	CTG CCA ATG GGA TCG AAT TT	1615, 1616
chr21	43032728	43032977	ATT CCT GAG GGT GAC ATG AA	GAG TGA CGC TGT TCA TTC TT	1617, 1618
chr21	43406661	43406910	CCT GCC CCA TCA ACT TAA AA	GAT AGT AAC CGG GTG TAG CA	1619, 1620
chr21	46521300	46521549	CTG GGT GCA GAG GAT CTC		1621, 1622
chr21	16323548	16323797	TTG AGT TGA ACT TTG CTT TAG A	TCT AAT AAG GGA TTG ATG GAG TT	1623, 1624
chr21	16330249	16330498	GAA CTA GGA GAC ACT GGG TT	GGG AGA TTT CCT GCT TGT AG	1625, 1626
chr21	16332622	16332871	CAA GAA TAG CTA ACT GGT GCT	AAT CCA TGC AGC TTC TCT CT	1627, 1628
chr21	16333315	16333564	AGT TAC ACA CTG AAT CAT GGG	TGC ATT GTC TCT GGT TTG AA	1629, 1630
chr21	16335355	16335604	CCC ATG TGG CTT CAC TAA TA	TCC TGA ATG CAT CCT TAA CC	1631, 1632

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	16344361	16344610	GAT TGT GGT CAC GTG GAG AG	TGG GAA AGG TGA GAA GGA TT	1633, 1634
chr21	16349606	16349855	CAG ATC CAG GTA TTC GGA GA	TTC GGG ACC CAT ACC TAA AA	1635, 1636
chr21	16358650	16358899	ACA ACA ACA ACC ATT ACC CA	TCT TTT CTG GAC CCA CAT GA	1637, 1638
chr21	15713251	15713500	TGG CCA TAG TAC TGC TTG TA	GAC CTC TAC ATC TGT ATC TTC C	1639, 1640
chr21	15714357	15714606	TGT CTC ACT GTT GGG AAC TT	CCC TTC ATT TTC TGT CCC AT	1641, 1642
chr21	15725977	15726226	TTT GTC TGT ATC CTA TGC CC	TCA GCC TTG AGT ATT AGC CTA	1643, 1644
chr21	15731366	15731615	GCT GAC AAA ATT GGA TCC CA	AAG TTT GCC ATG AAG GTC AT	1645, 1646
chr21	15816232	15816481	AAA ATC CTC CTG AGT CCT CT	GAC TTC TGG TTG TTT CCT CA	1647, 1648
chr21	15843963	15844212	TTC ACT GGG CTC TTC AGC TA	ATG TGT CTA TTG CCC TAC CT	1649, 1650
chr21	15858450	15858699	AAA GGG CAG GAG TTA GGT AA	TGC GGG AAG TTC ACA TGA A	1651, 1652
chr21	15859015	15859264	AGG CAT AAG AAA CCA GGT TG	GTG AAC TTC AGG CTG CTT A	1653, 1654
chr21	16002625	16002874	GTA GTT CGG TCC AAT GTC AG	TGG TTT CGT CCC GTA AAT AG	1655, 1656
chr21	16030414	16030663	GAT GGT CAC AAT TGC AGG TT	AAT TCC TTT CAA TGC TGG CT	1657, 1658
chr21	16063119	16063368	GCC AAA GAT CTC AAT TGC CA	AGA AAG ACA CAT ATG CCA TGG	1659, 1660
chr21	16068358	16068607	GAC CAA GAC TGT CTC TCC TT	TTG TTG GTG TCA GTT CTG AA	1661, 1662
chr21	16071495	16071744	TAA TGG TCA AAT CCC TCT CAA A	TGT AGG AAC AGA TTA GGG CA	1663, 1664

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	16081282	16081531	CAT GTA GGC TGA AGA CTC CT	CTC CAA GCT GAT ATG CCA TC	1665, 1666
chr21	16115078	16115327	TTT CTC TCC AAA CTG GTT GC	CCA CCC CTC ATT TCT TCC TT	1667, 1668
chr21	16122085	16122334	TAC AGG CAA GAA ATA GTG TCT	GGG TCC TTC GTT TTC TGT TT	1669, 1670
chr21	16130688	16130937	TTC AGC AAG AAT GGG GAT TC	TAG GAA ACA GGC TAA AAG GGA	1671, 1672
chr21	16133865	16134114	CAA AGA GAG AGC CAT CAC AG	TAC TGT GTA GAA GGC AGT GT	1673, 1674
chr21	16139969	16140218	TGA GAA CAC TGC TAT TTC TGC	GCT AAG GAC AAA GAA CCA CT	1675, 1676
chr21	16141592	16141841	GCA TGG TCA GGA CAT TGG		1677, 1678
chr21	16160517	16160766	GAT TGA ATC AGG AGG GAA GC	TGG CTA AGA CCA GGA TTG TT	1679, 1680
chr21	16165861	16166110	AGC TTA AAT GAT GAA GTG CTT TC		1681, 1682
chr21	16189746	16189995	TTC TCC TAC GTA TCT TGG CA	TGA TAG GCA GAT CAT TCC CC	1683, 1684
chr21	16190806	16191055	ACC TGG GAC ATA ACC TTG AT	GCA AAT GGC AAA GGG AAA AC	1685, 1686
chr21	16192618	16192867	CCA TTT TCC TAC TGC GTG TC	CAC GGC TAG TGC TCA TTT T	1687, 1688
chr21	16195809	16196058	GAA CAT ACC AAA CCC ACT GG	CCG TAA TAC CCA AGT CAT CTG	1689, 1690
chr21	16198337	16198586	ACC CAA TGA TGT ACA GTT CC	AGC AAA CTA AAA CAG CAA CTT C	1691, 1692
chr21	16203731	16203980	ACC TAT TCG ACT TGA AAC TCA G	AGC CTG CTA TCT TCA CTG G	1693, 1694
chr21	16207035	16207284	ATT TCT GCA CAA CTG TTC CA	ACC ACA TAT ATA GAG ACT TTG AAC	1695, 1696

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	16209341	16209590	GCT GTA ATG TGA CTA ACC CT	CTC CAG ACT CTG CAA GGA T	1697, 1698
chr21	16211270	16211519	TTT CCC GAG GTT CAC AGA TA	TTT CCT GTT GCT CTT GAT CA	1699, 1700
chr21	16219907	16220156	GAA CTT GTG TGA CCC AAA AC	CCA CAA AGA TGA AGG CCA AG	1701, 1702
chr21	16230785	16231034	CAT TGC ACT GTG ATG TCA TG	CCA TAC CTT AGT TCT CAG GGT	1703, 1704
chr21	16236026	16236275	GCA CTG GAA ATT GAC ATC AC	GTA AGA GAG AGC TGG GAC AA	1705, 1706
chr21	16241139	16241388	GGG AGA GGC TGA AAG AAG AA	TGT GAC CAT CCT ATC CAC AA	1707, 1708
chr21	16242812	16243061	TGT ATC ACT TCC TCA TGC CA	AGA ACC AGT TGT ACG AGT TC	1709, 1710
chr21	16246944	16247193	CCA AGA GTT TCC TGT TTC CA	AGT CTT CTC CCT TCC TTG TC	1711, 1712
chr21	16265484	16265733	ATG ACA CAT ACA TCC ATT TAC A	GCC CTT TTC TCT CTT TGA CC	1713, 1714
chr21	16273734	16273983	CTC AAA CTG CCC AGT GAT TT	TCT TGT TCC AAG TAT TCC TGG	1715, 1716
chr21	16275865	16276114	TCC TAG CTT GCC AAA GAA AT	ACC ACT TTA GCC CAT CTC TT	1717, 1718
chr21	16290234	16290483	CCT CCT CTC CAG GCA TTT TA	GTG GTG GAT TAT TGA GCT GG	1719, 1720
chr21	16295877	16296126	AAC AGA GTA GCA CAG AGA GT	TGC TTA ATG GGA TCA TTG ACC	1721, 1722
chr21	16296687	16296936	TTC AGA GAG ACA GAC AGC AT	CCC ACA TAG TGC AAA AGA CA	1723, 1724
chr21	16298842	16299091	AGA GGA AAA TCA CAA GCA GT	GTA TGT GTG AAG TAG CCG AG	1725, 1726
chr21	16316213	16316462	TTT GAA AAC CCA ACA GAC CT	TGG TCC TAA CTC AGA CCT TT	1727, 1728

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr21	16319836	16320085	AAT CCA CAC ACC AAC AGA GG	AAC ATG AAG GGA AGG TTG TG	1729, 1730
chr18	64634331	64634580	TGT TCA CAT CTG TTG GTT TGC	TAT ACT TCA ACT TGC AGG CAG	1731, 1732
chr18	64645925	64646174	GTT ACT CGG TGG GTG ATA TTT	GCC AAA GAC AAT GAG AGA GTC	1733, 1734
chr18	64975938	64976187	AAG AAA CAC CAG CAT CAG TTC	ATT TAG CAG CCA TGA CCA GTA	1735, 1736
chr18	64996573	64996822	AGG TTT AGA GGT GAG TGA ACA	TGG GCC AAT TCC TAA TCC ATT	1737, 1738
chr18	65066425	65066674	AGA AAC TTC ACT GTC TTC CAC T	CGA TCT CAT GAA TAA GTC TGA CC	1739, 1740
chr18	65073729	65073978	TGT GAT GGA CAT TGG TAC CTG	TGG AAA GCA GAC TAA CAG TGA	1741, 1742
chr18	65074170	65074419	ATA TCA TCT GCC TGT CCC AAC	GGG ACC CTA TGT AGA GAT TGT	1743, 1744
chr18	66343957	66344206	GAG TGC TCT GTG TTT GTT TCA	GGA GCC CAA GGA TGT ATT AGA	1745, 1746
chr18	66344406	66344655	TCA GTG GTG AGC TCT TGA ATA T	CTA ATA GCT GGT TCT GCA CAC	1747, 1748
chr18	66585247	66585496	ACT CTC TCT TCA CAC ATG CAA	GAT GAA ATG AAT GCT GAC TCT C	1749, 1750
chr18	66596489	66596738	GTG TTG AAG TCA GTA AAG CCT	AAA CTG AAG CTT CGA GAA CCC	1751, 1752
chr18	66601231	66601480	TGC TTT CAC ATG GCA CTA GAT	TGC CGA CAA CTA CTT TAG GTA	1753, 1754
chr18	69966208	69966457	GGA GAG AGA AAT CCC AAC TGA	TGT ATC CAA TCA CCT GTC AGA	1755, 1756
chr18	70088939	70089188	GGG AGA TGT CAA CAC TAG GTC	CAG CAC CTT AAG CAG AAA TCA	1757, 1758
chr18	70093364	70093613	ATA TGA CAT GGT GGC TCT CC	AAG CCC TTC ATC CAT TTC TCT	1759, 1760

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	70107935	70108184	AAC ATA GAG CCA TGG GAG GTA	ATC TTG GTG CCA TCT TAA GGT	1761, 1762
chr18	70198868	70199117	GAT AGC CTT CAA ATC ATG CCT	AGC AAA CCA ATC GCA AAC TAG	1763, 1764
chr18	70200128	70200377	GAA AGC GGG TGA ACA ACA ATA	AAT CAT CAT CTT CAT CAG CTC G	1765, 1766
chr18	70200907	70201156	GAT AGA GAG CAC AAA GAG CAT	CCT TTC GCC TTG CTT ATA TGG	1767, 1768
chr18	70674976	70675225	AAC AAG AGG AAT AGG AGC CAG	ACT ACT CAA CAG CCT ACC AAA	1769, 1770
chr18	70682967	70683216	TGG GTG CTG ATA GTA ACA AAG	TGG AGC TGG GAA CTT TAA TGT	1771, 1772
chr18	73541191	73541440	ACT ATT GAA CTG TTG GCT TCG	GAA GAG AGA GAG AAT GCG TGT	1773, 1774
chr18	73548315	73548564	TCC ACT AAA GAG CAA CCA AAC	GAC ATG GAT ATT CTG GTG CCA	1775, 1776
chr18	73605288	73605537	TGA AGT GGT CAG TAA CAA TGG	CTG TTG CAA GAT GAC CCA AAT	1777, 1778
chr18	73704485	73704734	ATT TCA GAG CTC CTT TGT CCT	AGA TAC ACA CAC GTT CAC AAA C	1779, 1780
chr18	73752546	73752795	GGC AAA GAA ATC TGG TGT TCA	GAT GGC AAT GCT TGA TAA CGA	1781, 1782
chr18	73764755	73765004	TTG CTG GTT GAT AGG CAT TTG	TTC CAT GAA GTT CCT CAA GAC T	1783, 1784
chr18	73768420	73768669	ATT CCC GCA ATT GTG AGA TTC	CCA CCC ACA TAC CCT GAA ATT	1785, 1786
chr18	73775027	73775276	AAA GAG GTA CAG AAC TCA GAC C	GTA ACA CAC GGA TGC TGA AG	1787, 1788
chr18	73788751	73789000	GTC TTC ATG AAC GTT GCC AAT	TGG ACA CGA GGC TAT TTG TAG	1789, 1790
chr18	73851108	73851357	CTT CTC AGG GCT CTT TGT GTA	GGC TTA AGA AGG AGA GTG GTT	1791, 1792

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	73854713	73854962	GCA ACA GAA ACC AAG ATT CCT	TAG TTT CCT TTG GCC TTC TCC	1793, 1794
chr18	73871422	73871671	TCA TTG TCT ACC TCA AAG AGC A	CTA GGG TTC CCA GTT CAC AAA	1795, 1796
chr18	73876103	73876352	CTC TGA AGG AAC AAA GGA TGG	GAA ACA ACA TTG AGG GCA TTG	1797, 1798
chr18	73879805	73880054	CAT TAG AAT GCG GTG GTT TCA	GAG CTC TTT GAA GTA GAA GCA	1799, 1800
chr18	73884248	73884497	GAT GTC TGG GCT GAG GTT TAA	CCA TTC CAA CAA AGC TTC CG	1801, 1802
chr18	73931865	73932114	TTC CTC TTG AAG ATG CAC TGG	CTA CTT GCC CTA TTG TGT CGA	1803, 1804
chr18	73938014	73938263	CCA GCA TGT GAG GAA TTG AAC	CCA GGG TGT TTG AAG GTA GAA	1805, 1806
chr18	73943212	73943461	CCC ACT TAG TCA TCC ACA CAT	CTG GAG GTA AGA AGG AAT GCA	1807, 1808
chr18	73947628	73947877	GTT TCA CAC ACC AGA AGA GAG	ATG TGG GAC TCT TTG CTC TC	1809, 1810
chr18	73964168	73964417	CCA TAC ACC TGC TCT GAC ATT	ATG AAT ACA GCT TTG CAT GGC	1811, 1812
chr18	73966321	73966570	ATC AGT AAC AGT CCC ATT GCT	GTT AAA GCA TTC ACA GCC CTC	1813, 1814
chr18	73974071	73974320	CAT GAG GCA TTT GAT CCA TGG	CTG GGA CTT GTC TAT CCT CCT	1815, 1816
chr18	73985549	73985798	ACC CTG TTT CAC TGA ACA ACT	ATG TCT GTC CAA GTG AAC AGT	1817, 1818
chr18	73987174	73987423	CCC TGT AAT GAG AGC GTT ATT	AAA GTA TCC AGA CCC AGA ACC	1819, 1820
chr18	48466086	48466335	GAG AGA ATG GGT TAA ATC TGC C	ATA GGC CAG CAC TCC AAA TAA	1821, 1822
chr18	48928528	48928777	CTG CCT GAC TTA GCC TTA AAT	CAA ATC AAG TCC CAT GGT AGG	1823, 1824

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	48929078	48929327	AAC CAG AAT GTT ACT AGC CCA	CTT TCC GTC TTT ATA GGC AGC	1825, 1826
chr18	48951524	48951773	CAA TCC TGT GTG TTT AGT GGA	AGA AAT CGT GTT CAC AGC CTA	1827, 1828
chr18	48951856	48952105	TGT GGG AAG CAT TGA CTC TT	CCG TAA ATG AAG TGG CTT GAA	1829, 1830
chr18	48959882	48960131	TCC TGT GAG AAA TGG AGC TTT	CCT TTC TTG CAA CCT TGA GAT	1831, 1832
chr18	48960304	48960553	GTT GCC AAG CTT AAA TAC CTG T	CTC TAG ATG CTC AAC CTC AGG	1833, 1834
chr18	49004516	49004765	CCA CAA CAA CAT AAA CAC TGC	AAT AAC AGT CCA CCA GAA CCA	1835, 1836
chr18	49006425	49006674	TCT TCC AGG CAT ATT CAT TGC	TCC AAG GAC CTG CAA ATG TTA	1837, 1838
chr18	49017695	49017944	ATC CAT TCT CTC TAC TTG GGA	AGG TTA CAT CAT TCA CCC ACA	1839, 1840
chr18	49148010	49148259	GGC TAG AGG GTG ATT ATA AGC T	ATG AAG ACA ATG ACA TCT GCG	1841, 1842
chr18	49149627	49149876	TAC TCA TCC CGA TTT CTT CCC	CAC TTG TCA TGG TTT AGG GAC	1843, 1844
chr18	49149933	49150182	TTC TCT TTC TCT TCT GGG CAG	ACC TCC ACC TTA TTG CTT CAA	1845, 1846
chr18	49150459	49150708	TTG TGA GAC TCA AGG CCA TTT	GAA TTG CAA AGG ATG GGT AGG	1847, 1848
chr18	50239403	50239652	TTG GTA GAG AGA GGC CAT TTG	CTG CAT TGT GAG TCC ATG TAA	1849, 1850
chr18	50242789	50243038	GGA GGA AGC TCT TGA AGA CAT	TCC TAT CTT CAT CCC TCT TCC	1851, 1852
chr18	50243069	50243318	AGC ATC TTC CGT TTA ACT CCA	TTC CAT TTA GCC TCC CAT CTG	1853, 1854
chr18	50243367	50243616	CTG CCT GCC AAG TAT GTT CT	TAG CTT TAT GGG CCT TGT TCT	1855, 1856

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	50247229	50247478	CTA CTC CTT GTG TCA TTG GCT	CCA CCT CTC AAA CCC AGA TTT	1857, 1858
chr18	50308727	50308976	TGA TTC TGA GAC ACG TGC TTA	GTC AGC GTA TTT GGG ATT GAA T	1859, 1860
chr18	50309031	50309280	CTT GAG GAC CTT TCA TGC TTG	AAA TCT GCC ACC CAT TTC TTC	1861, 1862
chr18	50338747	50338996	TTG TGA TTT CAG GTA GGA GGG	TAT TCT GAG TTC TAC CCA GGT	1863, 1864
chr18	50423284	50423533	TTC CCT CAG AGA CAG TAT CCT	TAC ATG AGA CCC AGA AAC AGA	1865, 1866
chr18	50424422	50424671	AAA GGA GGT CTG GCT TTG AA	CTT CTT GGC AGA CTA TCA GGA	1867, 1868
chr18	50424789	50425038	GCA TAA ACC TGG ACT GTG AAA	AAG CTG CTA AAT CTG TAG GGA	1869, 1870
chr18	50448837	50449086	CAC TCA GCG ATT CTC CTC AC	CTG ACC AGA CCT GTT GAC TAA	1871, 1872
chr18	50449492	50449741	AAA GCC AGA CAC AGA CTA GTT	TGA TAT GTT CAG TTT GCC TAC C	1873, 1874
chr18	50514079	50514328	ATT CCT GGG ACC ACA AGC AT	AGC TTG AGT TTC TTG CTG GG	1875, 1876
chr18	50519889	50520138	TAT TGC CTC ATG TGG TTG TG	TTC CCG CAA AGT AGA AGC TAT	1877, 1878
chr18	56064244	56064493	GTG TTG ACT TGA AAG GAA TCA C	AAA CGC ATA CAA ACA GGA GAC	1879, 1880
chr18	56148666	56148915	CAG GTT AGG AAT GAC AGT GGG	CTT CTA AAC CCA TCA CCT GCT	1881, 1882
chr18	56149037	56149286	TTA GGT TAC CCA GGG ACG TTA	CAC CCA AAC TCA CAG GTA CAA	1883, 1884
chr18	56244171	56244420	CGG TTT GCT TTC TGA ACA ACA	TGA ATT CTG AGA TCG AGA GCC	1885, 1886
chr18	56245242	56245491	CAT TTG GGA CCC TTT GAA ACT	AGA TTA ACT GTT GCC TCA CTG	1887, 1888

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	56247063	56247312	GGT TAT CTC TGG GCA AAG TTC	CAA GAC AGT GCA TTC CAT GG	1889, 1890
chr18	56527049	56527298	TAG AGA GCA GAG AAC AAA CCC	CCA AGG AAA GAG TTG AGA AGG	1891, 1892
chr18	56528011	56528260	ATA AGG GCA TTT GGA GGG AAA	TGA GTG CAG TCG ATA AGG AAG	1893, 1894
chr18	56532190	56532439	AAT GCA CAC TTA GAC ACC ACA	AAC ACC GAG AAA GAG AGA GAG	1895, 1896
chr18	56532882	56533131	GGA TTG CTA CCC AGG AGA TAA	TGT ACT GCT TTC GTC TTA TGC	1897, 1898
chr18	56534208	56534457	AGA GGT TCT GTG TAT GAG TGT	CAT ATT CGC ACT GTA TAG CCG	1899, 1900
chr18	56601487	56601736	TAG AGA ATT GTA CGC TGG ACA	CTG ATG GAT TCT CTG GTG TGA	1901, 1902
chr18	56633131	56633380	TCT GGA GAA ATG CAC AAG AGA	TCA AGG AGA AGA GAG AGG GTA	1903, 1904
chr18	56635259	56635508	TGG GTT AGA ACA TGG TGC TTA	TCT CCC AAA GCA GAC AAA GAC	1905, 1906
chr18	56719296	56719545	ATC GCA TCA CAC CCT TAC TAT	AGT CAG TTG TTA CGT GCA AAG	1907, 1908
chr18	58037828	58038077	GGA AAT TTA GCT TGA CAT GGC	AAA GGT TTG TTC ATC CTC CCT	1909, 1910
chr18	58038328	58038577	TTT GTA AAT CCA CAG TGC CTA C	ACA GAG CAC GCA ATA TAG GAA	1911, 1912
chr18	58039111	58039360	GGT ACT GGA GAG CAT AGA AGA	CTG CAT TCA CCC ATG TAC TTT	1913, 1914
chr18	58040290	58040539	AAA CAA GCT ATC TTC AGG CAG	TCC AGC CAT ACC ATG TCT ATC	1915, 1916
chr18	59424085	59424334	CGA ACA ATC AGA GAC TCG ACT	CTC ACT AGG GAA GAA CAG CAG	1917, 1918
chr18	59424765	59425014	CTG GTT GAC AAT CTG CAA GTT	TGC TGG GTC TGA GTG TTA TAA A	1919, 1920

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	59441324	59441573	ACC ATC CAA GTC GTC TTC ATA	GTT GTG TGA ATG GTG CTG TTA	1921, 1922
chr18	59499718	59499967	ATA GCT ACG CAT ACC CTG TAG	GGC CCA GAA GAC TCT TGT AAT	1923, 1924
chr18	59500226	59500475	AGC AGA AGA AAC AGT AAG GCA	CGA CCT ACA TCA GCT AAT GGT	1925, 1926
chr18	59507920	59508169	ACC CAG GGA CCT ATT TGT TC	AAG GGA AGA ATA ACA ATG GTG C	1927, 1928
chr18	59543044	59543293	CAA GGG CTC AGG TCT TCA TTA	ATG GGA GTA TGG GAG TAG GAA	1929, 1930
chr18	59556453	59556702	TCA CTG TGA CTT GGA GAC TAA	TTG GTG GCT TGC AGA GAT TT	1931, 1932
chr18	59614976	59615225	TGC TCT GCT TCA CTG TGA TTA	TCA CAC GAT CAT CAT ACT CAC A	1933, 1934
chr18	59625553	59625802	GAT GAA TGA CTA ATA GCC CAC G	GGT AGC AGA TGA CTA GAC GAT	1935, 1936
chr18	59631090	59631339	CCA TGT TTA GTT TGG TGC TGT	ACG CCT CTG TCA TTT CCT AAC	1937, 1938
chr18	59631779	59632028	AGC CAA GTG AGG TGC TAA AT	CAG TTG ACT CAA TGG TGC AAT	1939, 1940
chr18	59632388	59632637	TCA GGG AGA AAT GAT GTC ACC	ATA GGT TAC AGA TTG CCA CGT	1941, 1942
chr18	59695718	59695967	CAG TAG CTG GCA AGA ATC ATC	GAT GCT GCT ATC AAA GGA ACA	1943, 1944
chr18	59696087	59696336	CAA CAC TGC TAG AAT TCC CAA	AAG ACA AAG AGA TGG AAG GCA	1945, 1946
chr18	59696563	59696812	CCT GAG AAG CAC CTG ATT GTA	AAT ACC CTC TTC CCT TCC TCA	1947, 1948
chr18	59712419	59712668	TGT TGT CAG AAA TCC CAG GAA	CCA CCG TCA ATA TTT ATC AGC T	1949, 1950
chr18	59756979	59757228	AAC TGG AGC CAT ATA ACG ATG	GCC TCA GTC CAA ATC TTA GAT	1951, 1952

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	59770789	59771038	GAA ATG GTG CCC TAT TGT TGA	CCT TAG GAT TCT CAA AGA GTG T	1953, 1954
chr18	59805993	59806242	TCT TAC ATG CAG TCA TAC TCC T	GCA GTA CAG ATT CTT GAA CAG T	1955, 1956
chr18	59813876	59814125	GTC CCT CAG TAA CAC CAT CTT A	AGA GGA TTA GAT GTC TTG CTG T	1957, 1958
chr18	59855411	59855660	GAA GCA AGA GGA TCA GGC AAT	TAC TGC AGG CAA TTC AGG TAA	1959, 1960
chr18	59909054	59909303	AGT GTT TCA GAG GCT TGA AAG	GAA GAG GTC CAG TAA GTG AGG	1961, 1962
chr18	59909336	59909585	ATC CGG CAT CCT TTA AAC TCT	TTG TGA GTC CTT GTC TCC TTG	1963, 1964
chr18	59910140	59910389	AGG CTA GGA AGA AAT GGG AAA	GAC GAC TAA GAC ATT GCA TCA	1965, 1966
chr18	59910602	59910851	ACA CAT ATG CTC TGT CTC TCA	AGA AGT CTC TCT CCG TTG TTT	1967, 1968
chr18	59973803	59974052	AGT TAG TTA TCA CCT CGT CCC	CTT ATG TGC ATC AAC TGT GCT	1969, 1970
chr18	59996728	59996977	TGT GTA TTT CCC TCT AGT TGC A	AGT GTC TCT CAG AAT CAG GAC	1971, 1972
chr18	60010541	60010790	AGC CTC TTT CTA CAT CGT TCG	TTT ATT TCC CTA CGC AAA GCC	1973, 1974
chr18	60011381	60011630	TTA CCT GTG CAG AAG AGT GAC	AGC CTT TGA TGA CTG AGT TGA	1975, 1976
chr18	60240694	60240943	TCT TGT GTT CTA GCG TGT TTG	TTG GTT TCT ATT CTG CAC TGC	1977, 1978
chr18	60378353	60378602	TGT GGT TAG TCA GAA ATG TGG	TTA GAG CTT GCT AGT ATC GGG	1979, 1980
chr18	60384593	60384842	GGG TCC TGA TGA GTC TTT GTC	GAA ATC CCA AAC TGC CTG AAA	1981, 1982
chr18	60384979	60385228	TGG TGC CTT TGT TTA TTC AGC	ATC CTT CTT GTG AAC CTT CCT	1983, 1984

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	60389544	60389793	TGT TAT GTG CCA GGG TTT AAC	ACC AGA TGC ATG TGA TTA AAG G	1985, 1986
chr18	60401084	60401333	ATT GGT TCC AGA TAC AGT CGA	GTG TAC TCT AGG CTA CTG TCA	1987, 1988
chr18	60412199	60412448	GGA AGG AAG TAC AGC ATG GAT	GTC AGC AGC AAG TAA AGG TTC	1989, 1990
chr18	60420053	60420302	CCT TAT TGA AGC TGA CCA TGC	CAT CTA GTC AAG GGT TCC ACA	1991, 1992
chr18	60434561	60434810	CAG TGG GAA ATG TGC TTA CAT	CAA GTC ATG CTC CAA ACT GTT	1993, 1994
chr18	60449047	60449296	AAA GGG CCT ATA TTC ACC AGA	AGG ACT TAG GAC AAC AGA GAA	1995, 1996
chr18	60452471	60452720	AGA GCC ATT TAA GAC TCT CTG T	TGC CCA ACA CCA TCT CTA ATA	1997, 1998
chr18	60456757	60457006	TAC CTT GTT CTC TGC CTC AAT	GCC TTC ATC ACT CAG AAC TTC	1999, 2000
chr18	60467147	60467396	TTT AGA AGG ATG TGG ACA GGG	AGG GTA TCT ATT CTC CGG ACA	2001, 2002
chr18	60469757	60470006	AGC AGG ACA TGG ACT TCA AA	ATT TGC CCA AGT AAG TTC CAC	2003, 2004
chr18	60514168	60514417	GCG GCT CTT GTT TCT GAA ATC	TGG AAG TAC ATG GGA TGC ATT	2005, 2006
chr18	60537888	60538137	ACA GGT AGG AGT TCA GAG ACA	CTT GAA GAG TTC CAA TGC CAA	2007, 2008
chr18	60544380	60544629	TTT CCT ATT CTG CTC TTC TGC T	CGC TGC TGT TTA AAT CGA TCA	2009, 2010
chr18	60544742	60544991	AAA TGC TGC TCA GGG TTA GAG	GCT CTG CTT TGC TCA AAT TCT	2011, 2012
chr18	60545407	60545656	CAA CTT TAC TCT GCA CAG CTC	TGA GCT CCA GAA TTA GAT GTG T	2013, 2014
chr18	60611934	60612183	CAG TGC CAC TAC AAA GAA ATC A	AAC ACC TCC TTT CTC ACT ACA G	2015, 2016

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	60617081	60617330	AGA AGC AGA GGT TGG ATA TGG	ACA AAC TTC ATT CAC CGC AG	2017, 2018
chr18	60617641	60617890	CCA CAA TCC CAT AGT CAC CAT	AAC TAC GCC ACC CAA CTA AA	2019, 2020
chr18	60630438	60630687	GCT GGT TCT TGT TGC TGA TAA	GCT TCA GTG TAA CCA TGA CTC	2021, 2022
chr18	60630849	60631098	TCC CTC ACG ACT TAT GTT TGA	TGC ACA ATT AAG CTA CTT CTC C	2023, 2024
chr18	60639840	60640089	CTA CCT TCT CCA GTG CAC TAT	CAA CAC CAA ACT TGC CTG AAT	2025, 2026
chr18	60640300	60640549	GGA CCT CTC TTT GAA ATG GAC	TCC ACA ATT TCT ACA GCA ACC	2027, 2028
chr18	60776944	60777193	TGG GTT GTG TTT CTC TGA CTT	GTG TCA TTT GAT TGG TGC TCT	2029, 2030
chr18	60813738	60813987	TAC AAG CCT CCT TTA ACC CTT	GGG TGT TTC AGT AGG TTA GGA T	2031, 2032
chr18	60814585	60814834	AAA GGG TTT GAT ACA GTT GGG	TGG GAT TCT AAT GTC TGG TGC	2033, 2034
chr18	60938666	60938915	AGG TAT TGG AGA GCA AGA AAG A	AGA GGT GGT TGG TTG GTT	2035, 2036
chr18	60939202	60939451	GCA AAC TGG AGC TAA AGT CAT	GGG CAT CCT GTC TGA AAT ATG	2037, 2038
chr18	60950541	60950790	TTG GTT GCT AGC TCT CAA ATG	AAG AAG CGC AGA TAC AGT ACA	2039, 2040
chr18	60953225	60953474	TTC AAT GCC CTT ACT TCT CCT	CAC AGC AAG TTT GAA CCT AGT	2041, 2042
chr18	60958474	60958723	GCT TGA GGC GCA TAT GAT TG	AGT GCA AAT GAT GAC CTG TTG	2043, 2044
chr18	60960879	60961128	CAT ATG GTG CTT GTT CTG GG	GAA CTG TTG CAT GAG AGG TAC	2045, 2046
chr18	60965583	60965832	CAA ACT CAC CAC CCT TCA TTC	CTT TGT CCT TCT CTG TTG TGT	2047, 2048

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	60970051	60970300	CCT TAG CCC TGC AAA TAA CAC	TTT CTT CTA GAG TCC AGA GGT G	2049, 2050
chr18	60974839	60975088	TCA CAG ATA CGG ACA AGC TC	ATT CTC TTC TCT CTT CCA GCC	2051, 2052
chr18	60984378	60984627	GCC ACA GGT ATC AAT CAC TTC	TAT GGC TTT GCT ACC TTG TCA	2053, 2054
chr18	61055997	61056246	GCT TCT GTG GCA CTA ATC AAG	CTA TTT CTC TGG CTC TTG ACC	2055, 2056
chr18	61088669	61088918	GCC TTA TTG ACT TAC TGG ACT G	TTC TTA AAC CTC TGT GTG GCT	2057, 2058
chr18	61090462	61090711	GTC AGG GAT TAG AGG CAG AAC	TGG ACA AAC AAG AAC TGG GTA	2059, 2060
chr18	61100325	61100574	CAA TCA CTT GGT CAG ATA GTG T	CCA TGA TCA CTG AAA CCA ACA	2061, 2062
chr18	61154459	61154708	AGT GGA GAG GTT GAG TAT AGT G	CCA TAT GCC CTG CTC TTT AAG	2063, 2064
chr18	61162901	61163150	TGA GAA GGG AGG AAG AAT GTG	GGG TGG ACA AAG CAA TTC AAA	2065, 2066
chr18	61170629	61170878	AAG GTC AAA CTC TCC ATT CCA	CAG CAT TCA ATT CAT CCT TGT G	2067, 2068
chr18	61197492	61197741	GGA GAT GTC TTT GCC CTG ATT	AAG TAG AAG CAA GCC CTG AAT	2069, 2070
chr18	61213452	61213701	GGT CCT CGT TTG TCC TTA AGA	GTG TGG TAG GGA TGA GAA TTA T	2071, 2072
chr18	61213900	61214149	AGT ATT GCT TTG AGG GCT CTA	TCA GGT AAG CTT CCC TCC AC	2073, 2074
chr18	61216749	61216998	AGC TAT CAT GTA AGT CAC TCC C	ACT GGT TGT AGA AAG GAC CTC	2075, 2076
chr18	61232928	61233177	AGG AAA TTC AGT ACC TCA GCT	AGT AAG AGG CCA GAA GTC AGA	2077, 2078
chr18	61236079	61236328	CAA CCT TCT CAT TGT TGA AGC T	GCA GTG CAG GCC TAT ATA TAG	2079, 2080

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	61253277	61253526	GGT GAC TTT GCT TTC CCA AG	AAA TCT CTG AGT CGG CCA TAA	2081, 2082
chr18	61259478	61259727	GCT GAC AAA CGG AGG GAG AG	AGG CAT GGC AAA CTT ACT TG	2083, 2084
chr18	61264533	61264782	TGT TCT TCA TCA GGC ACA ATG	CAT TTC ACT TTC GAG GAT GGT	2085, 2086
chr18	61289440	61289689	ACC ATT TGT GTG ATC CAG AAC	GTC AGA CTA AAG TGA GGA CCA	2087, 2088
chr18	61368889	61369138	CTT GTC TTG AGT GCG GTA CA	CCA GCC CTA CCT AAA GTG AAT	2089, 2090
chr18	61370663	61370912	GCC TCT CAG TTT CCT CTT ATA G	CCC TTT CAC AAG ACT CTT CTC	2091, 2092
chr18	63685629	63685878	CCT AGA TCA GTG CAG AGA ATT TAG T		2093, 2094
chr18	63711583	63711832	CTT TAG TTT GGA GGC CTC ATT C	CCA AAT GTA GAA CAG GAT CAG C	2095, 2096
chr18	63721183	63721432	CTG ACA TCG ATG GAA TTC TGG	TAG CAG TAG GTG TGG CTT TC	2097, 2098
chr18	64379625	64379874	ACA CTG CAC GAA TGG AAG ATC	TTG GAT TCT CTT GGT TGT GAG	2099, 2100
chr18	64398368	64398617	ATA AAC TTG GTG CTC AGT GGT	CAA CGC TTT GGT ATA GTT TGT G	2101, 2102
chr18	64410092	64410341	ACA AGC ATT ACA GAA TTC GGC	CCA GGT GCC ATC GTT AAA GAA	2103, 2104
chr18	64412606	64412855	ACT GAC AGA TTC TCA CCT ATA TCA G		2105, 2106
chr18	64417267	64417516	GGA TCA AAG CCA CTC TAG ACT	GGA TAA GTC AAC TAC CAT GGT T	2107, 2108
chr18	64421562	64421811	TTG AGA TGG CAT CAA GTT CAA G	AAT GGA ATT ACT CAG CTG TGG	2109, 2110
chr18	64426871	64427120	ACT GGA TTC ATG CGT TAT CAA G	GGC AGA AAC TGA TAG AGA CTG	2111, 2112

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	64436287	64436536	TAT TCT TGT GTG GAC CCT GTG	ACC ATG TTC TGA GTA CCT CTT	2113, 2114
chr18	64593727	64593976	TGA TAT TGC ATG AAA GTC CCT G	AG TCC TGA ATC AAT GTC TAA CAC	2115, 2116
chr18	24962594	24962843	TCA CAC ATG AGG AGT AGA CA	TGG TCC CTG TGC TTT GAT AT	2117, 2118
chr18	24964748	24964997	CAC AGC AGG AGA CAT GAG AA	TGG CTT TTC TTT CCT CGG TA	2119, 2120
chr18	25003553	25003802	TTT CCT CCT GGC TTG ATC AC	CCA AGG CTG CTT TAA TTC CA	2121, 2122
chr18	25033345	25033594	CAA AGG AGA GAA GTG ACC CA	CAA ACT ATC GCT GAG GAC CT	2123, 2124
chr18	25037434	25037683	CTA CGA GTG AAA CAG AGT GC	GTC TGC TGC CAT TGA GTT AT	2125, 2126
chr18	25052992	25053241	AGA CTA AAA GCC TCC AAG CC	GCT CAC CCT CTC TTC TCT CT	2127, 2128
chr18	25080042	25080291	TAG AAT ATG TCA CCC AGC CC	ACC TGT TTC TCC CAG TTA CA	2129, 2130
chr18	25124915	25125164	ACA GAA TCA TCC CAT AGC CA	GCC CAT GAA AGA GAA ACC AG	2131, 2132
chr18	25128057	25128306	CGC CAT TCT GTG CTT AAT TTG	CTC CAT CAG TGC AGA AGT CC	2133, 2134
chr18	25140257	25140506	GTG AAG CAA GAG AAA GCA AGA	ACA GTC AGC AGC CCT AAA AT	2135, 2136
chr18	25140722	25140971	TGA TTC GGC TGC AGG TTA TT	GCC TCC TTC ACA TAA TGC AG	2137, 2138
chr18	25147759	25148008	CTG GCT TCA AAT GCA TCT GA	TTG TCA ACA GAG AGT CAG CT	2139, 2140
chr18	25157828	25158077	ACT GGG AAA TTG GAA TTC GC	CTG AAA TGG TCT GGG AGT CT	2141, 2142
chr18	25160244	25160493	GGT AAA ACT GCC TGG AAA CT	TCA AAG ACA GAG TGA GTG GA	2143, 2144

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	25166354	25166603	AGA CCA CGG GCT CTA TCT AT	TTT GGG GGT TAC ACT TCA TAG	2145, 2146
chr18	25172986	25173235	CAT CTT GCT TAT TGG CTT ACG A	AGC CAG CAG AAT AAT ACC AGG	2147, 2148
chr18	25174972	25175221	GAG GTA GAG GCA GTG TCT TG	ACC TCA TCT TTT GTC AGC CT	2149, 2150
chr18	25175640	25175889	TAG TCC TTG AAC TCC CTG GT	ACA GTT CCA TAG GCA GGT TT	2151, 2152
chr18	25182855	25183104	CCA GCT TAG CGT CTG TTT TT	GCA GTT CCA GAT CCA ATA TGC	2153, 2154
chr18	25198531	25198780	GAC CAC AAC TAT CAA GAG CAC	TTT GGG AAA GAT GGG AGA GC	2155, 2156
chr18	25201886	25202135	CCA AAG AAA GGT TGA AGC CC	TTG CAG GTA AGG TAC AGA AGA	2157, 2158
chr18	25209395	25209644	AAT AAT GTG CAC TGT GAT GGC	CTT CAG CTG CAT CTT GAG C	2159, 2160
chr18	25213880	25214129	TTT AAG GGT CTG ATG GTT GC	GGC ACT TCA AAA ACA AAC CC	2161, 2162
chr18	25216888	25217137	GAA AAT GCC CAT CGT CTC AA	AGG GTT TTA TGG TCT CCT GG	2163, 2164
chr18	25228617	25228866	ATT CCT TGT CTT TCC CCC TC	TCA ACA CGG AGA ACT GAA AAC	2165, 2166
chr18	25231535	25231784	TCA TTT CTC TAG CCC AAA GAT G	TCC GTG TAA ATG AAC AAA GCA C	2167, 2168
chr18	25245662	25245911	TTG GTT TGT TGA CTT CAG CC	TTC AGG GAA TGG TTT GCA TT	2169, 2170
chr18	25246300	25246549	GCT CAG TGA CAG TTG GGA TT	GAG ATG CCA TTC CCA AAA GG	2171, 2172
chr18	25254843	25255092	GTC TTG TCT CTC TTC TTC CAC T	CCC TAC CAT AGT GCC AGA TG	2173, 2174
chr18	25265476	25265725	TTT AGA GCA GGT GGA AAC GA	CAG CTT TCA GTG ACA GAG GA	2175, 2176

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	25278532	25278781	CTT GGG TTT TTA TCG GTT GCT	CCA AAG GCA GAT GAG TGT TT	2177, 2178
chr18	25279010	25279259	GTT GCC TGG ATT GCT CTA AA	GCC TGG GAT AGA AAT GGG AA	2179, 2180
chr18	25283817	25284066	AAA TCA CGA CGT AGG AAA CC	GGA AAG GAA AGG AAG CTG TG	2181, 2182
chr18	25292042	25292291	TCA CCT TGG AGC AGG TCA TA	TCA GAG AGG TCT TGC TGA AG	2183, 2184
chr18	25295429	25295678	CGA AGA AGG TCT GGG AGA TG	TCT CTC TGT TGC TTG TTT CCT	2185, 2186
chr18	25330345	25330594	CAC CAT TGT TTC ATC AGG ACT	CGC ATG TGG TAG ATC ATC AG	2187, 2188
chr18	25332963	25333212	TTG CAT CAT CAG CTC ACA TAC	TAT AAC ACC CTC ACC TCC CA	2189, 2190
chr18	25333284	25333533	AGA CCT GGA AAA TGA TGG GT	CAC CCA AAT CAC CTT GCT ATG	2191, 2192
chr18	25345252	25345501	CCT GGA AGT GTG TAA CAA GC	CAA CTA CCG TGG ATT CCG TT	2193, 2194
chr18	25384702	25384951	CTC CCT AGC AAA AAC TTC TCA	TCT ATC ATG AGT CGC TTC CA	2195, 2196
chr18	25510767	25511016	TGC CTT ATT CAC TGT GCA AC	TTT CTG TCA CTT TCT GGG CT	2197, 2198
chr18	25519108	25519357	TGA TGG CCT AGT GAG TTT CC	CGT GTG TTT CTA GTG CAT TGT	2199, 2200
chr18	25531724	25531973	TGC AAT GTA ACA AAA GCG TG	GGG CTC AGA GGG AAT ATC AG	2201, 2202
chr18	25543168	25543417	GCC ACA TTT GCT TTC ACA CA	CCG ACG AAT GGA TGA AAG AC	2203, 2204
chr18	25547425	25547674	ACG TCA TTG GGT TCA TGG C	ACC CAA ATT CCA TGC CTA CT	2205, 2206
chr18	25557343	25557592	ATT TTA CCC CCT TAG GCA CC	TGT ACA GCA GTC TCC AGA AA	2207, 2208

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	25564483	25564732	TCT TCT ACA CAG CCC TTC AG	GCC CTC TTA CCC TTT CTC AT	2209, 2210
chr18	25583087	25583336	ATT GGG ATC GTC AGC ATC AA	CAT TCA AAG ATC CAG ACC AGG	2211, 2212
chr18	25585083	25585332	TGA TTG TCT TGT CCA CTG GT	ATC TGT GAT TGC TGC CCT C	2213, 2214
chr18	25589902	25590151	ACA TCA CAC TTC ATG CCC TT	CTA GAA ACT CCC AGG ACA GA	2215, 2216
chr18	25601966	25602215	ATG AAG GCA TTA GGA GGG AG	CAT GTG TGG AAA GGA TTG GT	2217, 2218
chr18	25611986	25612235	AAA GTG GAG AAG TGG CAG AT	TAT GAA TGA ACC GTG GCT CA	2219, 2220
chr18	25617498	25617747	CCT TAG GAT TCT GAG AGG TGA G	AAG TTG AGT CGT TTG TCC CA	2221, 2222
chr18	25629105	25629354	AGG GCT TCT GAT TGA TTT GC	TAG TGT TTC AGG AGC GTG TT	2223, 2224
chr18	25631180	25631429	CAG GGT AGT CGG GAT TTC TC	CCA GGA CAA GCA GAC ATT TT	2225, 2226
chr18	25643639	25643888	CCC GGT AAT GAT CTA CAG CA	GCT GCT GGT GAT TTT TGA AGA	2227, 2228
chr18	25645371	25645620	AAC GGC ACT TGG TTC ACT A	CTC TGG GCA AAC AAG AAA CC	2229, 2230
chr18	25665025	25665274	ACT CTG AAC TCC TCC TCC TG	GTG TGT GTT TGT GGA AGT GT	2231, 2232
chr18	25666614	25666863	TTT GCT CAC ACA CAA GAC AC	AAA GCC CAA TCT CTC TGG TTA	2233, 2234
chr18	25667057	25667306	ACG ACT GCA TCC TTT TCA TG	TAG GCG GGC TTA TTG TGT TT	2235, 2236
chr18	25670741	25670990	CCA TCA TAG CCT ACA AAT ACC C	TCA ATG TAA ACT GCC CGG AG	2237, 2238
chr18	25673208	25673457	AGA GAG TTG AAA ATA TCC CCC A	ATG GGA GTC GAA TGG TGT AAA	2239, 2240

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	25681018	25681267	ACT TTC TTG AAC ACC CCA GT	TAA CAT TTG AGG GCA TGG GA	2241, 2242
chr18	25682079	25682328	TGC CTG TGT CCT ACT TTT CC	CAG AAT ACC CTC ACT GTG CT	2243, 2244
chr18	25689906	25690155	TGA CTG CCC AAG AAT GTA CA	GGA GAT AAC AGC AGA GGT CC	2245, 2246
chr18	25700540	25700789	CCG AAC ACG CTG TAT GTA TT	TGT GGG TGA TAT CTG TGT CT	2247, 2248
chr18	25703648	25703897	GGA AGG GAA TTG AAG CAC AG	GCC ATC CTG TAA CTG AAT GC	2249, 2250
chr18	25707188	25707437	TCA TCC TAT CCA CCA ACC TG	GTA TTT TCC CTT TGC CGC AG	2251, 2252
chr18	25716100	25716349	AAT GAA CTG GCC CTG ACT TA	ATT ACA GCA AAG AAC GTG GC	2253, 2254
chr18	25722951	25723200	TAA GAT ACC ATA CCG CAG CT	TCT GTG TGT TTT GCA TTG GT	2255, 2256
chr18	25725055	25725304	TCA GCG TTC ATG GTA CCA ATA	GTG ACA GTT TTC CAA GGC AT	2257, 2258
chr18	25748105	25748354	GTT TCC CAA CCA ACA AAC AAG	GCA TTA CTT TTT CGC ACA CT	2259, 2260
chr18	26214441	26214690	TCT ATC GGG ATG GAG AGT GA	GGG GAT TGT TTT AAG CAG GC	2261, 2262
chr18	26236788	26237037	GTT CAG ACA GGT GGA CTA GG	CCA TTG TTC TCA CCA ACT CT	2263, 2264
chr18	26261336	26261585	AAG CAA CCT GGG AAA TTG TG	CCT GAA ACA CAA GCA GCA G	2265, 2266
chr18	26265937	26266186	GGG TTA AGG TTG CTG GGT TA	AGC TGC CTA TTT GAT TGG TG	2267, 2268
chr18	26285286	26285535	GTG AAA TGT GGT TGT AGT GCA	CAG TAC AGT CAG CCT TCC TT	2269, 2270
chr18	26289006	26289255	GTG ACT GCC TTG CTT CAT TT	TGC ATT CAA ACT ACC CCA AG	2271, 2272

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	26308405	26308654	CAG TTC TGG AGC CTT CTA CT	CCG AAA AGA GGC AAG CAA TT	2273, 2274
chr18	26308962	26309211	TTA GGG CAG GAT GTA CAG AA	GTC ACC TCA ACC TAA CTC CA	2275, 2276
chr18	26312054	26312303	GCT GGT GAC CTT CAT TCA AG	GCA ACA GTC TAC CCG TCT AG	2277, 2278
chr18	26316149	26316398	TAA AAC CAT GTT CGG GGC A	ATC AAT GCT CTG ACC TCC TG	2279, 2280
chr18	47100905	47101154	CTA CCT GTC CGT TTC CCT TAC	ATA CAT CAG GCC TCC AGA ATT	2281, 2282
chr18	47130278	47130527	GAC AAA GAT GAC TGG AGG TGA	CAC ATC TTT AGA GCT CAG GTG A	2283, 2284
chr18	47366936	47367185	GGG TGG ATG GTG AGA TAT GTG	CCA TCA CTT CAC AAT CCA CAC	2285, 2286
chr18	47371131	47371380	CTT CAA ACC TGA TCC ATG TGC	AAT CCT GCA GTC ATC TTC CC	2287, 2288
chr18	47689868	47690117	GCA GAA ACA GCA TGA ATC TCC	CAA GTC TGG TTT GTG AGA AGC	2289, 2290
chr18	47690267	47690516	TGA CTT CAA ACA TCC CAT CCA	GGG AGC TTC TGT AGT CTT TGA	2291, 2292
chr18	47703689	47703938	GTG GGA TGA GTT CTA GAG GAA	TAA GGA GAG CAG GAC TTA CAG	2293, 2294
chr18	47708139	47708388	AGT CAC ACA CAT ACA CAC AGT	CCA ACG GTT CAT TTG TCG TAT	2295, 2296
chr18	47717445	47717694	ACG ACT TCC CTG TGT AAC TTA	CCT TGC TCT GTT AAT GGG TTT	2297, 2298
chr18	47738531	47738780	CCT TCT CTT GTC TCT AGT GCC	AAC AAT GCT TAA CGG GAA TCC	2299, 2300
chr18	47777438	47777687	CTG GAA ATA CAC ACA CAC CTG	GCA GAG TTC ATA GAA GGG TCA	2301, 2302
chr18	47794212	47794461	ATT TGT AAA CCA CCC ACT TCG	AAA CCG AGA CGA CCA CCT AAT	2303, 2304

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	47799400	47799649	TCA CTG TGC TGA CAA ATC CTA	GAG TGA TGA TGA GCC ATG ATG	2305, 2306
chr18	47802662	47802911	CCC ATC ATC ATC CCT TCA GAC	GGA TAT TGG AGA TAG CAG GCA	2307, 2308
chr18	39874986	39875235	TCA TCA CTT TAT CCT CCC AGT	GTT TGG CAC TGC AAC TAG ATA	2309, 2310
chr18	39891490	39891739	ATG GGC ACA GGT AAA GAG TTT	TGA AAT AAG GGA AGC CAC ACA	2311, 2312
chr18	42204240	42204489	TTT GTA AGC TGA GTG TGA GGT	TCC TTA GTG TGC CAA TTA GCC	2313, 2314
chr18	42313146	42313395	TTA CTG TTT GAA TGC CAG CTC	TTA ATG TGG AGA GAC AGG CC	2315, 2316
chr18	42313939	42314188	TCC CTT CTC CCA TCA CAA TTC	CAA TGC ATC TTA CTC ACC CTT	2317, 2318
chr18	42314302	42314551	GAA GAC TGC ATG TGT GTC CTA	AGT ATG GAA GTG GGA ATT GGA	2319, 2320
chr18	42349923	42350172	TTC TCA CTC TCA ACT GAA CCA	TTG CTT CCA CAG AAA CTC TTC	2321, 2322
chr18	42407165	42407414	TCA GGG AGC TTC TAA TTA AGG A	AAC CGA CCT ATT CCA AAG TCT	2323, 2324
chr18	42418033	42418282	AAG ATG ATC CCA GGC TTA AGG	TGT GAA GCG AAT ACA GCT CAA	2325, 2326
chr18	42449702	42449951	GTT GAG GTT TGC TGA TCT TGG	GTT CTA ACT ACA CCA GGC TCT	2327, 2328
chr18	42450400	42450649	CAA AGA TAG ATT CGC ACA CCA	GTA TGA GTG TAG GTG TGG AGG	2329, 2330
chr18	42461427	42461676	GAG CTG GAC AAA TTA AAT GGC	AAT CTG GAT CTA GCG AAG GAC	2331, 2332
chr18	42462834	42463083	CCA GTG CAT TTG GTT TGA CA	TAA TGA GAA GGC AGG ATG AGG	2333, 2334
chr18	42468595	42468844	TAT GTG AAT CCT CTG TGT GGC	AAG ACA ACT CTC TAG GCC TCA	2335, 2336

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	42468938	42469187	AGC TTC TCT CTC ATT CTG CTT	GTT TAT GGT TGT CCC TGG AGA	2337, 2338
chr18	42644003	42644252	GAC TCC CGA TTT CAT TTG CTG	TCC ACC TTC TGA TCA CAC AAT	2339, 2340
chr18	42644494	42644743	TCC CAA TCG TTG TGA AAC ATA C	AAG ATC AGG TAC CAA GGC ATT	2341, 2342
chr18	42645387	42645636	TCT TTA CAG GAA GTT GGG ACC	ATG ATG TGA AGT CCA TGG TGA	2343, 2344
chr18	42741059	42741308	TAA GTT CAG ATC AGG GAG CAG	TCC AAA TTG ACT TCC ATG AGC	2345, 2346
chr18	42745733	42745982	GTT GAA AGT CTT ACA GAA CGC T	GTG GTT TCA GGA ATT TGG AGG	2347, 2348
chr18	42746460	42746709	CAA CAT AGG CAC ATT GTC CTC	TAT TTG CTG CTT CAT TCT TCC C	2349, 2350
chr18	42746911	42747160	GTC AGG CCT CAT AAC TCT CTT	AAG TCA TTA CGT CCC ACA CTG	2351, 2352
chr18	42747473	42747722	GTT GTG TGG CTT TCC TTA TCA	AAA GTC ATC AGA AGG GTA GCA	2353, 2354
chr18	43796659	43796908	CCT GCA GCT CTG TGT AAA TTT	AAT GAT GCC GAA CAG TGA GTA	2355, 2356
chr18	43820471	43820720	CTT TGG CCA GTT CTT TCT CTC	AAT GTA AGA CAG GGA CAG AGA	2357, 2358
chr18	43841913	43842162	GTG GCC CAG CAT TAT TTG TT	TGA ATT CCA CAG TCC AGT CAA	2359, 2360
chr18	43842202	43842451	TCT TGG TGT GAC TTG CTA ACA	AAT GCC TTC AAA GAC AGT GAC	2361, 2362
chr18	43842647	43842896	TTT CTG GCT GAG ATA AGA CCC	CAC ACC TGC AAT TGA GAT GAA	2363, 2364
chr18	43845701	43845950	ACA ATT CCG TGG TAT ACA GCT	GTC ATG ATG ATG CAA CAG CTA	2365, 2366
chr18	43846353	43846602	TGA TTG TGC CCT AAC CAA ACT	TTT CAC GGT AAG AGG AGC AAA	2367, 2368

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	43917652	43917901	TGT CGT ATC CTG CTG TTT AGA	TTC TCT CTA GGC AGG TGA ACT	2369, 2370
chr18	43918519	43918768	TCA GGA GTA AAG TCA GGA CCT	ACC CTT TGA AAG AAC CAG GAA	2371, 2372
chr18	43918998	43919247	TGA GCT GAT TTA CTG TGA CAC	AAT GAG CCA CTG TTC TCT AGG	2373, 2374
chr18	43919544	43919793	TAG CAC CTT GAC TTC AGG ATT	ATT TGC ACA TTA GGG CCT CAA	2375, 2376
chr18	44086123	44086372	TGA GGT TGG AAA GGG TCA ATT	GAA CTT CCC TGC TTC CTT CT	2377, 2378
chr18	44094299	44094548	CAG CTT TCC TTC CTC TTC TCT	AAA TAC TTG GCT GTG ACC ATG	2379, 2380
chr18	44167214	44167463	ACA GTG AGA GGA AAG AAC AGC	AAG ACC CTT GAG AAC TTC CAA	2381, 2382
chr18	44167791	44168040	GGA AAT AAA TTG TGA GCT GGC	ATG TGT AAA GAC GTC CTG GAA	2383, 2384
chr18	44168296	44168545	AGA CAG CCC TTC AAT CCA TAC	TCT ATG TGG AGG GAT TTG ACA	2385, 2386
chr18	44173512	44173761	CCT ACA TCC CTT CCT CCT TTC	TTC CTG AAG TTT ATG GTG CAA C	2387, 2388
chr18	44174626	44174875	TCA CCC ATC TTC CAA TTA GCT	AAG GTT GAA TGA GGA TCA AGC	2389, 2390
chr18	44857765	44858014	TTT CTA AGC ACA AAC TGA CAC C	GAG GGA AGA ACA CAA CAC ATG	2391, 2392
chr18	45110525	45110774	GAT GTT TGC ACT GGA GGG ATA	CAG ACT AGC CTA CAA TCC TCC	2393, 2394
chr18	45197621	45197870	GAA GAC TAA ATG TTG GCC GAA	CAT TCA GGT TCT TAA GGG CTG	2395, 2396
chr18	45197871	45198120	GTA GAG AGA GGG AGG ATC ACA	GAA AGG CAG ACG ATG AAA GAG	2397, 2398
chr18	45198760	45199009	CTT GCC ATG AAG TTT GAC CAG	ATC TCC ATC AGC ACA GGA ATT	2399, 2400

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	45234886	45235135	AGG ATG AGC ATT TGT AAC CTG	GCA GTG TGA CAT CTG TGA ATG	2401, 2402
chr18	45235523	45235772	TGT CGC TTT CAA ATT ACC CAC	AGA CTT TCC TGT TCC TCT TCA	2403, 2404
chr18	45238370	45238619	CAT ACA AGT GCT CTG TTA GGC	TTT ACA ATG AAC TAG CCA GGC	2405, 2406
chr18	45335896	45336145	AGG ACT TGG AAC CAG AAA GAC	TAA CAT CTG CCT GAA AGC TTC	2407, 2408
chr18	45336353	45336602	AAA GAG GGC TGA TAT CGT CTG	CTC GTG TGT GCA ATT TGG AAT	2409, 2410
chr18	45357461	45357710	CTC ACT GCA AAC TAT GGA ACC	GGA GAG ATG GAG AAG ACC TTT	2411, 2412
chr18	45357745	45357994	TAT TCT GCC CAT CTT CTT CCT	AAT GTA TTA CTG TGC TCC CGT	2413, 2414
chr18	45358502	45358751	GGA GAC AGC CCA AAC ATA GA	ACC ACC TGC CAC TGT ATA AAT	2415, 2416
chr18	45362379	45362628	AGC AAT GGT GAA GTT CTG GAT	AAT TCT TCT TCT GGT GCA AGG	2417, 2418
chr18	45397588	45397837	CTG GTC AGT GAG AGA AGG GAA	TTG CCC TTT GAA CTG TTG ATC	2419, 2420
chr18	45864183	45864432	TTT CTC CAC TGG CAT GAA CT	AAG CAC ACT AAG GCC TGA TAA	2421, 2422
chr18	46464382	46464631	CAT GAT CAC AAT TCC AAG CCA	TCA GTA TCC ATT CAG CAT CCA	2423, 2424
chr18	46473428	46473677	ACC CAG TCA AGT TAC AGT CTT	TAA CCG AAG CCC ATA CTC TG	2425, 2426
chr18	46474545	46474794	TGT AAA GCA TAT CAA GGG AAC G	TCC TTA CTC CAG ATA CCC GAT	2427, 2428
chr18	46481797	46482046	TGC AGA GAT ATG TTC CCG TAT	TCT GGC TTC TTT CTT GGA GAG	2429, 2430
chr18	46483016	46483265	AGA AGA CAG TAC AAG GAA GGC	GCA ACA TTC ATT TCA TCC TGC	2431, 2432

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	46589014	46589263	TGT TTG CCA TTT GTT CTC CTC	CAT CAC GAC ATC CAT TTC CAC	2433, 2434
chr18	46599847	46600096	TTG AAG GCA AGA GAA GTT TGG	GTG GTT ATT ATC GGT GGG TGA	2435, 2436
chr18	46601866	46602115	GCC AAG GAA ATG TAG GGA AAG	CTG CCA TTC CTT GTT TCC AA	2437, 2438
chr18	46983278	46983527	AAC CTT CAC ACC TAG AGA CAG	ATT TGG ACT TGA AGC AGC CT	2439, 2440
chr18	47013103	47013352	GCA TAA CAG GGA AAG TCA CCT	CTG TAT TCT TTG TCC ACC ACC	2441, 2442
chr18	47016802	47017051	AGG ATG TTA GTG GTT TGG GTA	GAC AGG ACA CCT TGG ATT GAT	2443, 2444
chr18	286722	286971	TCT GAC ACT GAC CTT CAA CT	GAG TAA TTC CCC CGA TGC AG	2445, 2446
chr18	298393	298642	GAA ACA TTG CTT TCC CTC CA	TGA GAA TCA TTG AGC CAA ACC	2447, 2448
chr18	338127	338376	TTC CAC ACA TCT CTT CTC CG	AAT TGT GAG CGT TAG AGT GC	2449, 2450
chr18	340427	340676	GGG AGC CTT GAA AAC CTG AA	CCA GTG GGT CTT AAC ATT GAG	2451, 2452
chr18	387135	387384	ATT GGT AGC GTT GTC AGC A	AAA AGG CTA GTA GAG GGT GC	2453, 2454
chr18	391175	391424	TTC CTG CAT CTT GTA GAC CC	TCG CTG AAG AAC TGA GAC AC	2455, 2456
chr18	434440	434689	GGG CTA ATG TTT TGC TTC CA	GGG AAG TGA TTG GAG AGA GG	2457, 2458
chr18	457527	457776	TGT TGA TTA GAG CTT CCC CC	CCG CTT GGT ATA GAG TGC TG	2459, 2460
chr18	460384	460633	AGA GGT TTT CTT CCC CGT G	TTG AGG TTG TCA GGA AAG CT	2461, 2462
chr18	469281	469530	TAG TGC CCT CTA TTG TGC CT	TCT TGG GAA AGG GTC ATT CT	2463, 2464

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	572716	572965	ACT GCT GGA CTT TGA AAT GC	TCT CTT CCA TGC ACT CCA C	2465, 2466
chr18	598352	598601	CAA ACA GTG AGA TGT GGC TG	GAT CTG ACC CTC TGC TCA C	2467, 2468
chr18	615413	615662	TTG CTT CCT GAA AAC TGG TTC	CCT GAA AGA TGC ATG GTT GG	2469, 2470
chr18	618280	618529	ATT CCA ATC ACG TCT CTG CA	GCC ACA GAA CAA CCA GAT TC	2471, 2472
chr18	901111	901360	ACA ATC TCA CAG CCT GGA AA	TGG GGT TAA GAG CTC AAG AG	2473, 2474
chr18	939383	939632	TCA GAT GGG TGA GGT TCT TG	AGT GTG GAT GAC TTC TGC AA	2475, 2476
chr18	26321283	26321532	CTG CTC CTT CCC TCC AAT TA	GCC CCA TTA TCC TCC TTT GT	2477, 2478
chr18	26337865	26338114	AAA TGC CAG TCC TGT AAA GG	CAA ATC AGA CCC ACT AAG CAC	2479, 2480
chr18	26345717	26345966	TGT CCC ATT GCT TAG GAA GT	TGT TTT GGA CTG CTT CAC TC	2481, 2482
chr18	26365387	26365636	TGT GTG ATC CAG AGA CCC TA	ATT ATC ATG CTC ACT CCT CCA	2483, 2484
chr18	26367518	26367767	ACC AAT GTA GAC TTA GCG GG	GAC ATA GCA CGG GAG GAG TA	2485, 2486
chr18	26392500	26392749	ACT CTC ATA TTG CCC CAC TT	GCT AGT GGC GTT TTA GGA AA	2487, 2488
chr18	26406499	26406748	CCA GGG ATT GAT GTA CTG GT	TCG CTT GGA AGT CAT AGC C	2489, 2490
chr18	26711590	26711839	AAT TCT GGT CTA TCT GGC GT	CAG AGC TGC TTT GAA GAT AAT CC	2491, 2492
chr18	26729342	26729591	GCC AGC CCT TTT CAC ATA TT	GAA GCC TGA TAG ATG TGC CT	2493, 2494
chr18	26733981	26734230	AAC TAC ACC ATC CCC TGT TT	TAC CTC TGC CTC CAA TTG TC	2495, 2496

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	26739336	26739585	CCA TTT CAA ACA TGC TGG TC	CCC ATG TAG ACA AAG TGC TT	2497, 2498
chr18	26750685	26750934	AGA TTA TAA GAA GGC AGG GAA C	GGC AGG TTT GTC TTA CAG TT	2499, 2500
chr18	26752487	26752736	GTA GAG GGC TTA AAA CAT GTC C	TGT GAG ATT GCA TTC CCC TT	2501, 2502
chr18	26757573	26757822	ACA AGA ACA CAG TCG TTA AGC	CGG GTC AGA GAA AGA TCA GG	2503, 2504
chr18	27244230	27244479	TCT CTC CTT CAC TCC CTT CA	AAA CAT TTG TAA CCA CTC CCT G	2505, 2506
chr18	35076347	35076596	TGT CCA CCC CTC TTT GAT TG	CCT GTA ATA TGG GAC TCC TGG	2507, 2508
chr18	35147912	35148161	TTT AGC TTC TCC TGC CTT TG	CCA AAC CAC ACA CAC AAA CT	2509, 2510
chr18	35282655	35282904	AGA AGC AAT TCA CCA GGT CA	AGG AGA AGG ACA TTT CAC AGG	2511, 2512
chr18	35292141	35292390	TGG AGT CAG AAG TGT GTG TT	AGG CCG ATA AGA CAA GGT TC	2513, 2514
chr18	35304247	35304496	TCT GGT GTC AAA GCT TAG GG	GTT TCC CAT AGA GCC CTG G	2515, 2516
chr18	35311302	35311551	TGC CGA TGA TGT GTG TTT TG	CTC CAC TCT CTC CAA CCA AC	2517, 2518
chr18	36463709	36463958	TGT CCC TTC CTA ATC CCA AA	AAA CTG TGA AAG GAC GAG GA	2519, 2520
chr18	36473033	36473282	AGG ATG TTT AAG TTG CAG CA	TCC CTT GCT TTT GTA CCA GG	2521, 2522
chr18	36475403	36475652	TAT GCA GTT TTA CCC CCT CC	CAT GTG TGT ACT GTG CCT CA	2523, 2524
chr18	36479145	36479394	TTC TGT GTG GTC TCC TCT TG	TCT TTC ACT GTC ACT ATG GGG	2525, 2526
chr18	36482038	36482287	ATG GAG GGA CAA GTG AGA CA	AAA TGC AGC TTC CCA ACA TC	2527, 2528

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chromosome	start	stop	forward primer	reverse primer	SEQ ID NOs:
chr18	36483959	36484208	GGG GAA CAT GGA GCT GTA AA	GAG ACT TTC TGG AGG ACG AA	2529, 2530
chr18	36485761	36486010	GGA CCC CCT ACC ACA TTT AC	GAA ACG TAA TTT AGT GAC TGG C	2531, 2532
chr18	36487627	36487876	AGA TGG AGA AAT GTG CAG AGA	ATG TGT CTA TTG CTA CCT GTG A	2533, 2534
chr18	36489135	36489384	ATG ACT GCA TCC AAG AGC A	CTC CTT CAT TTT GCT GGT GG	2535, 2536
chr18	36531244	36531493	CAG AAT TTC CAG GCA GTT GT	GGT GAT CAT TTG TCT GCA CA	2537, 2538
chr18	36554006	36554255	GAA TCC AGA AGC TCA GTC CTT	TGA AGG GAT GAA GGC AGA AG	2539, 2540
chr18	36558050	36558299	AGC CCT GGA ATC TTG ACA TT	ATT GTG TTG TCC TTC CGT TT	2541, 2542
chr18	36569619	36569868	GTG CAT TAT ACG GAT GGC C	CTG AAG CAT CAC TGG CAT TG	2543, 2544
chr18	1017928	1018177	GGT AGA GGG TCC TGT GAT TC	AAG CTT GTA GTC TGG GTA GC	2545, 2546
chr18	241538	241787	GTA ACT GCT AGC CAC TGA GT	TCC CTC TGT ACT ATG TAG CAT G	2547, 2548
chr18	242831	243080	GCC TTT TTG GGA ATC CTA GT	TTC ATT TCC CTT TGT TGC CC	2549, 2550

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FIG 3

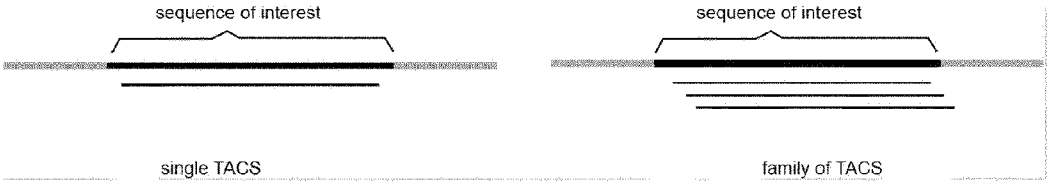

sequence of interest
sequence of interest
single TACS
family of TACS

FIG 4

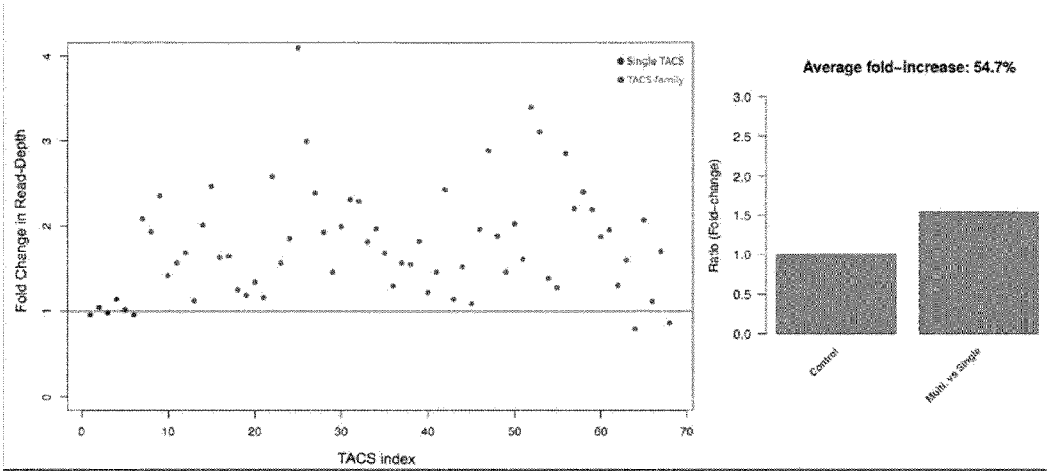

Single TACS
TACS family
Fold Change in Read-Depth
TACS index
Average fold-increase: 54.7%
Ratio (Fold-change)
Control
Multi. vs Single

FIG 5

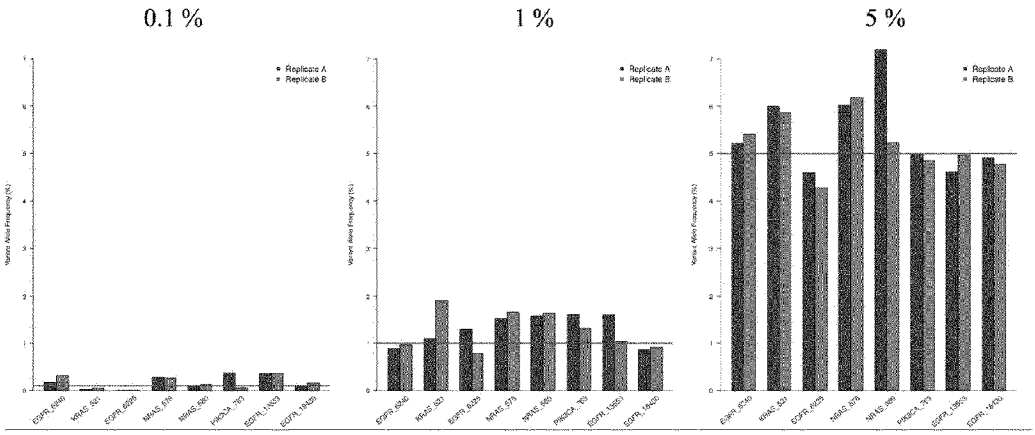

0.1 %
1 %
5 %
Replicate A
Replicate B

FIG 6

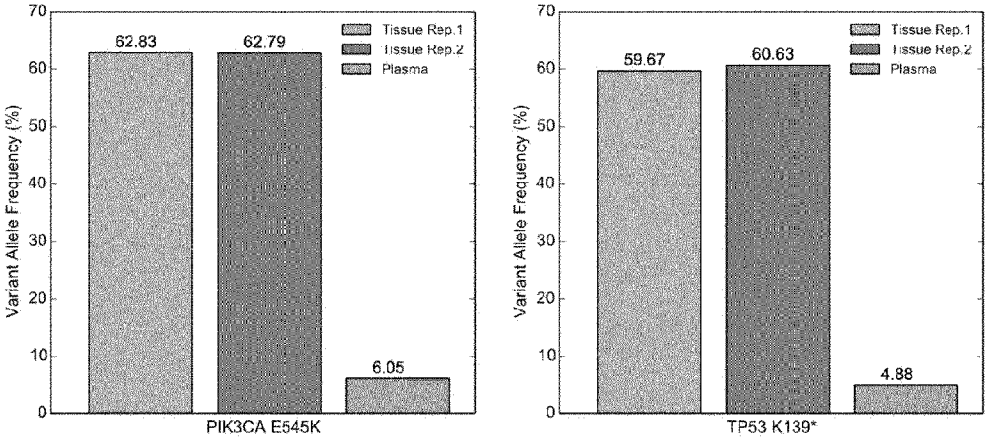

Variant Allele Frequency (%)
62.83
62.79
6.05
Tissue Rep.1
Tissue Rep.2
Plasma
PIK3CA E545K
59.67
60.63
4.88
TP53 K139*

FIG 7

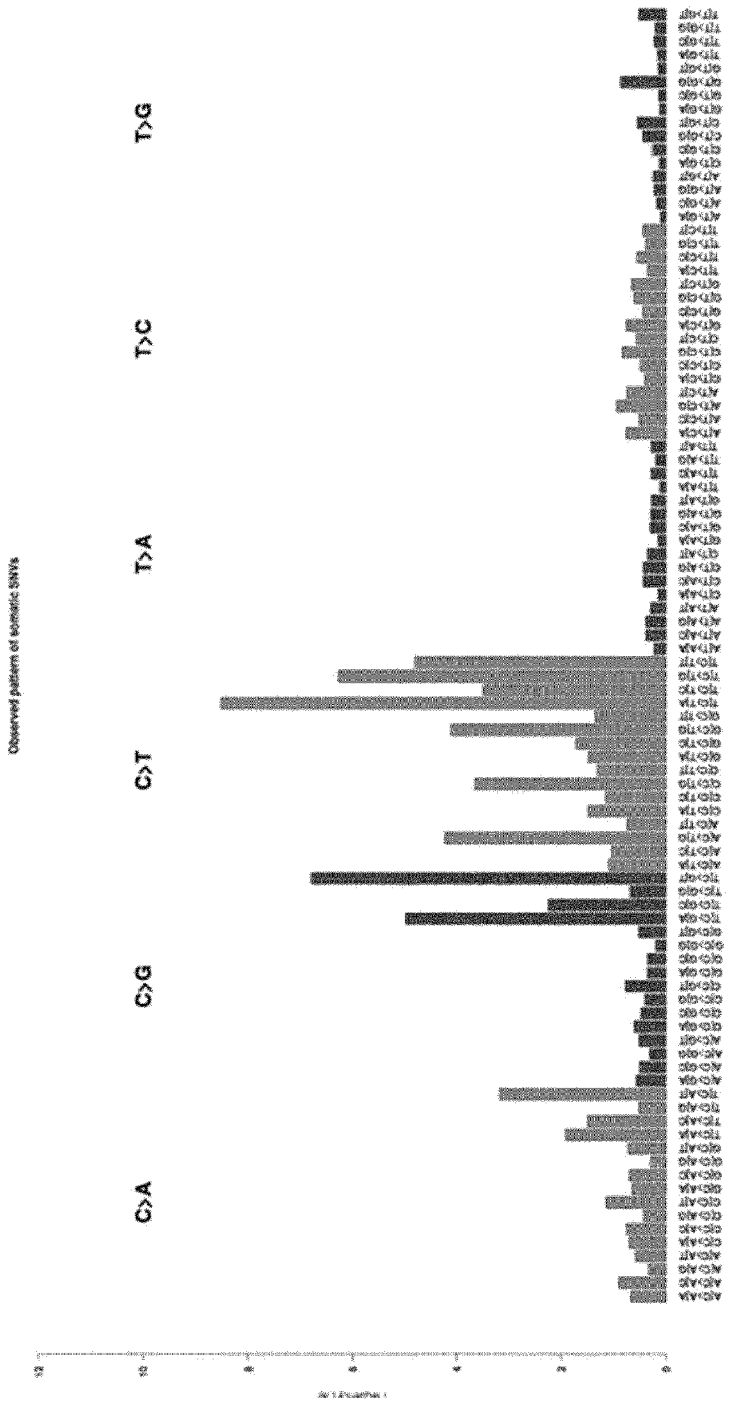


FIG 8

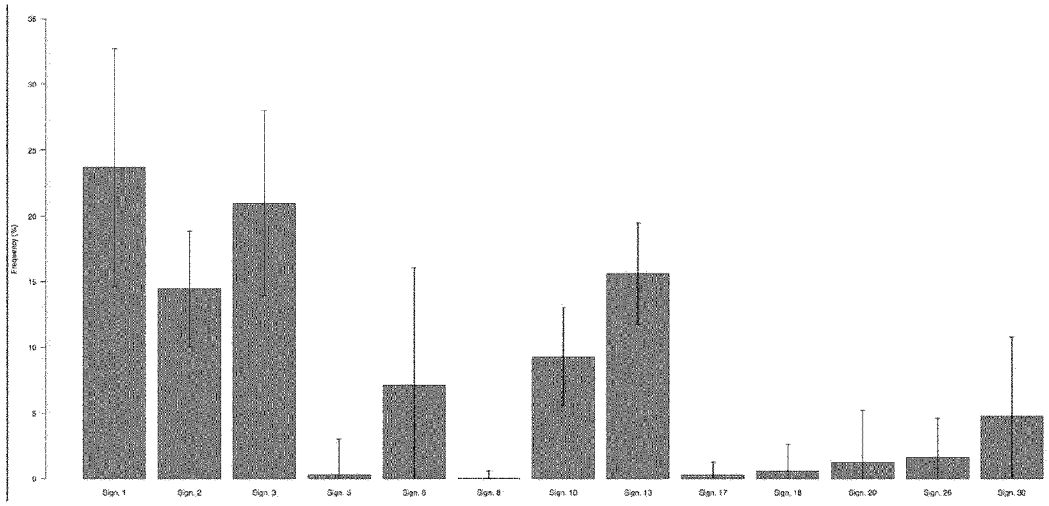

Frequency (%)
0
5
10
15
20
25
30
35
Sign. 1
Sign. 2
Sign. 3
Sign. 5
Sign. 6
Sign. 8
Sign. 10
Sign. 13
Sign. 17
Sign. 18
Sign. 20
Sign. 26
Sign. 30

FIG 9

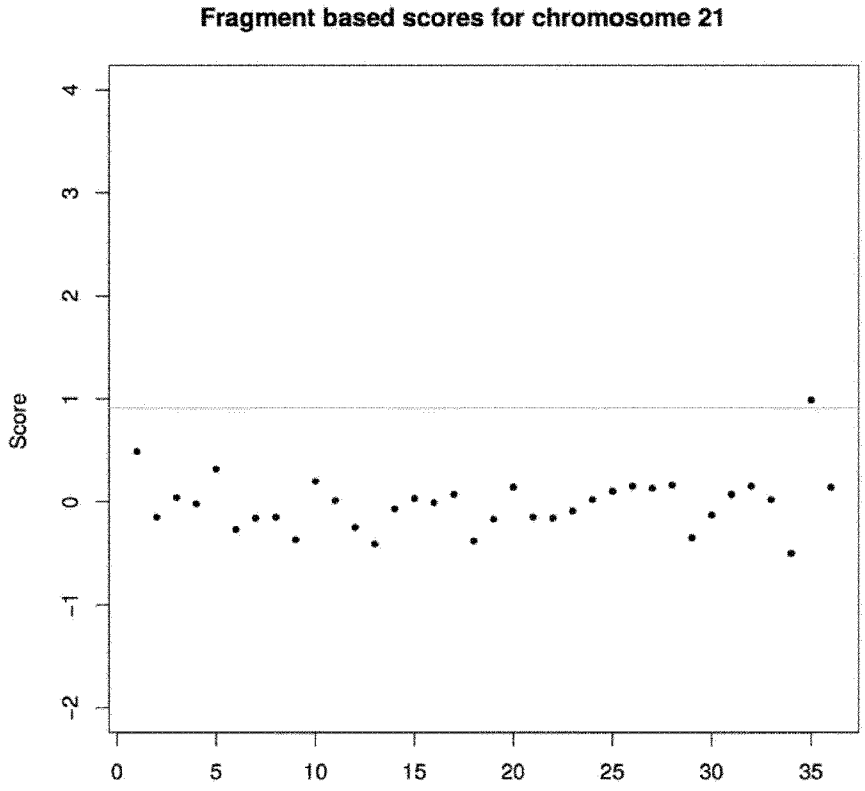

Fragment based scores for chromosome 21
Score
4
3
2
1
0
−1
−2
0
5
10
15
20
25
30
35

FIG 10

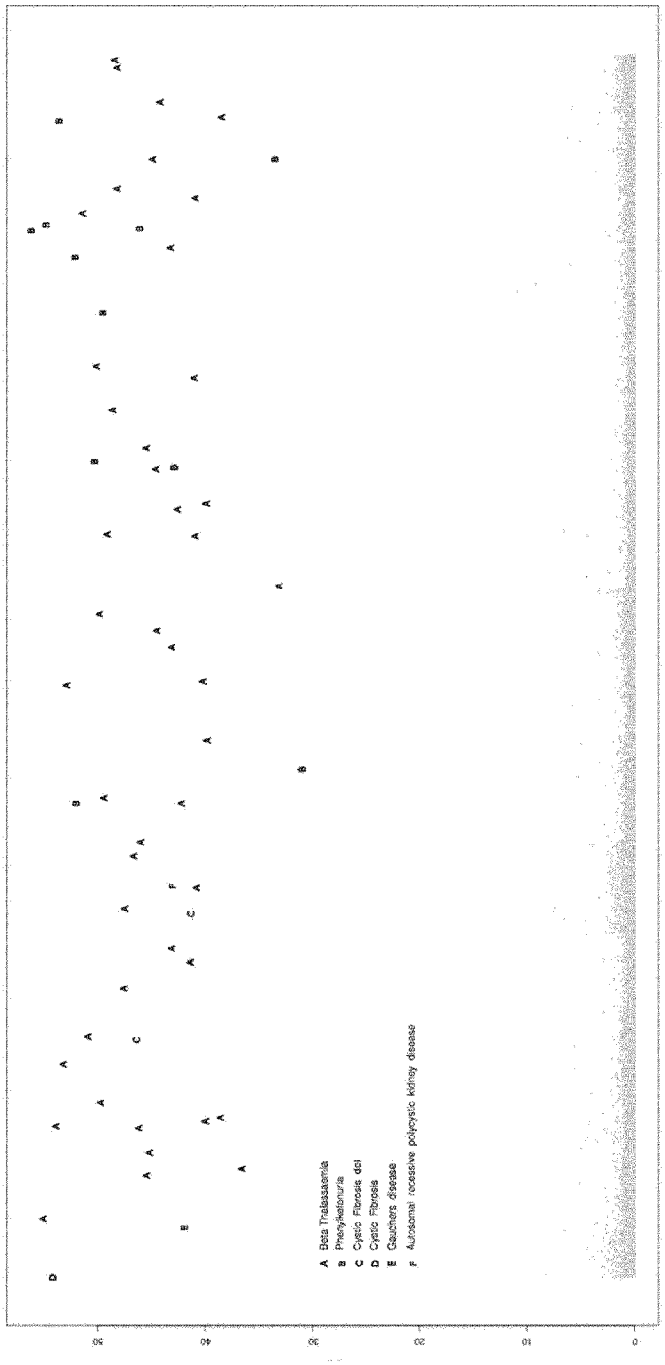

A Beta Thalassaemia
B Phenylketonuria
C Cystic Fibrosis del
D Cystic Fibrosis
E Gauchers disease
F Autosomal recessive polycystic kidney disease
50
40
30
20
10
0

FIG 11

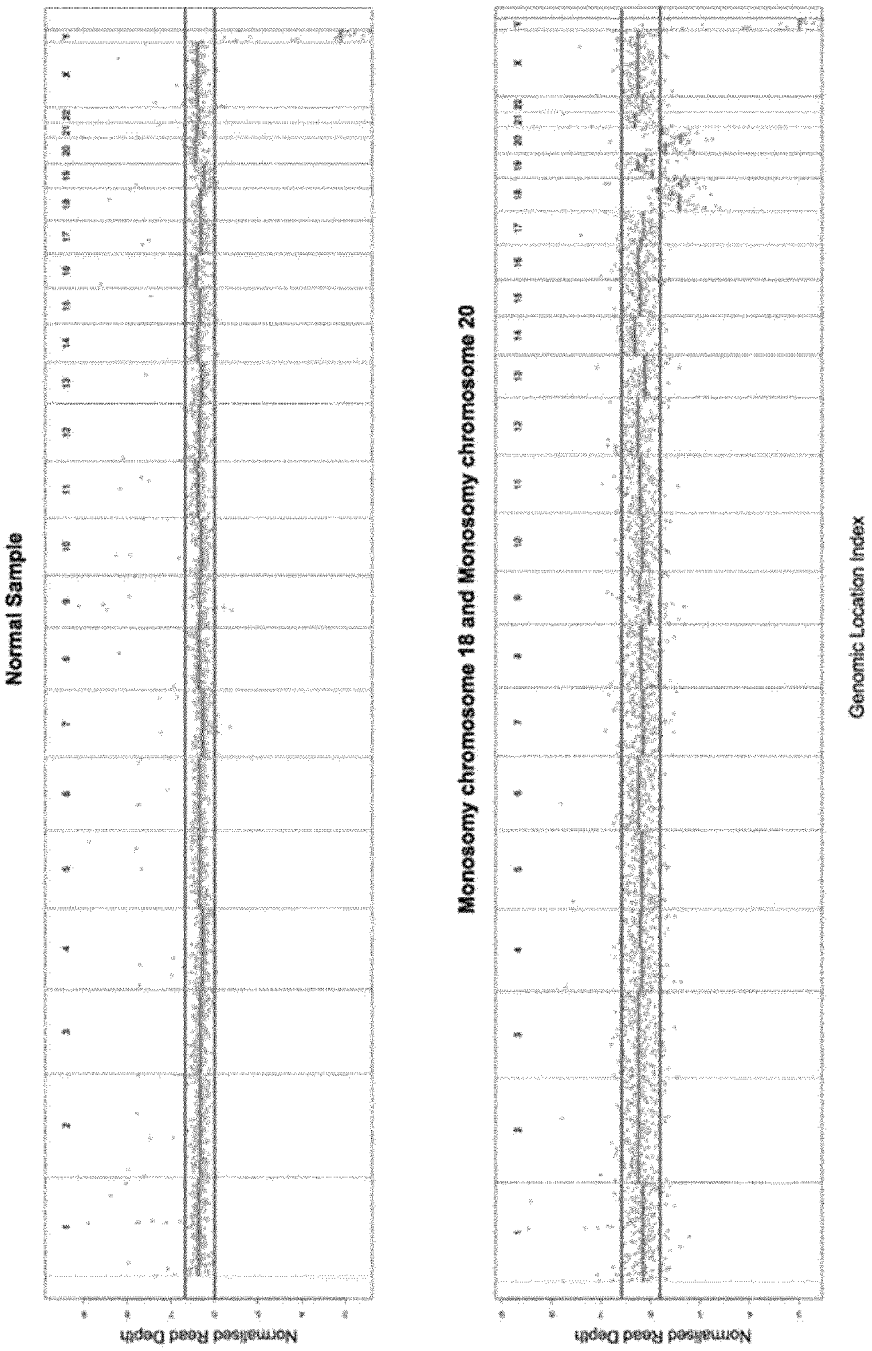

Normal Sample
Monosomy chromosome 18 and Monosomy chromosome 20
Normalised Read Depth
Normalised Read Depth
Genomic Location Index

FIG 12

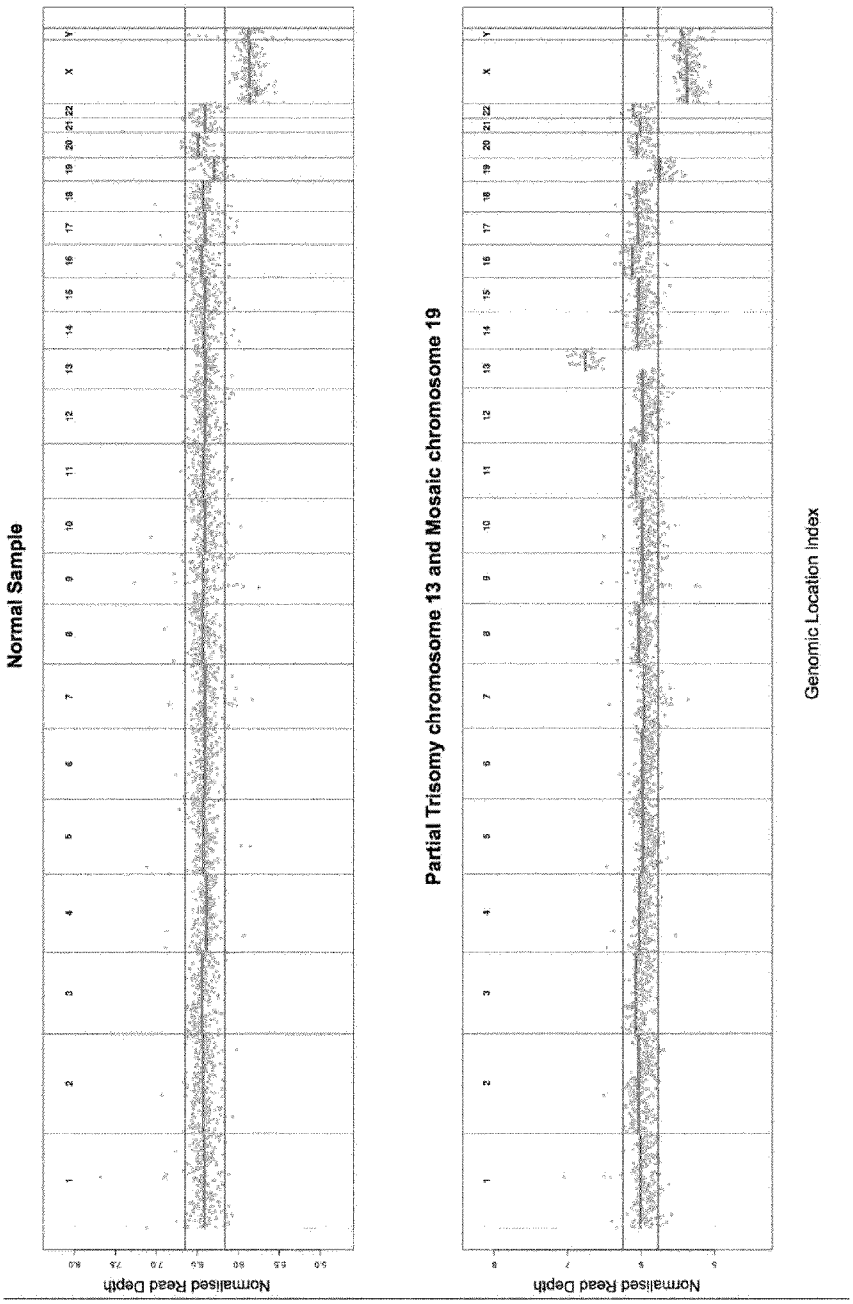

Normal Sample
Partial Trisomy chromosome 13 and Mosaic chromosome 19
Normalised Read Depth
Normalised Read Depth
Genomic Location Index

FIG 13

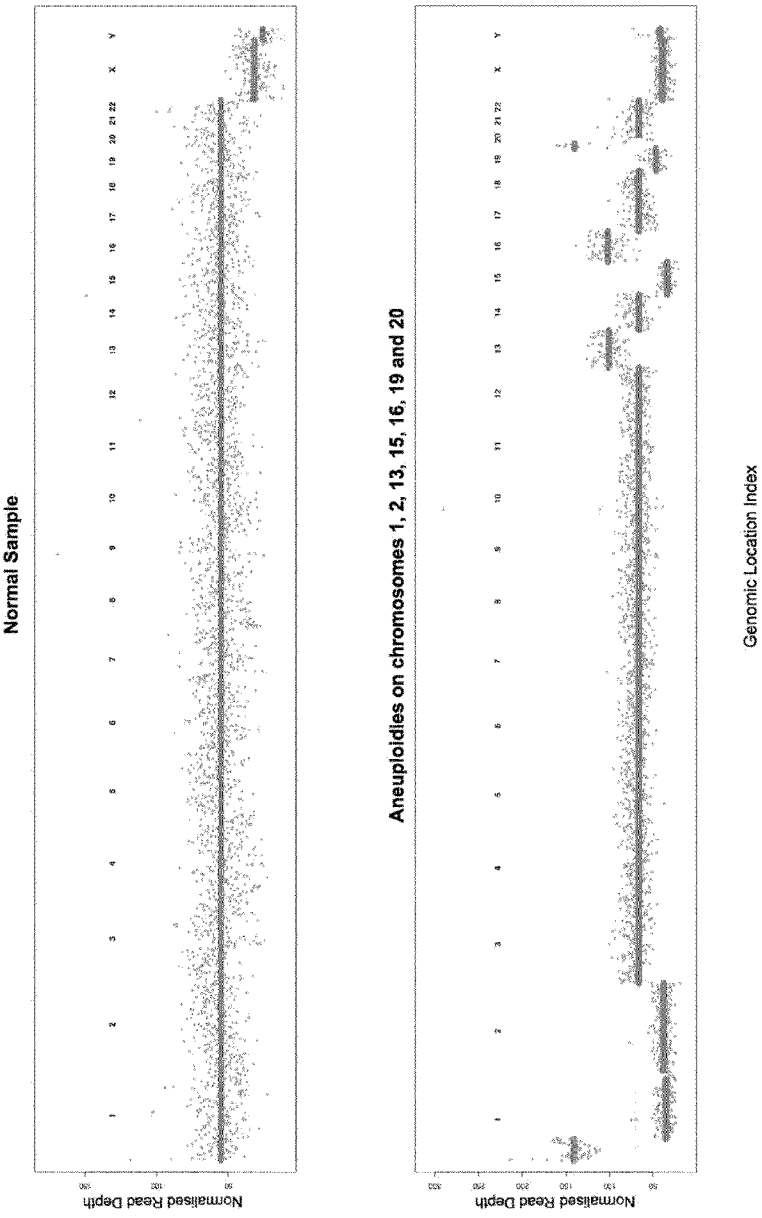

Normal Sample
Aneuploidies on chromosomes 1, 2, 13, 15, 16, 19 and 20
Normalised Read Depth
Normalised Read Depth
Genomic Location Index

FIG 14

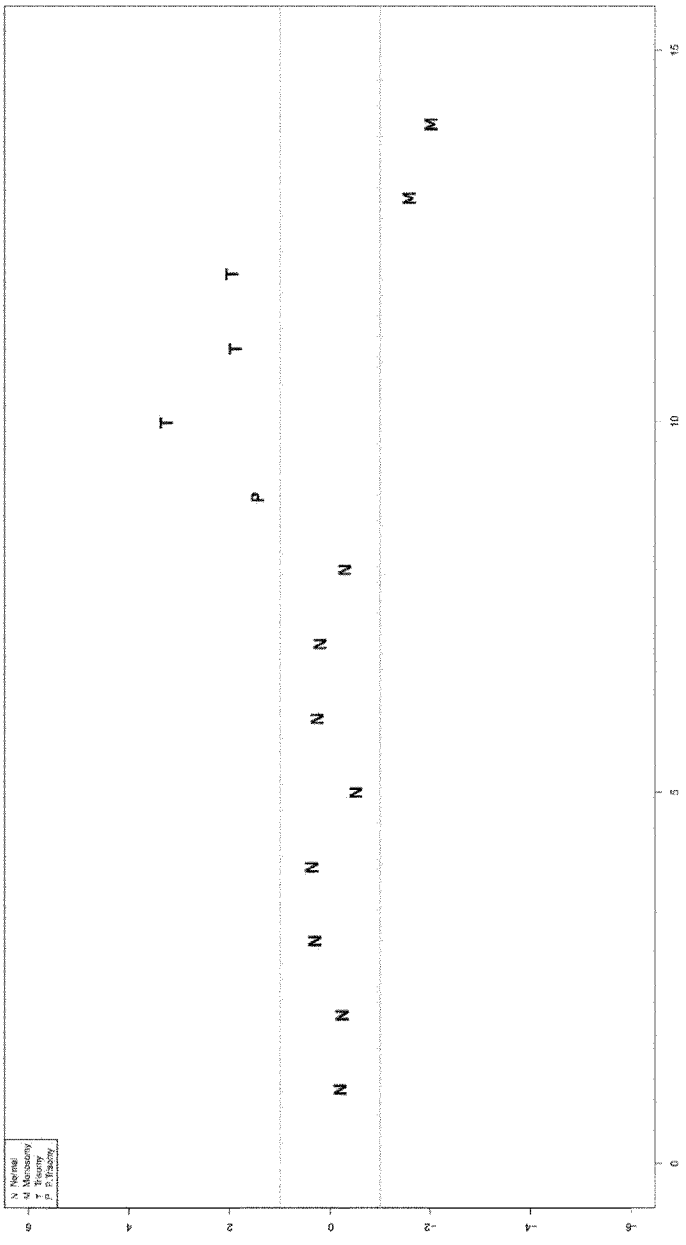


FIG 15

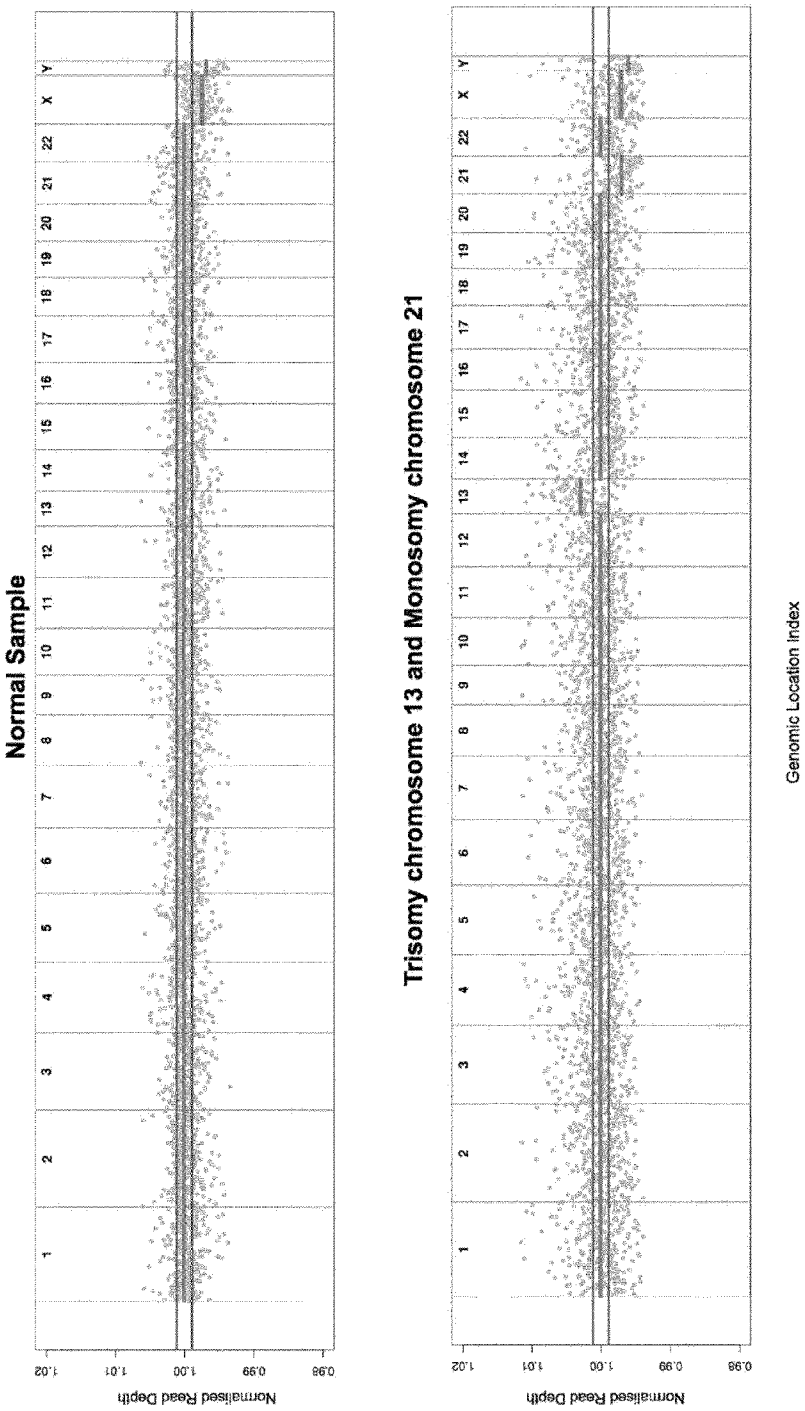

Normal Sample
Trisomy chromosome 13 and Monosomy chromosome 21
Normalised Read Depth
1.02
1.01
1.00
0.99
0.98
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Genomic Location Index

FIG 16

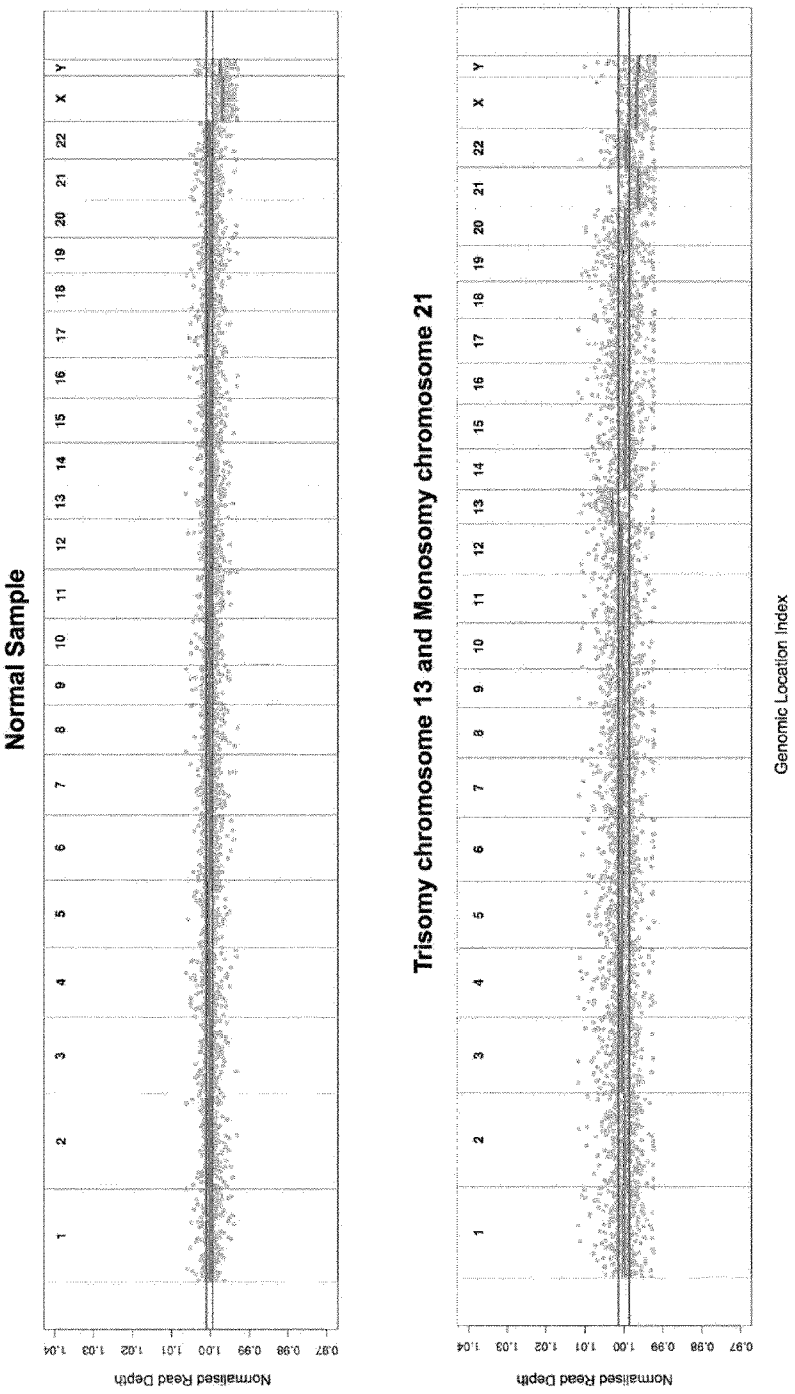

Normal Sample
Trisomy chromosome 13 and Monosomy chromosome 21
Normalised Read Depth
0.97
0.98
0.99
1.00
1.01
1.02
1.03
1.04
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Genomic Location Index

FIG 17

chromosome	start	stop	gene
chr1	115256347	115256588	NRAS
chr1	115256398	115256647	NRAS
chr1	115256410	115256649	NRAS
chr1	115256442	115256691	NRAS
chr1	115256467	115256726	NRAS
chr1	115256470	115256715	NRAS
chr1	115258538	115258778	NRAS
chr1	115258570	115258813	NRAS
chr1	115258607	115258846	NRAS
chr1	115258659	115258901	NRAS
chr3	178916725	178916970	PI3KCA
chr3	178916766	178917010	PI3KCA
chr3	178916782	178917028	PI3KCA
chr3	178916822	178917068	PI3KCA
chr3	178917417	178917566	PI3KCA
chr3	178917417	178917618	PI3KCA
chr3	178917420	178917590	PI3KCA
chr3	178917420	178917604	PI3KCA
chr3	178921331	178921578	PI3KCA
chr3	178921339	178921585	PI3KCA
chr3	178921347	178921596	PI3KCA
chr3	178921364	178921608	PI3KCA
chr3	178927888	178928114	PI3KCA
chr3	178927898	178928114	PI3KCA
chr3	178927974	178928182	PI3KCA
chr3	178935873	178936102	PI3KCA
chr3	178935930	178936133	PI3KCA

chr3	178935944	178936197	PIK3CA
chr3	178935995	178936197	PI3KCA
chr3	178936030	178936279	PI3KCA
chr3	178951914	178952159	PI3KCA
chr3	178951915	178952140	PI3KCA
chr3	178951921	178952140	PI3KCA
chr3	178951921	178952159	PI3KCA
chr3	178951948	178952109	PIK3CA
chr3	178952004	178952152	PIK3CA
chr3	178952010	178952109	PIK3CA
chr3	178952109	178952152	PIK3CA
chr7	55241468	55241710	EGFR
chr7	55241542	55241789	EGFR
chr7	55241576	55241746	EGFR
chr7	55242269	55242500	EGFR
chr7	55242272	55242520	EGFR
chr7	55242280	55242532	EGFR
chr7	55242319	55242558	EGFR
chr7	55242332	55242584	EGFR
chr7	55242505	55242271	EGFR
chr7	55248852	55249080	EGFR
chr7	55248896	55249120	EGFR
chr7	55248933	55249182	EGFR
chr7	55248937	55249187	EGFR
chr7	55248961	55249208	EGFR
chr7	55259335	55259571	EGFR
chr7	55259337	55259586	EGFR
chr7	55259356	55259602	EGFR
chr7	55259368	55259570	EGFR
chr7	55259391	55259640	EGFR

Fig. 17 cont.			
chromosome	start	stop	gene
chr12	25378488	25378688	KRAS
chr12	25378503	25378751	KRAS
chr12	25378546	25378778	KRAS
chr12	25378554	25378783	KRAS
chr12	25380153	25380359	KRAS
chr12	25380166	25380337	KRAS
chr12	25380167	25380326	KRAS
chr12	25380167	25380359	KRAS
chr12	25398080	25398329	KRAS
chr12	25398145	25398394	KRAS
chr12	25398153	25398397	KRAS
chr12	25398159	25398408	KRAS
chr12	25398186	25398433	KRAS