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(71) Applicant: **SEQUENOM, INC.** [US/US]; 3595 John Hopkins Court, San Diego, CA 92121 (US).

(72) Inventors: **VAN DEN BOMM, Dirk, Johannes**; 638 Bonair Way, Unit A, La Jolla, CA 92037 (US). **CANTOR, Charles, R.**; 526 Stratford Court, Unit E, Del Mar, CA 92014 (US). **KIM, Sung, Kyun**; 662 Glenmore Boulevard, Glendale, CA 91206 (US). **DZAKULA, Zeljko**; 12830 Sundance Avenue, San Diego, CA 92129 (US). **DECIU, Cosmin**; 10545 Sea Mist Way, San Diego, CA 92121 (US).

(74) Agents: **DICKINSON, Kari, A.** et al.; Grant Anderson LLP, c/o Portfolioip, P.O. Box 52050, Minneapolis, MN 55402 (US).

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(54) Title: METHODS AND PROCESSES FOR NON-INVASIVE ASSESSMENT OF GENETIC VARIATIONS

(57) Abstract: Technology provided herein relates in part to methods, processes and apparatuses for non-invasive assessment of genetic variations.

METHODS AND PROCESSES FOR NON-INVASIVE ASSESSMENT OF GENETIC VARIATIONS

Related Patent Applications

This patent application claims the benefit of U.S. Provisional Patent Application No. 61/545,053 filed on October 7, 2011, entitled METHODS AND PROCESSES FOR NON-INVASIVE ASSESSMENT OF GENETIC VARIATIONS, naming Dirk Johannes Van Den Boom and Charles R. Cantor as inventors, and designated by Attorney Docket No. SEQ-6036-PV. This patent application also claims the benefit of U.S. Provisional Patent Application No. 61/709,899 filed on October 4, 2012, entitled METHODS AND PROCESSES FOR NON-INVASIVE ASSESSMENT OF GENETIC VARIATIONS, naming Cosmin Deciu, Zeljko Dzakula, Mathias Ehrich and Sung Kyun Kim as inventors, and designated by Attorney Docket No. SEQ-6034-PV3; U.S. Provisional Patent Application No. 61/663,477 filed on June 22, 2012, entitled METHODS AND PROCESSES FOR NON-INVASIVE ASSESSMENT OF GENETIC VARIATIONS, naming Zeljko Dzakula and Mathias Ehrich as inventors, and designated by Attorney Docket No. SEQ-6034-PV2; and U.S. Provisional Patent Application No. 61/544,251 filed on October 6, 2011, entitled METHODS AND PROCESSES FOR NON-INVASIVE ASSESSMENT OF GENETIC VARIATIONS, naming Zeljko Dzakula and Mathias Ehrich as inventors, and designated by Attorney Docket No. SEQ-6034-PV. The entire content of the foregoing provisional applications are incorporated herein by reference, including all text, tables and drawings.

Field

Technology provided herein relates in part to methods, processes and apparatuses for non-invasive assessment of genetic variations.

Background

Genetic information of living organisms (e.g., animals, plants and microorganisms) and other forms o Genetic information of living organisms (e.g., animals, plants and microorganisms) and other forms of replicating genetic information (e.g., viruses) is encoded in deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Genetic information is a succession of nucleotides or modified nucleotides representing the primary structure of chemical or hypothetical nucleic acids. In humans, the complete genome contains about 30,000 genes located on twenty-four (24) chromosomes (see The Human Genome, T. Strachan, BIOS Scientific Publishers, 1992). Each gene encodes a

specific protein, which after expression via transcription and translation fulfills a specific biochemical function within a living cell.

Many medical conditions are caused by one or more genetic variations. Certain genetic variations cause medical conditions that include, for example, hemophilia, thalassemia, Duchenne Muscular Dystrophy (DMD), Huntington's Disease (HD), Alzheimer's Disease and Cystic Fibrosis (CF) (Human Genome Mutations, D. N. Cooper and M. Krawczak, BIOS Publishers, 1993). Such genetic diseases can result from an addition, substitution, or deletion of a single nucleotide in DNA of a particular gene. Certain birth defects are caused by a chromosomal abnormality, also referred to as an aneuploidy, such as Trisomy 21 (Down's Syndrome), Trisomy 13 (Patau Syndrome), Trisomy 18 (Edward's Syndrome), Monosomy X (Turner's Syndrome) and certain sex chromosome aneuploidies such as Klinefelter's Syndrome (XXY), for example. Another genetic variation is fetal gender, which can often be determined based on sex chromosomes X and Y. Some genetic variations may predispose an individual to, or cause, any of a number of diseases such as, for example, diabetes, arteriosclerosis, obesity, various autoimmune diseases and cancer (e.g., colorectal, breast, ovarian, lung).

Identifying one or more genetic variations or variances can lead to diagnosis of, or determining predisposition to, a particular medical condition. Identifying a genetic variance can result in facilitating a medical decision and/or employing a helpful medical procedure. In some cases, identification of one or more genetic variations or variances involves the analysis of cell-free DNA.

Cell-free DNA (CF-DNA) is composed of DNA fragments that originate from cell death and circulate in peripheral blood. High concentrations of CF-DNA can be indicative of certain clinical conditions such as cancer, trauma, burns, myocardial infarction, stroke, sepsis, infection, and other illnesses. Additionally, cell-free fetal DNA (CFF-DNA) can be detected in the maternal bloodstream and used for various noninvasive prenatal diagnostics.

The presence of fetal nucleic acid in maternal plasma allows for non-invasive prenatal diagnosis through the analysis of a maternal blood sample. For example, quantitative abnormalities of fetal DNA in maternal plasma can be associated with a number of pregnancy-associated disorders, including preeclampsia, preterm labor, antepartum hemorrhage, invasive placentation, fetal Down syndrome, and other fetal chromosomal aneuploidies. Hence, fetal nucleic acid analysis in maternal plasma can be a useful mechanism for the monitoring of fetomaternal well-being.

Early detection of pregnancy-related conditions, including complications during pregnancy and genetic defects of the fetus is important, as it allows early medical intervention necessary for the safety of both the mother and the fetus. Prenatal diagnosis traditionally has been conducted using cells isolated from the fetus through procedures such as chorionic villus sampling (CVS) or amniocentesis. However, these conventional methods are invasive and present an appreciable risk to both the mother and the fetus. The National Health Service currently cites a miscarriage rate of between 1 and 2 per cent following the invasive amniocentesis and chorionic villus sampling (CVS) tests. The use of non-invasive screening techniques that utilize circulating CFF-DNA can be an alternative to these invasive approaches.

Summary

Provided in some aspects are methods for determining the presence or absence of a genetic variation and methods for determining the presence or absence of a fetal aneuploidy using partial nucleotide sequence reads, and computer program products and systems for implementing methods discussed herein.

Also provided, in some aspects, are methods for detecting the presence or absence of a genetic variation, comprising: (a) obtaining counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, where at least some of the partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii), (b) normalizing the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and (c) detecting the presence or absence of a genetic variation based on the normalized counts. In some cases, the genetic variation is a nucleic acid sequence variation. In some cases, the genetic variation is a copy number variation. In some embodiments, the test sample is from a pregnant female and the genetic variation is a fetal aneuploidy.

In some embodiments, a method comprises comparing the normalized counts to a reference, thereby making a comparison, where determining the presence or absence of the genetic variation in (c) is based on the normalized counts and the comparison. In some cases, the reference is counts of sequence reads mapped to a reference chromosome or segment thereof. In some

cases, the reference chromosome is chromosome 1, chromosome 14, chromosome 19 or combination thereof.

In some embodiments, the counts of the partial nucleotide sequence reads obtained in (a) comprise counts of partial nucleotide sequence reads mapped to a test chromosome or segment thereof. In some cases, the test chromosome is chromosome 13, chromosome 18, chromosome 21 or combination thereof. In some cases, the counts are expressed as a ratio of counts for genomic sections in a test chromosome or segment thereof to counts for genomic sections in autosomes or segment thereof, thereby providing a count representation.

In some embodiments, the normalizing in (b) comprises normalizing according to guanine and cytosine (GC) content of the genomic sections, and providing calculated genomic section levels. In some embodiments, the normalizing in (b) comprises: (i) determining a guanine and cytosine (GC) bias for each of the genomic sections for multiple samples from a fitted relation for each sample between (1) the counts of the partial nucleotide sequence reads mapped to each of the genomic sections, and (2) GC content for each of the genomic sections; and (ii) calculating a genomic section level for each of the genomic sections from a fitted relation between (1) the GC bias and (2) the counts of the partial nucleotide sequence reads mapped to each of the genomic sections, thereby providing calculated genomic section levels, whereby bias in the counts of the partial nucleotide sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels, and where the normalized counts in (b) comprise the calculated genomic section levels. In some cases, the normalized counts in (b) are the calculated genomic section levels.

In some embodiments, the normalized counts are adjusted for a first level for a first set of genomic sections which first level is significantly different than a second level for a second set of genomic sections, thereby providing adjusted normalized counts, where determining the presence or absence of the genetic variation in (c) is based on the adjusted normalized counts.

In some embodiments, a method comprises (i) identifying a first elevation of the normalized counts significantly different than a second elevation of the normalized counts in a normalized counts profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections; (ii) determining an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the

genome; and (iii) adjusting the first elevation by a predetermined value, or adjusting the first elevation to the second elevation, when the first elevation is within one of the expected elevation ranges, thereby providing an adjustment of the first elevation. In some cases, the segment of the genome comprises the first elevation or the second elevation, or the first elevation and the second elevation.

In some embodiments, the normalizing in (b) comprises performing a local regression on the counts of the partial nucleotide sequence reads or the calculated genomic section levels, or the counts of the partial nucleotide sequence reads and the calculated genomic section levels. Sometimes the local regression comprises a weighted least squares fit. Sometimes the local regression comprises a LOESS regression.

In some embodiments, the partial nucleotide sequence reads are unary partial reads, for which unary partial reads one nucleotide is known at known positions and the other positions can be any one of three other nucleotides. Sometimes the partial nucleotide sequence reads are about 30 base pairs or more.

In some embodiments, the partial nucleotide sequence reads are binary partial reads, for which binary partial reads a first nucleotide class consisting of two possible bases is known at known positions and a second nucleotide class consisting of two possible bases is known at known positions, where the bases of the first nucleotide class are different than the bases of the second nucleotide class. Sometimes the partial nucleotide sequence reads are about 30 base pairs or more.

In some embodiments, the partial nucleotide sequence reads are ternary partial reads, for which ternary partial reads a first nucleotide is known at known positions, a second nucleotide is known at other known positions and the other positions are any one of two nucleotides other than the first nucleotide and the second nucleotide. Sometimes the partial nucleotide sequence reads are about 20 base pairs or more.

In some embodiments, a method comprises determining partial nucleotide sequence reads of the nucleic acid from the test sample. In some cases, the partial nucleotide sequence reads are determined using a method comprising a massively parallel sequencing (MPS) process or a nanopore process, or a massively parallel sequencing (MPS) process and a nanopore process. In

some embodiments, a method comprises mapping the partial nucleotide sequence reads to genomic sections of the reference genome.

In some embodiments, a method comprises isolating the nucleic acid from the test sample. In some embodiments, a method comprises obtaining the test sample. In some cases, the test sample is obtained from a pregnant female. The test sample sometimes is blood plasma, blood serum or urine.

Also provided, in some aspects, are systems comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, where at least some of the partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii); and which instructions executable by the one or more processors are configured to: (a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and (b) detect the presence or absence of a genetic variation based on the normalized counts.

Also provided, in some aspects, are apparatuses comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, where at least some of the partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii); and which instructions executable by the one or more processors are configured to: (a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and (b) detect the presence or absence of a genetic variation based on the normalized counts.

Also provided, in some aspects, are computer program products tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are

configured to: (a) access counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, where at least some of the partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii), (b) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and (c) detect the presence or absence of a genetic variation based on the normalized counts.

Also provided, in some aspects, are methods for detecting the presence or absence of a fetal aneuploidy comprising: (a) obtaining partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (b) mapping the partial nucleotide sequence reads to reference genome sections, (c) counting the number of partial nucleotide sequence reads mapped to each reference genome section, (d) comparing the number of counts of the partial nucleotide sequence reads mapped in (c), or derivative thereof, to a reference, thereby making a comparison, and (e) determining the presence or absence of a fetal aneuploidy based on the comparison.

Also provided, in some aspects, are methods for detecting the presence or absence of a fetal aneuploidy comprising: (a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (b) counting the number of partial nucleotide sequence reads mapped to each reference genome section, (c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, thereby making a comparison, and (d) determining the presence or absence of a fetal aneuploidy based on the comparison.

Also provided, in some aspects, are methods for detecting the presence or absence of a fetal aneuploidy comprising: (a) obtaining a sample comprising circulating, cell-free nucleic acid from a

pregnant female, (b) isolating sample nucleic acid from the sample, (c) obtaining partial nucleotide sequence reads from the sample nucleic acid, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (d) mapping the partial nucleotide sequence reads to reference genome sections, (e) counting the number of partial nucleotide sequence reads mapped to each reference genome section, (f) comparing the number of counts of the partial nucleotide sequence reads mapped in (e), or derivative thereof, to a reference, thereby making a comparison, and (g) determining the presence or absence of a fetal aneuploidy based on the comparison.

Also provided, in some aspects, are methods for detecting the presence or absence of a genetic variation comprising: (a) obtaining partial nucleotide sequence reads from a sample comprising nucleic acid from a subject, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (b) mapping the partial nucleotide sequence reads to reference genome sections, (c) comparing the partial nucleotide sequence reads mapped in (b) to a reference, thereby making a comparison, and (d) determining the presence or absence of a genetic variation based on the comparison.

Also provided, in some aspects, are methods for detecting the presence or absence of a genetic variation comprising: (a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising nucleic acid from a subject, to reference genome sections, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (b) comparing the partial nucleotide sequence reads mapped in (a) to a reference, thereby making a comparison, and (c) determining the presence or absence of a genetic variation based on the comparison.

Also provided, in some aspects, are methods for detecting the presence or absence of a genetic variation comprising: (a) obtaining a sample comprising nucleic acid from a subject, (b) isolating sample nucleic acid from the sample, (c) obtaining partial nucleotide sequence reads from the

sample nucleic acid, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (d) mapping the partial nucleotide sequence reads to reference genome sections, (e) comparing the partial nucleotide sequence reads mapped in (d) to a reference, thereby making a comparison, and (f) determining the presence or absence of a genetic variation based on the comparison.

In some embodiments, the genetic variation is a nucleic acid sequence variation. In some cases, nucleotide sequences of the partial nucleotide sequence reads are compared to a reference and sometimes a sequence match or mismatch is determined.

In some embodiments, the genetic variation is a nucleic acid copy number variation. In some embodiments, a method further comprises after the mapping of partial nucleotide sequence reads, counting the number of partial nucleotide sequence reads mapped to each reference genome section. Often, the number of counts of the partial nucleotide sequence reads, or derivative thereof, are compared to a reference.

In some embodiments, the subject is a fetus and the sample is from a pregnant female that bears a fetus. In some cases, the sample comprises circulating, cell-free nucleic acid and sometimes the sample nucleic acid comprises maternal and fetal nucleic acid.

In some embodiments, the sample is blood, urine, saliva, cervical swab, serum, and/or plasma.

In some embodiments, the partial nucleotide sequence reads comprise relative positional information for one or more nucleobase species. In some cases, the partial nucleotide sequence reads contain relative positional information for adenine. In some cases, the partial nucleotide sequence reads contain relative positional information for guanine. In some cases, the partial nucleotide sequence reads contain relative positional information for thymine. In some cases, the partial nucleotide sequence reads contain relative positional information for cytosine. In some cases, the partial nucleotide sequence reads contain relative positional information for methyl-cytosine.

In some embodiments, the partial nucleotide sequence reads contain relative positional information for two nucleobase species selected from the group consisting of adenine, guanine, thymine, cytosine or methyl-cytosine. In some embodiments, the partial nucleotide sequence reads contain relative positional information for three nucleobase species selected from the group consisting of adenine, guanine, thymine, cytosine or methyl-cytosine.

In some embodiments, the one or more nucleobase species comprise one or more detectable labels. In some embodiments, the partial nucleotide sequence reads contain relative positional information for sequences complementary to one or more hybridized probe species. In some cases, the one or more hybridized probe species comprise one or more detectable labels.

In some embodiments, the nucleobase class is purine. In some embodiments, the nucleobase class is pyrimidine. In some cases, purines are distinguished from pyrimidines in the partial nucleotide sequence reads.

In some embodiments, the sample nucleic acid comprises single stranded nucleic acid. In some embodiments, the sample nucleic acid comprises double stranded nucleic acid. In some cases, the nucleobase class is a nucleobase pair species in a duplex nucleic acid.

In some embodiments, the obtaining partial nucleotide sequence reads includes subjecting the sample nucleic acid to a sequencing process using a sequencing device. In some cases, the partial nucleotide sequence reads are obtained by nanopore sequencing. In some cases, the partial nucleotide sequence reads are obtained by reversible terminator-based sequencing. In some cases, the partial nucleotide sequence reads are obtained by pyrosequencing. In some cases, the partial nucleotide sequence reads are obtained by real time sequencing. In some cases, the partial nucleotide sequence reads are obtained by oligonucleotide probe ligation sequencing. In some cases, the partial nucleotide sequence reads are obtained by sequencing by hybridization.

In some embodiments, the partial nucleotide sequence reads comprise a number of discrete position identities sufficient to map to a reference genome section. In some embodiments, the partial nucleotide sequence read is of sufficient length to map to a reference genome section. In some cases, the partial nucleotide sequence read length is at least about 36 nucleobases. In some cases, the partial nucleotide sequence read length is at least about 72 nucleobases. In

some cases, the partial nucleotide sequence read length is at least about 108 nucleobases. In some embodiments, the nucleobase gaps in each partial nucleotide sequence read independently are about 1 to about 100 sequential nucleobases.

In some embodiments, a method comprises obtaining full nucleotide sequence reads, which nucleotide sequence reads do not contain nucleobase gaps between identified nucleobases or a nucleobase class comprising a subset of nucleobases present in the sample nucleic acid.

In some embodiments, the genetic variation is associated with a medical condition. In some cases, the medical condition is cancer. In some cases, the medical condition is an aneuploidy and is sometimes a fetal aneuploidy. In some embodiments, the fetal aneuploidy is trisomy 13, trisomy 18 or trisomy 21.

Also provided, in some aspects, are computer program products, comprising a computer usable medium having a computer readable program code embodied therein, the computer readable program code comprising distinct software modules comprising a sequence receiving module, a logic processing module, and a data display organization module, the computer readable program code adapted to be executed to implement a method for identifying the presence or absence of a fetal aneuploidy, the method comprising: (a) obtaining, by the sequence receiving module, partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii); (b) receiving, by the logic processing module, the partial nucleotide sequence reads; (c) mapping, by the logic processing module, the partial nucleotide sequence reads to reference genome sections; (d) counting, by the logic processing module, the number of partial nucleotide sequence reads mapped to each reference genome section; (e) comparing, by the logic processing module, the number of counts of the partial nucleotide sequence reads, or derivative thereof, to a reference, or portion thereof, thereby making a comparison; (f) providing, by the logic processing module, an outcome determinative of the presence or absence of a fetal aneuploidy based on the comparison; and (g) organizing, by the data display organization module in response to being determined by the logic processing module, a data display indicating the presence or absence of a fetal aneuploidy.

In some embodiments, a computer program product is stored in an apparatus comprising memory. In some cases, the apparatus comprises a processor that implements one or more functions of the computer program product specified in any of the above embodiments.

Also provided, in some aspects, are systems comprising a nucleic acid sequencing apparatus and a processing apparatus, where the sequencing apparatus obtains sequence reads from a sample, and the processing apparatus obtains the sequence reads from the sequencing apparatus and carries out a method comprising: (a) mapping partial nucleotide sequence reads from the sequencing apparatus that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, where at least some partial nucleotide sequence reads comprise: i) multiple nucleobase gaps between identified nucleobases, or ii) one or more nucleobase classes, where each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii), (b) counting the number of partial nucleotide sequence reads mapped to each reference genome section, (c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, or portion thereof, thereby making a comparison, and (d) providing an outcome determinative of the presence or absence of a fetal aneuploidy based on the comparison.

Certain aspects of the technology are described further in the following description, examples, claims and drawings.

Brief Description of the Drawings

The drawings illustrate embodiments of the technology and are not limiting. For clarity and ease of illustration, the drawings are not made to scale and, in some instances, various aspects may be shown exaggerated or enlarged to facilitate an understanding of particular embodiments.

FIG. 1 graphically illustrates how increased uncertainty in bin counts within a genomic region sometimes reduces gaps between euploid and trisomy Z-values. FIG. 2 graphically illustrates how decreased differences between triploid and euploid number of counts within a genomic region sometimes reduces predictive power of Z-scores. See Example 1 for experimental details and results.

FIG. 3 graphically illustrates the dependence of p-values on the position of genomic bins within chromosome 21. FIG. 4 schematically represents a bin filtering procedure. A large number of euploid samples are lined up, bin count uncertainties (SD or MAD values) are evaluated, and bins with largest uncertainties sometimes are filtered out. FIG. 5 graphically illustrates count profiles for chromosome 21 in two patients. FIG. 6 graphically illustrates count profiles for patients used to filter out uninformative bins from chromosome 18. In FIG. 6, the two bottom traces show a patient with a large deletion in chromosome 18. See Example 1 for experimental details and results.

FIG. 7 graphically illustrates the dependence of p-values on the position of genomic bins within chromosome 18. FIG. 8 schematically represents bin count normalization. The procedure first lines up known euploid count profiles, from a data set, and normalizes them with respect to total counts. For each bin, the median counts and deviations from the medians are evaluated. Bins with too much variability (exceeding 3 mean absolute deviations (e.g., MAD)) sometimes are eliminated. The remaining bins are normalized again with respect to residual total counts, and medians are re-evaluated following the renormalization, in some embodiments. Finally, the resulting reference profile (see bottom trace, left panel) is used to normalize bin counts in test samples (see top trace, left panel), smoothing the count contour (see trace on the right) and leaving gaps where uninformative bins have been excluded from consideration. FIG. 9 graphically illustrates the expected behavior of normalized count profiles. The majority of normalized bin counts often will center on 1, with random noise superimposed. Deletions and duplications (e.g., maternal or fetal, or maternal and fetal, deletions and duplications) sometimes shifts the elevation to an integer multiple of 0.5. Profile elevations corresponding to a triploid fetal chromosome often shifts upward in proportion to the fetal fraction. See Example 1 for experimental details and results.

FIG. 10 graphically illustrates a normalized T18 count profile with a heterozygous maternal deletion in chromosome 18. The light gray segment of the graph tracing shows a higher average elevation than the black segment of the graph tracing. See Example 1 for experimental details and results.

FIG. 11 graphically illustrates normalized binwise count profiles for two samples collected from the same patient with heterozygous maternal deletion in chromosome 18. The substantially identical tracings can be used to determine if two samples are from the same donor. FIG. 12 graphically illustrates normalized binwise count profiles of a sample from one study, compared with two samples from a previous study. The duplication in chromosome 22 unambiguously points out the patient's identity. FIG. 13 graphically illustrates normalized binwise count profiles of chromosome

4 in the same three patients presented in FIG. 12. The duplication in chromosome 4 confirms the patient's identity established in FIG. 12. See Example 1 for experimental details and results.

FIG. 14 graphically illustrates the distribution of normalized bin counts in chromosome 5 from a euploid sample. FIG. 15 graphically illustrates two samples with different levels of noise in their normalized count profiles. FIG. 16 schematically represents factors determining the confidence in peak elevation: noise standard deviation (e.g., σ) and average deviation from the reference baseline (e.g., Δ). See Example 1 for experimental details and results.

FIG. 17 graphically illustrates the results of applying a correlation function to normalized bin counts. The correlation function shown in FIG. 17 was used to normalize bin counts in chromosome 5 of an arbitrarily chosen euploid patient. FIG. 18 graphically illustrates the standard deviation for the average stretch elevation in chromosome 5, evaluated as a sample estimate (square data points) and compared with the standard error of the mean (triangle data points) and with the estimate corrected for auto-correlation $\rho = 0.5$ (circular data points). The aberration depicted in FIG. 18 is about 18 bins long. See Example 1 for experimental details and results.

FIG. 19 graphically illustrates Z-values calculated for average peak elevation in chromosome 4. The patient has a heterozygous maternal duplication in chromosome 4 (see FIG. 13). FIG. 20 graphically illustrates p-values for average peak elevation, based on a t-test and the Z-values from FIG. 19. The order of the t-distribution is determined by the length of the aberration. See Example 1 for experimental details and results.

FIG. 21 schematically represents edge comparisons between matching aberrations from different samples. Illustrated in FIG. 21 are overlaps, containment, and neighboring deviations. FIG. 22 graphically illustrates matching heterozygous duplications in chromosome 4 (dark gray top trace and black bottom trace), contrasted with a marginally touching aberration in an unrelated sample (light gray middle trace). See Example 1 for experimental details and results.

FIG. 23 schematically represents edge detection by means of numerically evaluated first derivatives of count profiles. FIG. 24 graphically illustrates that first derivative of count profiles, obtained from real data, are difficult to distinguish from noise. FIG. 25 graphically illustrates the third power of the count profile, shifted by 1 to suppress noise and enhance signal (see top trace). Also illustrated in FIG. 25 (see bottom trace) is a first derivative of the top trace. Edges are unmistakably detectable. See Example 1 for experimental details and results.

FIG. 26 graphically illustrates histograms of median chromosome 21 elevations for various patients. The black histogram illustrates median chromosome 21 elevations for 86 euploid patients. The gray histogram illustrates median chromosome 21 elevations for 35 trisomy 21 patients. The count profiles were normalized with respect to a euploid reference set prior to evaluating median elevations. FIG. 27 graphically illustrates a distribution of normalized counts for chromosome 21 in a trisomy sample. FIG. 28 graphically represents area ratios for various patients. The dark gray histogram illustrates chromosome 21 area ratios for 86 euploid patients. The light gray histogram illustrates chromosome 21 area ratios for 35 trisomy 21 patients. The count profiles were normalized with respect to a euploid reference set prior to evaluating area ratios. See Example 1 for experimental details and results.

FIG. 29 graphically illustrates area ratio in chromosome 21 plotted against median normalized count elevations. The light gray data points represent about 86 euploid samples. The dark gray data points represent about 35 trisomy patients. See Example 1 for experimental details and results.

FIG. 30 graphically illustrates relationships among 9 different classification criteria, as evaluated for a set of trisomy patients. The criteria involve Z-scores, median normalized count elevations, area ratios, measured fetal fractions, fitted fetal fractions, the ratio between fitted and measured fetal fractions, sum of squared residuals for fitted fetal fractions, sum of squared residuals with fixed fetal fractions and fixed ploidy, and fitted ploidy values. See Example 1 for experimental details and results.

FIG. 31 graphically illustrates simulated functional Phi profiles for trisomy (light gray) and euploid cases (dark gray). FIG. 32 graphically illustrates functional Phi values derived from measured trisomy (dark gray) and euploid data sets (light gray). See Example 2 for experimental details and results.

FIG. 33 graphically illustrates linearized sum of squared differences as a function of measured fetal fraction. FIG. 34 graphically illustrates fetal fraction estimates based on Y-counts plotted against values obtained from a fetal quantifier assay (e.g., FQA) fetal fraction values. FIG. 35 graphically illustrates Z-values for T21 patients plotted against FQA fetal fraction measurements. For FIG. 33-35 see Example 2 for experimental details and results.

FIG. 36 graphically illustrates fetal fraction estimates based on chromosome Y plotted against measured fetal fractions. FIG. 37 graphically illustrates fetal fraction estimates based on chromosome 21 (Chr21) plotted against measured fetal fractions. FIG. 38 graphically illustrates fetal fraction estimates derived from chromosome X counts plotted against measured fetal fractions. FIG. 39 graphically illustrates medians of normalized bin counts for T21 cases plotted against measured fetal fractions. For FIG. 36-39 see Example 2 for experimental details and results.

FIG. 40 graphically illustrates simulated profiles of fitted triploid ploidy (e.g., X) as a function of F_0 with fixed errors ΔF = +/- 0.2%. FIG. 41 graphically illustrates fitted triploid ploidy values as a function of measured fetal fractions. For FIG. 40 and 41 see Example 2 for experimental details and results.

FIG. 42 graphically illustrates probability distributions for fitted ploidy at different levels of errors in measured fetal fractions. The top panel in FIG. 42 sets measured fetal fraction error to 0.2%. The middle panel in FIG. 42 sets measured fetal fraction error to 0.4%. The bottom panel in FIG. 42 sets measured fetal fraction error to 0.6%. See Example 2 for experimental details and results.

FIG. 43 graphically illustrates euploid and trisomy distributions of fitted ploidy values for a data set derived from patient samples. FIG. 44 graphically illustrates fitted fetal fractions plotted against measured fetal fractions. For FIG. 43 and 44 see Example 2 for experimental details and results.

FIG. 45 schematically illustrates the predicted difference between euploid and trisomy sums of squared residuals for fitted fetal fraction as a function of the measured fetal fraction. FIG. 46 graphically illustrates the difference between euploid and trisomy sums of squared residuals as a function of the measured fetal fraction using a data set derived from patient samples. The data points are obtained by fitting fetal fraction values assuming fixed uncertainties in fetal fraction measurements. FIG. 47 graphically illustrates the difference between euploid and trisomy sums of squared residuals as a function of the measured fetal fraction. The data points are obtained by fitting fetal fraction values assuming that uncertainties in fetal fraction measurements are proportional to fetal fractions: $\Delta F = 2/3 + F_0/6$. For FIG. 45-47 see Example 2 for experimental details and results.

FIG. 48 schematically illustrates the predicted dependence of the fitted fetal fraction plotted against measured fetal fraction profiles on systematic offsets in reference counts. The lower and upper branches represent euploid and triploids cases, respectively. FIG. 49 graphically represents the effects of simulated systematic errors Δ artificially imposed on actual data. The main diagonal in the upper panel and the upper diagonal in the lower right panel represent ideal agreement. The dark gray line in all panels represents equations (51) and (53) for euploid and triploid cases, respectively. The data points represent actual measurements incorporating various levels of artificial systematic shifts. The systematic shifts are given as the offset above each panel. For FIG. 48 and 49 see Example 2 for experimental details and results.

FIG. 50 graphically illustrates fitted fetal fraction as a function of the systematic offset, obtained for a euploid and for a triploid data set. FIG. 51 graphically illustrates simulations based on equation (61), along with fitted fetal fractions for actual data. Black lines represent two standard deviations (obtained as square root of equation (61)) above and below equation (40). ΔF is set to $2/3 + F_0/6$. For FIG. 50 and 51 see Example 2 for experimental details and results.

Example 3 addresses FIG. 52 to 61F. FIG. 52 graphically illustrates an example of application of the cumulative sum algorithm to a heterozygous maternal microdeletion in chromosome 12, bin 1457. The difference between the intercepts associated with the left and the right linear models is 2.92, indicating that the heterozygous deletion is 6 bins wide.

FIG. 53 graphically illustrates a hypothetical heterozygous deletion, approximately 2 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is -1.

FIG. 54 graphically illustrates a hypothetical homozygous deletion, approximately 2 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is -2.

FIG. 55 graphically illustrates a hypothetical heterozygous deletion, approximately 6 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is -3.

FIG. 56 graphically illustrates a hypothetical homozygous deletion, approximately 6 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is -6.

FIG. 57 graphically illustrates a hypothetical heterozygous duplication, approximately 2 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is 1.

FIG. 58 graphically illustrates a hypothetical homozygous duplication, approximately 2 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is 2.

FIG. 59 graphically illustrates a hypothetical heterozygous duplication, approximately 6 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is 3.

FIG. 60 graphically illustrates a hypothetical homozygous duplication, approximately 6 genomic sections wide, and its associated cumulative sum profile. The difference between the left and the right intercepts is 6.

FIG. 61A-F graphically illustrate candidates for fetal heterozygous duplications in data obtained from women and infant clinical studies with high fetal fraction values (40-50%). To rule out the possibility that the aberrations originate from the mother and not the fetus, independent maternal profiles were used. The profile elevation in the affected regions is approximately 1.25, in accordance with the fetal fraction estimates.

FIG. 62 to FIG. 111 are described in Example 4 herein.

FIG. 112A-C illustrates padding of a normalized autosomal profile for a euploid WI sample. FIG. 112A is an example of an unpadded profile. FIG. 112B is an example of a padded profile. FIG. 112C is an example of a padding correction (e.g., an adjusted profile, an adjusted elevation).

FIG. 113A-C illustrates padding of a normalized autosomal profile for a euploid WI sample. FIG. 113A is an example of an unpadded profile. FIG. 113B is an example of a padded profile. FIG. 113C is an example of a padding correction (e.g., an adjusted profile, an adjusted elevation).

FIG. 114A-C illustrates padding of a normalized autosomal profile for a trisomy 13 WI sample. FIG. 114A is an example of an unpadded profile. FIG. 114B is an example of a padded profile. FIG. 114C is an example of a padding correction (e.g., an adjusted profile, an adjusted elevation).

FIG. 115A-C illustrates padding of a normalized autosomal profile for a trisomy 18 WI sample. FIG. 115A is an example of an unpadded profile. FIG. 115B is an example of a padded profile. FIG. 115C is an example of a padding correction (e.g., an adjusted profile, an adjusted elevation).

FIG. 116-120, 122, 123, 126, 128, 129 and 131 show a maternal duplication within a profile.

FIG. 121, 124, 125, 127 and 130 show a maternal deletion within a profile.

FIG. 132 shows GC coefficient for a binary genome.

FIG. 133 shows GC curve for a binary genome.

FIG. 134 shows relative coverage for a binary genome.

FIG. 135 shows standard deviation of counts per bin for a binary genome.

FIG. 136 shows total autosomal counts for a binary genome.

FIG. 137 presents a representative counts profile illustrating the observed aligned reads per 50kb bin and their respective GC content. Top row represents the original four base genome and the bottom row represents a binary genome. Raw (i.e. no normalization); GCN (i.e. a multiplicative normalization that rescales each counts per bin according to an expectation derived from its GC content); RM (i.e. repeat masked); GCRM (i.e. GCN on RM data); and PERUN (i.e. PERUN with secondary LOESS with Padding)

FIG. 138 shows raw chromosome 21 representation for a binary genome.

FIG. 139 shows PERUN chromosome 21 representation for a binary genome.

FIG. 140 shows raw chromosome 13 representation for a binary genome.

FIG. 141 shows PERUN chromosome 13 representation for a binary genome.

FIG. 142 shows raw chromosome 18 representation for a binary genome.

FIG. 143 shows PERUN chromosome 18 representation for a binary genome.

FIG. 144 shows WGSIM raw chromosome 21 representation for a binary genome.

FIG. 145 shows WGSIM PERUN chromosome 21 representation for a binary genome.

FIG. 146 shows logarithmic number of unique sequences possible for a 4 base genome and for a 2 base genome as functions of read length. The horizontal line is the length of the human reference genome.

FIG. 147 shows GC coefficient for unary genome A.

FIG. 148 shows GC coefficient for unary genome C.

FIG. 149 shows GC coefficient for unary genome G.

FIG. 150 shows GC coefficient for unary genome T.

FIG. 151 shows total autosomal counts for unary genome A.

FIG. 152 shows total autosomal counts for unary genome C.

FIG. 153 shows total autosomal counts for unary genome G.

FIG. 154 shows total autosomal counts for unary genome T.

FIG. 155 shows raw chromosome 21 representation for unary genome A.

FIG. 156 shows raw chromosome 21 representation for unary genome C.

FIG. 157 shows raw chromosome 21 representation for unary genome G.

FIG. 158 shows raw chromosome 21 representation for unary genome T.

FIG. 159 shows PERUN chromosome 21 representation for unary genome A.

FIG. 160 shows PERUN chromosome 21 representation for unary genome C.

FIG. 161 shows PERUN chromosome 21 representation for unary genome G.

FIG. 162 shows PERUN chromosome 21 representation for unary genome T.

FIG. 163 shows PERUN chromosome 13 representation for unary genome A.

FIG. 164 shows PERUN chromosome 13 representation for unary genome C.

FIG. 165 shows PERUN chromosome 13 representation for unary genome G.

FIG. 166 shows PERUN chromosome 13 representation for unary genome T.

FIG. 167 shows PERUN chromosome 18 representation for unary genome A.

FIG. 168 shows PERUN chromosome 18 representation for unary genome C.

FIG. 169 shows PERUN chromosome 18 representation for unary genome G.

FIG. 170 shows PERUN chromosome 18 representation for unary genome T.

FIG. 171 presents a representative counts profile illustrating the observed aligned reads per 50 kb bin and their respective GC content for unary genome guanine. Top row represents the original four base genome and the bottom row represents unary genome guanine. Raw (i.e. no normalization); GCN (i.e. a multiplicative normalization that rescales each counts per bin according to an expectation derived from its GC content); RM (i.e. repeat masked); GCRM (i.e. GCN on RM data); and PERUN (i.e. PERUN with secondary LOESS with Padding)

FIG. 172 presents a representative counts profile illustrating the observed aligned reads per 50 kb bin and their respective GC content for unary genome cytosine. Top row represents the original four base genome and the bottom row represents unary genome cytosine. Raw (i.e. no normalization); GCN (i.e. a multiplicative normalization that rescales each counts per bin according to an expectation derived from its GC content); RM (i.e. repeat masked); GCRM (i.e. GCN on RM data); and PERUN (i.e. PERUN with secondary LOESS with Padding)

FIG. 173 shows WGSIM total autosomal counts for unary genome A.

FIG. 174 shows WGSIM total autosomal counts for unary genome C.

FIG. 175 shows WGSIM total autosomal counts for unary genome G.

FIG. 176 shows WGSIM total autosomal counts for unary genome T.

FIG. 177 shows WGSIM raw chromosome 21 representation for unary genome A.

FIG. 178 shows WGSIM raw chromosome 21 representation for unary genome C.

FIG. 179 shows WGSIM raw chromosome 21 representation for unary genome G.

FIG. 180 shows WGSIM raw chromosome 21 representation for unary genome T.

FIG. 181 shows WGSIM PERUN chromosome 21 representation for unary genome A.

FIG. 182 shows WGSIM PERUN chromosome 21 representation for unary genome C.

FIG. 183 shows WGSIM PERUN chromosome 21 representation for unary genome G.

FIG. 184 shows WGSIM PERUN chromosome 21 representation for unary genome T.

Detailed Description

Provided are methods, processes and apparatuses useful for identifying a genetic variation. Identifying a genetic variation sometimes comprises detecting a copy number variation and/or sometimes comprises adjusting an elevation comprising a copy number variation. In some embodiments, an elevation is adjusted providing an identification of one or more genetic variations or variances with a reduced likelihood of a false positive or false negative diagnosis. In some embodiments, identifying a genetic variation by a method described herein can lead to a diagnosis of, or determining a predisposition to, a particular medical condition. Identifying a genetic variance can result in facilitating a medical decision and/or employing a helpful medical procedure.

Also provided herein are methods for determining the presence or absence of a genetic variation and methods for determining the presence or absence of a fetal aneuploidy using partial nucleotide sequence reads, and computer program products and systems for implementing the methods discussed herein. Assessment of a genetic variation, such as, for example, a fetal aneuploidy, from a maternal sample typically involves sequencing of the nucleic acid present in the sample, mapping sequence reads to certain regions in the genome, quantifying the sequence reads for the sample, and analyzing the quantification. Sequencing using existing methods which generate complete nucleotide sequence reads, however, can be expensive and often is the rate-limiting step in the assessment of genetic variations. Generation of partial nucleotide sequence reads can be faster and less expensive than generation of complete sequence reads. Provided herein are methods for rapidly assessing the presence or absence of a genetic variation using partial nucleotide sequence reads.

Samples

Provided herein are methods and compositions for analyzing nucleic acid. In some embodiments, nucleic acid fragments in a mixture of nucleic acid fragments are analyzed. A mixture of nucleic acids can comprise two or more nucleic acid fragment species having different nucleotide sequences, different fragment lengths, different origins (e.g., genomic origins, fetal vs. maternal origins, cell or tissue origins, sample origins, subject origins, and the like), or combinations thereof.

Nucleic acid or a nucleic acid mixture utilized in methods and apparatuses described herein often is isolated from a sample obtained from a subject. A subject can be any living or non-living

organism, including but not limited to a human, a non-human animal, a plant, a bacterium, a fungus or a protist. Any human or non-human animal can be selected, including but not limited to mammal, reptile, avian, amphibian, fish, ungulate, ruminant, bovine (e.g., cattle), equine (e.g., horse), caprine and ovine (e.g., sheep, goat), swine (e.g., pig), camelid (e.g., camel, llama, alpaca), monkey, ape (e.g., gorilla, chimpanzee), ursid (e.g., bear), poultry, dog, cat, mouse, rat, fish, dolphin, whale and shark. A subject may be a male or female (e.g., woman).

Nucleic acid may be isolated from any type of suitable biological specimen or sample (e.g., a test sample). A sample or test sample can be any specimen that is isolated or obtained from a subject (e.g., a human subject, a pregnant female). Non-limiting examples of specimens include fluid or tissue from a subject, including, without limitation, umbilical cord blood, chorionic villi, amniotic fluid, cerebrospinal fluid, spinal fluid, lavage fluid (e.g., bronchoalveolar, gastric, peritoneal, ductal, ear, arthroscopic), biopsy sample (e.g., from pre-implantation embryo), celocentesis sample, fetal nucleated cells or fetal cellular remnants, washings of female reproductive tract, urine, feces, sputum, saliva, nasal mucous, prostate fluid, lavage, semen, lymphatic fluid, bile, tears, sweat, breast milk, breast fluid, embryonic cells and fetal cells (e.g. placental cells). In some embodiments, a biological sample is a cervical swab from a subject. In some embodiments, a biological sample may be blood and sometimes plasma or serum. As used herein, the term “blood” encompasses whole blood or any fractions of blood, such as serum and plasma as conventionally defined, for example. Blood or fractions thereof often comprise nucleosomes (e.g., maternal and/or fetal nucleosomes). Nucleosomes comprise nucleic acids and are sometimes cell-free or intracellular. Blood also comprises buffy coats. Buffy coats are sometimes isolated by utilizing a ficoll gradient. Buffy coats can comprise white blood cells (e.g., leukocytes, T-cells, B-cells, platelets, and the like). Sometimes buffy coats comprise maternal and/or fetal nucleic acid. Blood plasma refers to the fraction of whole blood resulting from centrifugation of blood treated with anticoagulants. Blood serum refers to the watery portion of fluid remaining after a blood sample has coagulated. Fluid or tissue samples often are collected in accordance with standard protocols hospitals or clinics generally follow. For blood, an appropriate amount of peripheral blood (e.g., between 3-40 milliliters) often is collected and can be stored according to standard procedures prior to or after preparation. A fluid or tissue sample from which nucleic acid is extracted may be acellular (e.g., cell-free). In some embodiments, a fluid or tissue sample may contain cellular elements or cellular remnants. In some embodiments fetal cells or cancer cells may be included in the sample.

A sample often is heterogeneous, by which is meant that more than one type of nucleic acid species is present in the sample. For example, heterogeneous nucleic acid can include, but is not limited to, (i) fetal derived and maternal derived nucleic acid, (ii) cancer and non-cancer nucleic acid, (iii) pathogen and host nucleic acid, and more generally, (iv) mutated and wild-type nucleic acid. A sample may be heterogeneous because more than one cell type is present, such as a fetal cell and a maternal cell, a cancer and non-cancer cell, or a pathogenic and host cell. In some embodiments, a minority nucleic acid species and a majority nucleic acid species is present.

For prenatal applications of technology described herein, fluid or tissue sample may be collected from a female at a gestational age suitable for testing, or from a female who is being tested for possible pregnancy. Suitable gestational age may vary depending on the prenatal test being performed. In certain embodiments, a pregnant female subject sometimes is in the first trimester of pregnancy, at times in the second trimester of pregnancy, or sometimes in the third trimester of pregnancy. In certain embodiments, a fluid or tissue is collected from a pregnant female between about 1 to about 45 weeks of fetal gestation (e.g., at 1-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-28, 28-32, 32-36, 36-40 or 40-44 weeks of fetal gestation), and sometimes between about 5 to about 28 weeks of fetal gestation (e.g., at 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27 weeks of fetal gestation). Sometimes a fluid or tissue sample is collected from a pregnant female during or just after (e.g., 0 to 72 hours after) giving birth (e.g., vaginal or non-vaginal birth (e.g., surgical delivery)).

Nucleic Acid Isolation and Processing

Nucleic acid may be derived from one or more sources (e.g., cells, serum, plasma, buffy coat, lymphatic fluid, skin, soil, and the like) by methods known in the art. Cell lysis procedures and reagents are known in the art and may generally be performed by chemical (e.g., detergent, hypotonic solutions, enzymatic procedures, and the like, or combination thereof), physical (e.g., French press, sonication, and the like), or electrolytic lysis methods. Any suitable lysis procedure can be utilized. For example, chemical methods generally employ lysing agents to disrupt cells and extract the nucleic acids from the cells, followed by treatment with chaotropic salts. Physical methods such as freeze/thaw followed by grinding, the use of cell presses and the like also are useful. High salt lysis procedures also are commonly used. For example, an alkaline lysis procedure may be utilized. The latter procedure traditionally incorporates the use of phenol-chloroform solutions, and an alternative phenol-chloroform-free procedure involving three solutions

can be utilized. In the latter procedures, one solution can contain 15mM Tris, pH 8.0; 10mM EDTA and 100 ug/ml Rnase A; a second solution can contain 0.2N NaOH and 1% SDS; and a third solution can contain 3M KOAc, pH 5.5. These procedures can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6 (1989), incorporated herein in its entirety.

The terms “nucleic acid” and “nucleic acid molecule” are used interchangeably. The terms refer to nucleic acids of any composition form, such as deoxyribonucleic acid (DNA, e.g., complementary DNA (cDNA), genomic DNA (gDNA) and the like), ribonucleic acid (RNA, e.g., message RNA (mRNA), short inhibitory RNA (siRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), microRNA, RNA highly expressed by the fetus or placenta, and the like), and/or DNA or RNA analogs (e.g., containing base analogs, sugar analogs and/or a non-native backbone and the like), RNA/DNA hybrids and polyamide nucleic acids (PNAs), all of which can be in single- or double-stranded form. Unless otherwise limited, a nucleic acid can comprise known analogs of natural nucleotides, some of which can function in a similar manner as naturally occurring nucleotides. A nucleic acid can be in any form useful for conducting processes herein (e.g., linear, circular, supercoiled, single-stranded, double-stranded and the like). A nucleic acid may be, or may be from, a plasmid, phage, autonomously replicating sequence (ARS), centromere, artificial chromosome, chromosome, or other nucleic acid able to replicate or be replicated in vitro or in a host cell, a cell, a cell nucleus or cytoplasm of a cell in certain embodiments. A nucleic acid in some embodiments can be from a single chromosome or fragment thereof (e.g., a nucleic acid sample may be from one chromosome of a sample obtained from a diploid organism). Sometimes nucleic acids comprise nucleosomes, fragments or parts of nucleosomes or nucleosome-like structures. Nucleic acids sometimes comprise protein (e.g., histones, DNA binding proteins, and the like). Nucleic acids analyzed by processes described herein sometimes are substantially isolated and are not substantially associated with protein or other molecules. Nucleic acids also include derivatives, variants and analogs of RNA or DNA synthesized, replicated or amplified from single-stranded ("sense" or "antisense", "plus" strand or "minus" strand, "forward" reading frame or "reverse" reading frame) and double-stranded polynucleotides. Deoxyribonucleotides include deoxyadenosine, deoxycytidine, deoxyguanosine and deoxythymidine. For RNA, the base cytosine is replaced with uracil and the sugar 2’ position includes a hydroxyl moiety. A nucleic acid may be prepared using a nucleic acid obtained from a subject as a template.

Nucleic acid may be isolated at a different time point as compared to another nucleic acid, where each of the samples is from the same or a different source. A nucleic acid may be from a nucleic

acid library, such as a cDNA or RNA library, for example. A nucleic acid may be a result of nucleic acid purification or isolation and/or amplification of nucleic acid molecules from the sample. Nucleic acid provided for processes described herein may contain nucleic acid from one sample or from two or more samples (e.g., from 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, or 20 or more samples).

Nucleic acids can include extracellular nucleic acid in certain embodiments. The term "extracellular nucleic acid" as used herein can refer to nucleic acid isolated from a source having substantially no cells and also is referred to as “cell-free” nucleic acid and/or “cell-free circulating” nucleic acid. Extracellular nucleic acid can be present in and obtained from blood (e.g., from the blood of a pregnant female). Extracellular nucleic acid often includes no detectable cells and may contain cellular elements or cellular remnants. Non-limiting examples of acellular sources for extracellular nucleic acid are blood, blood plasma, blood serum and urine. As used herein, the term “obtain cell-free circulating sample nucleic acid” includes obtaining a sample directly (e.g., collecting a sample, e.g., a test sample) or obtaining a sample from another who has collected a sample. Without being limited by theory, extracellular nucleic acid may be a product of cell apoptosis and cell breakdown, which provides basis for extracellular nucleic acid often having a series of lengths across a spectrum (e.g., a "ladder").

Extracellular nucleic acid can include different nucleic acid species, and therefore is referred to herein as "heterogeneous" in certain embodiments. For example, blood serum or plasma from a person having cancer can include nucleic acid from cancer cells and nucleic acid from non-cancer cells. In another example, blood serum or plasma from a pregnant female can include maternal nucleic acid and fetal nucleic acid. In some instances, fetal nucleic acid sometimes is about 5% to about 50% of the overall nucleic acid (e.g., about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, or 49% of the total nucleic acid is fetal nucleic acid). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 500 base pairs or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 500 base pairs or less). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 250 base pairs or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 250 base pairs or less). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 200 base pairs

or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 200 base pairs or less). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 150 base pairs or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 150 base pairs or less). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 100 base pairs or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 100 base pairs or less). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 50 base pairs or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 50 base pairs or less). In some embodiments, the majority of fetal nucleic acid in nucleic acid is of a length of about 25 base pairs or less (e.g., about 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of fetal nucleic acid is of a length of about 25 base pairs or less).

Nucleic acid may be provided for conducting methods described herein without processing of the sample(s) containing the nucleic acid, in certain embodiments. In some embodiments, nucleic acid is provided for conducting methods described herein after processing of the sample(s) containing the nucleic acid. For example, a nucleic acid can be extracted, isolated, purified, partially purified or amplified from the sample(s). The term “isolated” as used herein refers to nucleic acid removed from its original environment (e.g., the natural environment if it is naturally occurring, or a host cell if expressed exogenously), and thus is altered by human intervention (e.g., "by the hand of man") from its original environment. The term “isolated nucleic acid” as used herein can refer to a nucleic acid removed from a subject (e.g., a human subject). An isolated nucleic acid can be provided with fewer non-nucleic acid components (e.g., protein, lipid) than the amount of components present in a source sample. A composition comprising isolated nucleic acid can be about 50% to greater than 99% free of non-nucleic acid components. A composition comprising isolated nucleic acid can be about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% free of non-nucleic acid components. The term “purified” as used herein can refer to a nucleic acid provided that contains fewer non-nucleic acid components (e.g., protein, lipid, carbohydrate) than the amount of non-nucleic acid components present prior to subjecting the nucleic acid to a purification procedure. A composition comprising purified nucleic acid may be about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% free of other non-nucleic acid components. The term “purified” as used herein can refer to a nucleic acid provided that contains fewer nucleic acid species than in the sample source from which the nucleic acid is derived. A composition comprising purified nucleic

acid may be about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater than 99% free of other nucleic acid species. For example, fetal nucleic acid can be purified from a mixture comprising maternal and fetal nucleic acid. In certain examples, nucleosomes comprising small fragments of fetal nucleic acid can be purified from a mixture of larger nucleosome complexes comprising larger fragments of maternal nucleic acid.

The term "amplified" as used herein refers to subjecting a target nucleic acid in a sample to a process that linearly or exponentially generates amplicon nucleic acids having the same or substantially the same nucleotide sequence as the target nucleic acid, or segment thereof. The term "amplified" as used herein can refer to subjecting a target nucleic acid (e.g., in a sample comprising other nucleic acids) to a process that selectively and linearly or exponentially generates amplicon nucleic acids having the same or substantially the same nucleotide sequence as the target nucleic acid, or segment thereof. The term "amplified" as used herein can refer to subjecting a population of nucleic acids to a process that non-selectively and linearly or exponentially generates amplicon nucleic acids having the same or substantially the same nucleotide sequence as nucleic acids, or portions thereof, that were present in the sample prior to amplification. Sometimes the term "amplified" refers to a method that comprises a polymerase chain reaction (PCR).

Nucleic acid also may be processed by subjecting nucleic acid to a method that generates nucleic acid fragments, in certain embodiments, before providing nucleic acid for a process described herein. In some embodiments, nucleic acid subjected to fragmentation or cleavage may have a nominal, average or mean length of about 5 to about 10,000 base pairs, about 100 to about 1,000 base pairs, about 100 to about 500 base pairs, or about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000 or 9000 base pairs. Fragments can be generated by a suitable method known in the art, and the average, mean or nominal length of nucleic acid fragments can be controlled by selecting an appropriate fragment-generating procedure. In certain embodiments, nucleic acid of a relatively shorter length can be utilized to analyze sequences that contain little sequence variation and/or contain relatively large amounts of known nucleotide sequence information. In some embodiments, nucleic acid of a relatively longer length can be utilized to analyze sequences that contain greater sequence variation and/or contain relatively small amounts of nucleotide sequence information.

Nucleic acid fragments may contain overlapping nucleotide sequences, and such overlapping sequences can facilitate construction of a nucleotide sequence of the non-fragmented counterpart nucleic acid, or a segment thereof. For example, one fragment may have subsequences x and y and another fragment may have subsequences y and z, where x, y and z are nucleotide sequences that can be 5 nucleotides in length or greater. Overlap sequence y can be utilized to facilitate construction of the x-y-z nucleotide sequence in nucleic acid from a sample in certain embodiments. Nucleic acid may be partially fragmented (e.g., from an incomplete or terminated specific cleavage reaction) or fully fragmented in certain embodiments.

Nucleic acid can be fragmented by various methods known in the art, which include without limitation, physical, chemical and enzymatic processes. Non-limiting examples of such processes are described in U.S. Patent Application Publication No. 20050112590 (published on May 26, 2005, entitled "Fragmentation-based methods and systems for sequence variation detection and discovery," naming Van Den Boom et al.). Certain processes can be selected to generate non-specifically cleaved fragments or specifically cleaved fragments. Non-limiting examples of processes that can generate non-specifically cleaved fragment nucleic acid include, without limitation, contacting nucleic acid with apparatus that expose nucleic acid to shearing force (e.g., passing nucleic acid through a syringe needle; use of a French press); exposing nucleic acid to irradiation (e.g., gamma, x-ray, UV irradiation; fragment sizes can be controlled by irradiation intensity); boiling nucleic acid in water (e.g., yields about 500 base pair fragments) and exposing nucleic acid to an acid and base hydrolysis process.

As used herein, "fragmentation" or "cleavage" refers to a procedure or conditions in which a nucleic acid molecule, such as a nucleic acid template gene molecule or amplified product thereof, may be severed into two or more smaller nucleic acid molecules. Such fragmentation or cleavage can be sequence specific, base specific, or nonspecific, and can be accomplished by any of a variety of methods, reagents or conditions, including, for example, chemical, enzymatic, physical fragmentation.

As used herein, "fragments", "cleavage products", "cleaved products" or grammatical variants thereof, refers to nucleic acid molecules resultant from a fragmentation or cleavage of a nucleic acid template gene molecule or amplified product thereof. While such fragments or cleaved products can refer to all nucleic acid molecules resultant from a cleavage reaction, typically such fragments or cleaved products refer only to nucleic acid molecules resultant from a fragmentation

or cleavage of a nucleic acid template gene molecule or the segment of an amplified product thereof containing the corresponding nucleotide sequence of a nucleic acid template gene molecule. For example, an amplified product can contain one or more nucleotides more than the amplified nucleotide region of a nucleic acid template sequence (e.g., a primer can contain "extra" nucleotides such as a transcriptional initiation sequence, in addition to nucleotides complementary to a nucleic acid template gene molecule, resulting in an amplified product containing "extra" nucleotides or nucleotides not corresponding to the amplified nucleotide region of the nucleic acid template gene molecule). Accordingly, fragments can include fragments arising from portions of amplified nucleic acid molecules containing, at least in part, nucleotide sequence information from or based on the representative nucleic acid template molecule.

As used herein, the term “complementary cleavage reactions” refers to cleavage reactions that are carried out on the same nucleic acid using different cleavage reagents or by altering the cleavage specificity of the same cleavage reagent such that alternate cleavage patterns of the same target or reference nucleic acid or protein are generated. In certain embodiments, nucleic acid may be treated with one or more specific cleavage agents (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more specific cleavage agents) in one or more reaction vessels (e.g., nucleic acid is treated with each specific cleavage agent in a separate vessel).

Nucleic acid may be specifically cleaved or non-specifically cleaved by contacting the nucleic acid with one or more enzymatic cleavage agents (e.g., nucleases, restriction enzymes). The term “specific cleavage agent” as used herein refers to an agent, sometimes a chemical or an enzyme that can cleave a nucleic acid at one or more specific sites. Specific cleavage agents often cleave specifically according to a particular nucleotide sequence at a particular site. Non-specific cleavage agents often cleave nucleic acids at non-specific sites or degrade nucleic acids. Non-specific cleavage agents often degrade nucleic acids by removal of nucleotides from the end (either the 5’ end, 3’ end or both) of a nucleic acid strand.

Any suitable non-specific or specific enzymatic cleavage agent can be used to cleave or fragment nucleic acids. A suitable restriction enzyme can be used to cleave nucleic acids, in some embodiments. Examples of enzymatic cleavage agents include without limitation endonucleases (e.g., DNase (e.g., DNase I, II); RNase (e.g., RNase E, F, H, P); Cleavase™ enzyme; Taq DNA polymerase; E. coli DNA polymerase I and eukaryotic structure-specific endonucleases; murine FEN-1 endonucleases; type I, II or III restriction endonucleases such as Acc I, Afl III, Alu I, Alw44 I,

Apa I, Asn I, Ava I, Ava II, BamH I, Ban II, Bcl I, Bgl I. Bgl II, Bln I, Bsm I, BssH II, BstE II, Cfo I, Cla I, Dde I, Dpn I, Dra I, EclX I, EcoR I, EcoR I, EcoR II, EcoR V, Hae II, Hae II, Hind II, Hind III, Hpa I, Hpa II, Kpn I, Ksp I, Mlu I, MluN I, Msp I, Nci I, Nco I, Nde I, Nde II, Nhe I, Not I, Nru I, Nsi I, Pst I, Pvu I, Pvu II, Rsa I, Sac I, Sal I, Sau3A I, Sca I, ScrF I, Sfi I, Sma I, Spe I, Sph I, Ssp I, Stu I, Sty I, Swa I, Taq I, Xba I, Xho I; glycosylases (e.g., uracil-DNA glycosylase (UDG), 3-methyladenine DNA glycosylase, 3-methyladenine DNA glycosylase II, pyrimidine hydrate-DNA glycosylase, FaPy-DNA glycosylase, thymine mismatch-DNA glycosylase, hypoxanthine-DNA glycosylase, 5-Hydroxymethyluracil DNA glycosylase (HmUDG), 5-Hydroxymethylcytosine DNA glycosylase, or 1,N6-etheno-adenine DNA glycosylase); exonucleases (e.g., exonuclease III); ribozymes, and DNAzymes. Nucleic acid may be treated with a chemical agent, and the modified nucleic acid may be cleaved. In non-limiting examples, nucleic acid may be treated with (i) alkylating agents such as methylnitrosourea that generate several alkylated bases, including N3-methyladenine and N3-methylguanine, which are recognized and cleaved by alkyl purine DNA-glycosylase; (ii) sodium bisulfite, which causes deamination of cytosine residues in DNA to form uracil residues that can be cleaved by uracil N-glycosylase; and (iii) a chemical agent that converts guanine to its oxidized form, 8-hydroxyguanine, which can be cleaved by formamidopyrimidine DNA N-glycosylase. Examples of chemical cleavage processes include without limitation alkylation, (e.g., alkylation of phosphorothioate-modified nucleic acid); cleavage of acid lability of P3'-N5'-phosphoroamidate-containing nucleic acid; and osmium tetroxide and piperidine treatment of nucleic acid.

Nucleic acid also may be exposed to a process that modifies certain nucleotides in the nucleic acid before providing nucleic acid for a method described herein. A process that selectively modifies nucleic acid based upon the methylation state of nucleotides therein can be applied to nucleic acid, for example. In addition, conditions such as high temperature, ultraviolet radiation, x-radiation, can induce changes in the sequence of a nucleic acid molecule. Nucleic acid may be provided in any form useful for conducting a sequence analysis or manufacture process described herein, such as solid or liquid form, for example. In certain embodiments, nucleic acid may be provided in a liquid form optionally comprising one or more other components, including without limitation one or more buffers or salts.

Nucleic acid may be single or double stranded. Single stranded DNA, for example, can be generated by denaturing double stranded DNA by heating or by treatment with alkali, for example. In some cases, nucleic acid is in a D-loop structure, formed by strand invasion of a duplex DNA molecule by an oligonucleotide or a DNA-like molecule such as peptide nucleic acid (PNA). D loop

formation can be facilitated by addition of E. Coli RecA protein and/or by alteration of salt concentration, for example, using methods known in the art.

Determining Fetal Nucleic Acid Content

The amount of fetal nucleic acid (e.g., concentration, relative amount, absolute amount, copy number, and the like) in nucleic acid is determined in some embodiments. In some cases, the amount of fetal nucleic acid in a sample is referred to as "fetal fraction". Sometimes "fetal fraction" refers to the fraction of fetal nucleic acid in circulating cell-free nucleic acid in a sample (e.g., a blood sample, a serum sample, a plasma sample) obtained from a pregnant female. In certain embodiments, the amount of fetal nucleic acid is determined according to markers specific to a male fetus (e.g., Y-chromosome STR markers (e.g., DYS 19, DYS 385, DYS 392 markers); RhD marker in RhD-negative females), allelic ratios of polymorphic sequences, or according to one or more markers specific to fetal nucleic acid and not maternal nucleic acid (e.g., differential epigenetic biomarkers (e.g., methylation; described in further detail below) between mother and fetus, or fetal RNA markers in maternal blood plasma (see e.g., Lo, 2005, Journal of Histochemistry and Cytochemistry 53 (3): 293-296)).

Determination of fetal nucleic acid content (e.g., fetal fraction) sometimes is performed using a fetal quantifier assay (FQA) as described, for example, in U.S. Patent Application Publication No. 2010/0105049, which is hereby incorporated by reference. This type of assay allows for the detection and quantification of fetal nucleic acid in a maternal sample based on the methylation status of the nucleic acid in the sample. In some cases, the amount of fetal nucleic acid from a maternal sample can be determined relative to the total amount of nucleic acid present, thereby providing the percentage of fetal nucleic acid in the sample. In some cases, the copy number of fetal nucleic acid can be determined in a maternal sample. In some cases, the amount of fetal nucleic acid can be determined in a sequence-specific (or locus-specific) manner and sometimes with sufficient sensitivity to allow for accurate chromosomal dosage analysis (for example, to detect the presence or absence of a fetal aneuploidy).

A fetal quantifier assay (FQA) can be performed in conjunction with any of the methods described herein. Such an assay can be performed by any method known in the art and/or described in U.S. Patent Application Publication No. 2010/0105049, such as, for example, by a method that can distinguish between maternal and fetal DNA based on differential methylation status, and quantify

(i.e. determine the amount of) the fetal DNA. Methods for differentiating nucleic acid based on methylation status include, but are not limited to, methylation sensitive capture, for example, using a MBD2-Fc fragment in which the methyl binding domain of MBD2 is fused to the Fc fragment of an antibody (MBD-FC) (Gebhard et al. (2006) Cancer Res. 66(12):6118-28); methylation specific antibodies; bisulfite conversion methods, for example, MSP (methylation-sensitive PCR), COBRA, methylation-sensitive single nucleotide primer extension (Ms-SNuPE) or Sequenom MassCLEAVE™ technology; and the use of methylation sensitive restriction enzymes (e.g., digestion of maternal DNA in a maternal sample using one or more methylation sensitive restriction enzymes thereby enriching the fetal DNA). Methyl-sensitive enzymes also can be used to differentiate nucleic acid based on methylation status, which, for example, can preferentially or substantially cleave or digest at their DNA recognition sequence if the latter is non-methylated. Thus, an unmethylated DNA sample will be cut into smaller fragments than a methylated DNA sample and a hypermethylated DNA sample will not be cleaved. Except where explicitly stated, any method for differentiating nucleic acid based on methylation status can be used with the compositions and methods of the technology herein. The amount of fetal DNA can be determined, for example, by introducing one or more competitors at known concentrations during an amplification reaction. Determining the amount of fetal DNA also can be done, for example, by RT-PCR, primer extension, sequencing and/or counting. In certain instances, the amount of nucleic acid can be determined using BEAMing technology as described in U.S. Patent Application Publication No. 2007/0065823. In some cases, the restriction efficiency can be determined and the efficiency rate is used to further determine the amount of fetal DNA.

In some cases, a fetal quantifier assay (FQA) can be used to determine the concentration of fetal DNA in a maternal sample, for example, by the following method: a) determine the total amount of DNA present in a maternal sample; b) selectively digest the maternal DNA in a maternal sample using one or more methylation sensitive restriction enzymes thereby enriching the fetal DNA; c) determine the amount of fetal DNA from step b); and d) compare the amount of fetal DNA from step c) to the total amount of DNA from step a), thereby determining the concentration of fetal DNA in the maternal sample. In some cases, the absolute copy number of fetal nucleic acid in a maternal sample can be determined, for example, using mass spectrometry and/or a system that uses a competitive PCR approach for absolute copy number measurements. See for example, Ding and Cantor (2003) Proc Natl Acad Sci USA 100:3059-3064, and U.S. Patent Application Publication No. 2004/0081993, both of which are hereby incorporated by reference.

In some cases, fetal fraction can be determined based on allelic ratios of polymorphic sequences (e.g., single nucleotide polymorphisms (SNPs)), such as, for example, using a method described in U.S. Patent Application Publication No. 2011/0224087, which is hereby incorporated by reference. In such a method, nucleotide sequence reads are obtained for a maternal sample and fetal fraction is determined by comparing the total number of nucleotide sequence reads that map to a first allele and the total number of nucleotide sequence reads that map to a second allele at an informative polymorphic site (e.g., SNP) in a reference genome. In some cases, fetal alleles are identified, for example, by their relative minor contribution to the mixture of fetal and maternal nucleic acids in the sample when compared to the major contribution to the mixture by the maternal nucleic acids. Accordingly, the relative abundance of fetal nucleic acid in a maternal sample can be determined as a parameter of the total number of unique sequence reads mapped to a target nucleic acid sequence on a reference genome for each of the two alleles of a polymorphic site.

The amount of fetal nucleic acid in extracellular nucleic acid can be quantified and used in conjunction with a method provided herein. Thus, in certain embodiments, methods of the technology described herein comprise an additional step of determining the amount of fetal nucleic acid. The amount of fetal nucleic acid can be determined in a nucleic acid sample from a subject before or after processing to prepare sample nucleic acid. In certain embodiments, the amount of fetal nucleic acid is determined in a sample after sample nucleic acid is processed and prepared, which amount is utilized for further assessment. In some embodiments, an outcome comprises factoring the fraction of fetal nucleic acid in the sample nucleic acid (e.g., adjusting counts, removing samples, making a call or not making a call).

The determination step can be performed before, during, at any one point in a method described herein, or after certain (e.g., aneuploidy detection, fetal gender determination) methods described herein. For example, to achieve a fetal gender or aneuploidy determination method with a given sensitivity or specificity, a fetal nucleic acid quantification method may be implemented prior to, during or after fetal gender or aneuploidy determination to identify those samples with greater than about 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%,15%,16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25% or more fetal nucleic acid. In some embodiments, samples determined as having a certain threshold amount of fetal nucleic acid (e.g., about 15% or more fetal nucleic acid; about 4% or more fetal nucleic acid) are further analyzed for fetal gender or aneuploidy determination, or the presence or absence of aneuploidy or genetic variation, for example. In certain embodiments, determinations of, for example, fetal gender or the presence or

absence of aneuploidy are selected (e.g., selected and communicated to a patient) only for samples having a certain threshold amount of fetal nucleic acid (e.g., about 15% or more fetal nucleic acid; about 4% or more fetal nucleic acid).

In some embodiments, the determination of fetal fraction or determining the amount of fetal nucleic acid is not required or necessary for identifying the presence or absence of a chromosome aneuploidy. In some embodiments, identifying the presence or absence of a chromosome aneuploidy does not require the sequence differentiation of fetal versus maternal DNA. In some cases this is because the summed contribution of both maternal and fetal sequences in a particular chromosome, chromosome portion or segment thereof is analyzed. In some embodiments, identifying the presence or absence of a chromosome aneuploidy does not rely on a priori sequence information that would distinguish fetal DNA from maternal DNA.

Enriching for a subpopulation of nucleic acid

In some embodiments, nucleic acid (e.g., extracellular nucleic acid) is enriched or relatively enriched for a subpopulation or species of nucleic acid. Nucleic acid subpopulations can include, for example, fetal nucleic acid, maternal nucleic acid, nucleic acid comprising fragments of a particular length or range of lengths, or nucleic acid from a particular genome region (e.g., single chromosome, set of chromosomes, and/or certain chromosome regions). Such enriched samples can be used in conjunction with a method provided herein. Thus, in certain embodiments, methods of the technology comprise an additional step of enriching for a subpopulation of nucleic acid in a sample, such as, for example, fetal nucleic acid. In some cases, a method for determining fetal fraction described above also can be used to enrich for fetal nucleic acid. In certain embodiments, maternal nucleic acid is selectively removed (partially, substantially, almost completely or completely) from the sample. In some cases, enriching for a particular low copy number species nucleic acid (e.g., fetal nucleic acid) may improve quantitative sensitivity. Methods for enriching a sample for a particular species of nucleic acid are described, for example, in United States Patent No. 6,927,028, International Patent Application Publication No. WO2007/140417, International Patent Application Publication No. WO2007/147063, International Patent Application Publication No. WO2009/032779, International Patent Application Publication No. WO2009/032781, International Patent Application Publication No. WO2010/033639, International Patent Application Publication No. WO2011/034631, International Patent Application Publication

No. WO2006/056480, and International Patent Application Publication No. WO2011/143659, all of which are incorporated by reference herein.

In some embodiments, nucleic acid is enriched for certain target fragment species and/or reference fragment species. In some cases, nucleic acid is enriched for a specific nucleic acid fragment length or range of fragment lengths using one or more length-based separation methods described below. In some cases, nucleic acid is enriched for fragments from a select genomic region (e.g., chromosome) using one or more sequence-based separation methods described herein and/or known in the art. Certain methods for enriching for a nucleic acid subpopulation (e.g., fetal nucleic acid) in a sample are described in detail below.

Some methods for enriching for a nucleic acid subpopulation (e.g., fetal nucleic acid) that can be used with a method described herein include methods that exploit epigenetic differences between maternal and fetal nucleic acid. For example, fetal nucleic acid can be differentiated and separated from maternal nucleic acid based on methylation differences. Methylation-based fetal nucleic acid enrichment methods are described in U.S. Patent Application Publication No. 2010/0105049, which is incorporated by reference herein. Such methods sometimes involve binding a sample nucleic acid to a methylation-specific binding agent (methyl-CpG binding protein (MBD), methylation specific antibodies, and the like) and separating bound nucleic acid from unbound nucleic acid based on differential methylation status. Such methods also can include the use of methylation-sensitive restriction enzymes (as described above; e.g., HhaI and HpaII), which allow for the enrichment of fetal nucleic acid regions in a maternal sample by selectively digesting nucleic acid from the maternal sample with an enzyme that selectively and completely or substantially digests the maternal nucleic acid to enrich the sample for at least one fetal nucleic acid region.

Another method for enriching for a nucleic acid subpopulation (e.g., fetal nucleic acid) that can be used with a method described herein is a restriction endonuclease enhanced polymorphic sequence approach, such as a method described in U.S. Patent Application Publication No. 2009/0317818, which is incorporated by reference herein. Such methods include cleavage of nucleic acid comprising a non-target allele with a restriction endonuclease that recognizes the nucleic acid comprising the non-target allele but not the target allele; and amplification of uncleaved nucleic acid but not cleaved nucleic acid, where the uncleaved, amplified nucleic acid represents enriched target nucleic acid (e.g., fetal nucleic acid) relative to non-target nucleic acid

(e.g., maternal nucleic acid). In some cases, nucleic acid may be selected such that it comprises an allele having a polymorphic site that is susceptible to selective digestion by a cleavage agent, for example.

Some methods for enriching for a nucleic acid subpopulation (e.g., fetal nucleic acid) that can be used with a method described herein include selective enzymatic degradation approaches. Such methods involve protecting target sequences from exonuclease digestion thereby facilitating the elimination in a sample of undesired sequences (e.g., maternal DNA). For example, in one approach, sample nucleic acid is denatured to generate single stranded nucleic acid, single stranded nucleic acid is contacted with at least one target-specific primer pair under suitable annealing conditions, annealed primers are extended by nucleotide polymerization generating double stranded target sequences, and digesting single stranded nucleic acid using a nuclease that digests single stranded (i.e. non-target) nucleic acid. In some cases, the method can be repeated for at least one additional cycle. In some cases, the same target-specific primer pair is used to prime each of the first and second cycles of extension, and in some cases, different target-specific primer pairs are used for the first and second cycles.

Some methods for enriching for a nucleic acid subpopulation (e.g., fetal nucleic acid) that can be used with a method described herein include massively parallel signature sequencing (MPSS) approaches. MPSS typically is a solid phase method that uses adapter (i.e. tag) ligation, followed by adapter decoding, and reading of the nucleic acid sequence in small increments. Tagged PCR products are typically amplified such that each nucleic acid generates a PCR product with a unique tag. Tags are often used to attach the PCR products to microbeads. After several rounds of ligation-based sequence determination, for example, a sequence signature can be identified from each bead. Each signature sequence (MPSS tag) in a MPSS dataset is analyzed, compared with all other signatures, and all identical signatures are counted.

In some cases, certain MPSS-based enrichment methods can include amplification (e.g., PCR)-based approaches. In some cases, loci-specific amplification methods can be used (e.g., using loci-specific amplification primers). In some cases, a multiplex SNP allele PCR approach can be used. In some cases, a multiplex SNP allele PCR approach can be used in combination with uniplex sequencing. For example, such an approach can involve the use of multiplex PCR (e.g., MASSARRAY system) and incorporation of capture probe sequences into the amplicons followed by sequencing using, for example, the Illumina MPSS system. In some cases, a multiplex SNP

allele PCR approach can be used in combination with a three-primer system and indexed sequencing. For example, such an approach can involve the use of multiplex PCR (e.g., MASSARRAY system) with primers having a first capture probe incorporated into certain loci-specific forward PCR primers and adapter sequences incorporated into loci-specific reverse PCR primers, to thereby generate amplicons, followed by a secondary PCR to incorporate reverse capture sequences and molecular index barcodes for sequencing using, for example, the Illumina MPSS system. In some cases, a multiplex SNP allele PCR approach can be used in combination with a four-primer system and indexed sequencing. For example, such an approach can involve the use of multiplex PCR (e.g., MASSARRAY system) with primers having adaptor sequences incorporated into both loci-specific forward and loci-specific reverse PCR primers, followed by a secondary PCR to incorporate both forward and reverse capture sequences and molecular index barcodes for sequencing using, for example, the Illumina MPSS system. In some cases, a microfluidics approach can be used. In some cases, an array-based microfluidics approach can be used. For example, such an approach can involve the use of a microfluidics array (e.g., Fluidigm) for amplification at low plex and incorporation of index and capture probes, followed by sequencing. In some cases, an emulsion microfluidics approach can be used, such as, for example, digital droplet PCR.

In some cases, universal amplification methods can be used (e.g., using universal or non-loci-specific amplification primers). In some cases, universal amplification methods can be used in combination with pull-down approaches. In some cases, a method can include biotinylated ultramer pull-down (e.g., biotinylated pull-down assays from Agilent or IDT) from a universally amplified sequencing library. For example, such an approach can involve preparation of a standard library, enrichment for selected regions by a pull-down assay, and a secondary universal amplification step. In some cases, pull-down approaches can be used in combination with ligation-based methods. In some cases, a method can include biotinylated ultramer pull down with sequence specific adapter ligation (e.g., HALOPLEX PCR, Halo Genomics). For example, such an approach can involve the use of selector probes to capture restriction enzyme-digested fragments, followed by ligation of captured products to an adaptor, and universal amplification followed by sequencing. In some cases, pull-down approaches can be used in combination with extension and ligation-based methods. In some cases, a method can include molecular inversion probe (MIP) extension and ligation. For example, such an approach can involve the use of molecular inversion probes in combination with sequence adapters followed by universal amplification and sequencing. In some cases, complementary DNA can be synthesized and sequenced without amplification.

In some cases, extension and ligation approaches can be performed without a pull-down component. In some cases, a method can include loci-specific forward and reverse primer hybridization, extension and ligation. Such methods can further include universal amplification or complementary DNA synthesis without amplification, followed by sequencing. Such methods can reduce or exclude background sequences during analysis, in some cases.

In some cases, pull-down approaches can be used with an optional amplification component or with no amplification component. In some cases, a method can include a modified pull-down assay and ligation with full incorporation of capture probes without universal amplification. For example, such an approach can involve the use of modified selector probes to capture restriction enzyme-digested fragments, followed by ligation of captured products to an adaptor, optional amplification, and sequencing. In some cases, a method can include a biotinylated pull-down assay with extension and ligation of adaptor sequence in combination with circular single stranded ligation. For example, such an approach can involve the use of selector probes to capture regions of interest (i.e. target sequences), extension of the probes, adaptor ligation, single stranded circular ligation, optional amplification, and sequencing. In some cases, the analysis of the sequencing result can separate target sequences form background.

In some embodiments, nucleic acid is enriched for fragments from a select genomic region (e.g., chromosome) using one or more sequence-based separation methods described herein. Sequence-based separation generally is based on nucleotide sequences present in the fragments of interest (e.g., target and/or reference fragments) and substantially not present in other fragments of the sample or present in an insubstantial amount of the other fragments (e.g., 5% or less). In some embodiments, sequence-based separation can generate separated target fragments and/or separated reference fragments. Separated target fragments and/or separated reference fragments typically are isolated away from the remaining fragments in the nucleic acid sample. In some cases, the separated target fragments and the separated reference fragments also are isolated away from each other (e.g., isolated in separate assay compartments). In some cases, the separated target fragments and the separated reference fragments are isolated together (e.g., isolated in the same assay compartment). In some embodiments, unbound fragments can be differentially removed or degraded or digested.

In some embodiments, a selective nucleic acid capture process is used to separate target and/or reference fragments away from the nucleic acid sample. Commercially available nucleic acid

capture systems include, for example, Nimblegen sequence capture system (Roche NimbleGen, Madison, WI); Illumina BEADARRAY platform (Illumina, San Diego, CA); Affymetrix GENECHIP platform (Affymetrix, Santa Clara, CA); Agilent SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA); and related platforms. Such methods typically involve hybridization of a capture oligonucleotide to a segment or all of the nucleotide sequence of a target or reference fragment and can include use of a solid phase (e.g., solid phase array) and/or a solution based platform. Capture oligonucleotides (sometimes referred to as "bait") can be selected or designed such that they preferentially hybridize to nucleic acid fragments from selected genomic regions or loci (e.g., one of chromosomes 21, 18, 13, X or Y, or a reference chromosome).

In some embodiments, nucleic acid is enriched for a particular nucleic acid fragment length, range of lengths, or lengths under or over a particular threshold or cutoff using one or more length-based separation methods. Nucleic acid fragment length typically refers to the number of nucleotides in the fragment. Nucleic acid fragment length also is sometimes referred to as nucleic acid fragment size. In some embodiments, a length-based separation method is performed without measuring lengths of individual fragments. In some embodiments, a length based separation method is performed in conjunction with a method for determining length of individual fragments. In some embodiments, length-based separation refers to a size fractionation procedure where all or part of the fractionated pool can be isolated (e.g., retained) and/or analyzed. Size fractionation procedures are known in the art (e.g., separation on an array, separation by a molecular sieve, separation by gel electrophoresis, separation by column chromatography (e.g., size-exclusion columns), and microfluidics-based approaches). In some cases, length-based separation approaches can include fragment circularization, chemical treatment (e.g., formaldehyde, polyethylene glycol (PEG)), mass spectrometry and/or size-specific nucleic acid amplification, for example.

Certain length-based separation methods that can be used with methods described herein employ a selective sequence tagging approach, for example. The term "sequence tagging" refers to incorporating a recognizable and distinct sequence into a nucleic acid or population of nucleic acids. The term "sequence tagging" as used herein has a different meaning than the term "sequence tag" described later herein. In such sequence tagging methods, a fragment size species (e.g., short fragments) nucleic acids are subjected to selective sequence tagging in a sample that includes long and short nucleic acids. Such methods typically involve performing a

nucleic acid amplification reaction using a set of nested primers which include inner primers and outer primers. In some cases, one or both of the inner can be tagged to thereby introduce a tag onto the target amplification product. The outer primers generally do not anneal to the short fragments that carry the (inner) target sequence. The inner primers can anneal to the short fragments and generate an amplification product that carries a tag and the target sequence. Typically, tagging of the long fragments is inhibited through a combination of mechanisms which include, for example, blocked extension of the inner primers by the prior annealing and extension of the outer primers. Enrichment for tagged fragments can be accomplished by any of a variety of methods, including for example, exonuclease digestion of single stranded nucleic acid and amplification of the tagged fragments using amplification primers specific for at least one tag.

Another length-based separation method that can be used with methods described herein involves subjecting a nucleic acid sample to polyethylene glycol (PEG) precipitation. Examples of methods include those described in International Patent Application Publication Nos. WO2007/140417 and WO2010/115016. This method in general entails contacting a nucleic acid sample with PEG in the presence of one or more monovalent salts under conditions sufficient to substantially precipitate large nucleic acids without substantially precipitating small (e.g., less than 300 nucleotides) nucleic acids.

Another size-based enrichment method that can be used with methods described herein involves circularization by ligation, for example, using circligase. Short nucleic acid fragments typically can be circularized with higher efficiency than long fragments. Non-circularized sequences can be separated from circularized sequences, and the enriched short fragments can be used for further analysis.

Obtaining sequence reads

In some embodiments, nucleic acids (e.g., nucleic acid fragments, sample nucleic acid, cell-free nucleic acid) may be sequenced. In some cases, a full or substantially full sequence is obtained and sometimes a partial sequence is obtained. Sequencing, mapping and related analytical methods are known in the art (e.g., United States Patent Application Publication US2009/0029377, incorporated by reference). Certain aspects of such processes are described hereafter.

As used herein, “reads” (i.e., “a read”, “a sequence read”) are short nucleotide sequences produced by any sequencing process described herein or known in the art. Reads can be generated from one end of nucleic acid fragments ("single-end reads"), and sometimes are generated from both ends of nucleic acids (e.g., paired-end reads, double-end reads).

In some embodiments the nominal, average, mean or absolute length of single-end reads sometimes is about 20 contiguous nucleotides to about 50 contiguous nucleotides, sometimes about 30 contiguous nucleotides to about 40 contiguous nucleotides, and sometimes about 35 contiguous nucleotides or about 36 contiguous nucleotides. Sometimes the nominal, average, mean or absolute length of single-end reads is about 20 to about 30 bases in length. Sometimes the nominal, average, mean or absolute length of single-end reads is about 24 to about 28 bases in length. Sometimes the nominal, average, mean or absolute length of single-end reads is about 21, 22, 23, 24, 25, 26, 27, 28 or about 29 bases in length.

In certain embodiments, the nominal, average, mean or absolute length of the paired-end reads sometimes is about 10 contiguous nucleotides to about 25 contiguous nucleotides (e.g., about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides in length), sometimes is about 15 contiguous nucleotides to about 20 contiguous nucleotides, and sometimes is about 17 contiguous nucleotides or about 18 contiguous nucleotides.

Reads generally are representations of nucleotide sequences in a physical nucleic acid. For example, in a read containing an ATGC depiction of a sequence, "A" represents an adenine nucleotide, "T" represents a thymine nucleotide, "G" represents a guanine nucleotide and "C" represents a cytosine nucleotide, in a physical nucleic acid. Sequence reads obtained from the blood of a pregnant female can be reads from a mixture of fetal and maternal nucleic acid. A mixture of relatively short reads can be transformed by processes described herein into a representation of a genomic nucleic acid present in the pregnant female and/or in the fetus. A mixture of relatively short reads can be transformed into a representation of a copy number variation (e.g., a maternal and/or fetal copy number variation), genetic variation or an aneuploidy, for example. Reads of a mixture of maternal and fetal nucleic acid can be transformed into a representation of a composite chromosome or a segment thereof comprising features of one or both maternal and fetal chromosomes. In certain embodiments, “obtaining” nucleic acid sequence reads of a sample from a subject and/or “obtaining” nucleic acid sequence reads of a biological specimen from one or more reference persons can involve directly sequencing nucleic acid to

obtain the sequence information. In some embodiments, “obtaining” can involve receiving sequence information obtained directly from a nucleic acid by another.

Sequence reads can be mapped and the number of reads or sequence tags mapping to a specified nucleic acid region (e.g., a chromosome, a bin, a genomic section) are referred to as counts. In some embodiments, counts can be manipulated or transformed (e.g., normalized, combined, added, filtered, selected, averaged, derived as a mean, the like, or a combination thereof). In some embodiments, counts can be transformed to produce normalized counts. Normalized counts for multiple genomic sections can be provided in a profile (e.g., a genomic profile, a chromosome profile, a profile of a segment or portion of a chromosome). One or more different elevations in a profile also can be manipulated or transformed (e.g., counts associated with elevations can be normalized) and elevations can be adjusted.

In some embodiments, one nucleic acid sample from one individual is sequenced. In certain embodiments, nucleic acid samples from two or more biological samples, where each biological sample is from one individual or two or more individuals, are pooled and the pool is sequenced. In the latter embodiments, a nucleic acid sample from each biological sample often is identified by one or more unique identification tags.

In some embodiments, a fraction of the genome is sequenced, which sometimes is expressed in the amount of the genome covered by the determined nucleotide sequences (e.g., "fold" coverage less than 1). When a genome is sequenced with about 1-fold coverage, roughly 100% of the nucleotide sequence of the genome is represented by reads. A genome also can be sequenced with redundancy, where a given region of the genome can be covered by two or more reads or overlapping reads (e.g., "fold" coverage greater than 1). In some embodiments, a genome is sequenced with about 0.1-fold to about 100-fold coverage, about 0.2-fold to 20-fold coverage, or about 0.2-fold to about 1-fold coverage (e.g., about 0.2-, 0.3-, 0.4-, 0.5-, 0.6-, 0.7-, 0.8-, 0.9-, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-fold coverage).

In certain embodiments, a fraction of a nucleic acid pool that is sequenced in a run is further sub-selected prior to sequencing. In certain embodiments, hybridization-based techniques (e.g., using oligonucleotide arrays) can be used to first sub-select for nucleic acid sequences from certain chromosomes (e.g., a potentially aneuploid chromosome and other chromosome(s) not involved in the aneuploidy tested). In some embodiments, nucleic acid can be fractionated by size (e.g., by

gel electrophoresis, size exclusion chromatography or by microfluidics-based approach) and in certain instances, fetal nucleic acid can be enriched by selecting for nucleic acid having a lower molecular weight (e.g., less than 300 base pairs, less than 200 base pairs, less than 150 base pairs, less than 100 base pairs). In some embodiments, fetal nucleic acid can be enriched by suppressing maternal background nucleic acid, such as by the addition of formaldehyde. In some embodiments, a portion or subset of a pre-selected pool of nucleic acids is sequenced randomly. In some embodiments, the nucleic acid is amplified prior to sequencing. In some embodiments, a portion or subset of the nucleic acid is amplified prior to sequencing.

In some cases, a sequencing library is prepared prior to or during a sequencing process. Methods for preparing a sequencing library are known in the art and commercially available platforms may be used for certain applications. Certain commercially available library platforms may be compatible with certain nucleotide sequencing processes described herein. For example, one or more commercially available library platforms may be compatible with a sequencing by synthesis process. In some cases, a ligation-based library preparation method is used (e.g., ILLUMINA TRUSEQ, Illumina, San Diego CA). Ligation-based library preparation methods typically use a methylated adaptor design which can incorporate an index sequence at the initial ligation step and often can be used to prepare samples for single-read sequencing, paired-end sequencing and multiplexed sequencing. In some cases, a transposon-based library preparation method is used (e.g., EPICENTRE NEXTERA, Epicentre, Madison WI). Transposon-based methods typically use in vitro transposition to simultaneously fragment and tag DNA in a single-tube reaction (often allowing incorporation of platform-specific tags and optional barcodes), and prepare sequencer-ready libraries.

Any sequencing method suitable for conducting methods described herein can be utilized. In some embodiments, a high-throughput sequencing method is used. High-throughput sequencing methods generally involve clonally amplified DNA templates or single DNA molecules that are sequenced in a massively parallel fashion within a flow cell (e.g. as described in Metzker M Nature Rev 11:31-46 (2010); Volkerding et al. Clin Chem 55:641-658 (2009)). Such sequencing methods also can provide digital quantitative information, where each sequence read is a countable "sequence tag" or "count" representing an individual clonal DNA template, a single DNA molecule, bin or chromosome. Next generation sequencing techniques capable of sequencing DNA in a massively parallel fashion are collectively referred to herein as "massively parallel sequencing" (MPS). High-throughput sequencing technologies include, for example, sequencing-by-synthesis

with reversible dye terminators, sequencing by oligonucleotide probe ligation, pyrosequencing and real time sequencing. Non-limiting examples of MPS include Massively Parallel Signature Sequencing (MPSS), Polony sequencing, Pyrosequencing, Illumina (Solexa) sequencing, SOLiD sequencing, Ion semiconductor sequencing, DNA nanoball sequencing, Helioscope single molecule sequencing, single molecule real time (SMRT) sequencing, nanopore sequencing, ION Torrent and RNA polymerase (RNAP) sequencing.

Systems utilized for high-throughput sequencing methods are commercially available and include, for example, the Roche 454 platform, the Applied Biosystems SOLID platform, the Helicos True Single Molecule DNA sequencing technology, the sequencing-by-hybridization platform from Affymetrix Inc., the single molecule, real-time (SMRT) technology of Pacific Biosciences, the sequencing-by-synthesis platforms from 454 Life Sciences, Illumina/Solexa and Helicos Biosciences, and the sequencing-by-ligation platform from Applied Biosystems. The ION TORRENT technology from Life technologies and nanopore sequencing also can be used in high-throughput sequencing approaches.

In some embodiments, first generation technology, such as, for example, Sanger sequencing including the automated Sanger sequencing, can be used in a method provided herein. Additional sequencing technologies that include the use of developing nucleic acid imaging technologies (e.g. transmission electron microscopy (TEM) and atomic force microscopy (AFM)), also are contemplated herein. Examples of various sequencing technologies are described below.

A nucleic acid sequencing technology that may be used in a method described herein is sequencing-by-synthesis and reversible terminator-based sequencing (e.g. Illumina's Genome Analyzer; Genome Analyzer II; HISEQ 2000; HISEQ 2500 (Illumina, San Diego CA)). With this technology, millions of nucleic acid (e.g. DNA) fragments can be sequenced in parallel. In one example of this type of sequencing technology, a flow cell is used which contains an optically transparent slide with 8 individual lanes on the surfaces of which are bound oligonucleotide anchors (e.g., adaptor primers). A flow cell often is a solid support that can be configured to retain and/or allow the orderly passage of reagent solutions over bound analytes. Flow cells frequently are planar in shape, optically transparent, generally in the millimeter or sub-millimeter scale, and often have channels or lanes in which the analyte/reagent interaction occurs.

In certain sequencing by synthesis procedures, for example, template DNA (e.g., circulating cell-free DNA (ccfDNA)) sometimes can be fragmented into lengths of several hundred base pairs in preparation for library generation. In some embodiments, library preparation can be performed without further fragmentation or size selection of the template DNA (e.g., ccfDNA). Sample isolation and library generation may be performed using automated methods and apparatus, in certain embodiments. Briefly, template DNA is end repaired by a fill-in reaction, exonuclease reaction or a combination of a fill-in reaction and exonuclease reaction. The resulting blunt-end repaired template DNA is extended by a single nucleotide, which is complementary to a single nucleotide overhang on the 3' end of an adapter primer, and often increases ligation efficiency. Any complementary nucleotides can be used for the extension/overhang nucleotides (e.g., A/T, C/G), however adenine frequently is used to extend the end-repaired DNA, and thymine often is used as the 3' end overhang nucleotide.

In certain sequencing by synthesis procedures, for example, adapter oligonucleotides are complementary to the flow-cell anchors, and sometimes are utilized to associate the modified template DNA (e.g., end-repaired and single nucleotide extended) with a solid support, such as the inside surface of a flow cell, for example. In some embodiments, the adapter also includes identifiers (i.e., indexing nucleotides, or "barcode" nucleotides (e.g., a unique sequence of nucleotides usable as an identifier to allow unambiguous identification of a sample and/or chromosome)), one or more sequencing primer hybridization sites (e.g., sequences complementary to universal sequencing primers, single end sequencing primers, paired end sequencing primers, multiplexed sequencing primers, and the like), or combinations thereof (e.g., adapter/sequencing, adapter/identifier, adapter/identifier/sequencing). Identifiers or nucleotides contained in an adapter often are six or more nucleotides in length, and frequently are positioned in the adaptor such that the identifier nucleotides are the first nucleotides sequenced during the sequencing reaction. In certain embodiments, identifier nucleotides are associated with a sample but are sequenced in a separate sequencing reaction to avoid compromising the quality of sequence reads. Subsequently, the reads from the identifier sequencing and the DNA template sequencing are linked together and the reads de-multiplexed. After linking and de-multiplexing the sequence reads and/or identifiers can be further adjusted or processed as described herein.

In certain sequencing by synthesis procedures, utilization of identifiers allows multiplexing of sequence reactions in a flow cell lane, thereby allowing analysis of multiple samples per flow cell lane. The number of samples that can be analyzed in a given flow cell lane often is dependent on

the number of unique identifiers utilized during library preparation and/or probe design. Non limiting examples of commercially available multiplex sequencing kits include Illumina's multiplexing sample preparation oligonucleotide kit and multiplexing sequencing primers and PhiX control kit (e.g., Illumina's catalog numbers PE-400-1001 and PE-400-1002, respectively). A method described herein can be performed using any number of unique identifiers (e.g., 4, 8, 12, 24, 48, 96, or more). The greater the number of unique identifiers, the greater the number of samples and/or chromosomes, for example, that can be multiplexed in a single flow cell lane. Multiplexing using 12 identifiers, for example, allows simultaneous analysis of 96 samples (e.g., equal to the number of wells in a 96 well microwell plate) in an 8 lane flow cell. Similarly, multiplexing using 48 identifiers, for example, allows simultaneous analysis of 384 samples (e.g., equal to the number of wells in a 384 well microwell plate) in an 8 lane flow cell.

In certain sequencing by synthesis procedures, adapter-modified, single-stranded template DNA is added to the flow cell and immobilized by hybridization to the anchors under limiting-dilution conditions. In contrast to emulsion PCR, DNA templates are amplified in the flow cell by "bridge" amplification, which relies on captured DNA strands "arching" over and hybridizing to an adjacent anchor oligonucleotide. Multiple amplification cycles convert the single-molecule DNA template to a clonally amplified arching "cluster," with each cluster containing approximately 1000 clonal molecules. Approximately 50 x 10^6 separate clusters can be generated per flow cell. For sequencing, the clusters are denatured, and a subsequent chemical cleavage reaction and wash leave only forward strands for single-end sequencing. Sequencing of the forward strands is initiated by hybridizing a primer complementary to the adapter sequences, which is followed by addition of polymerase and a mixture of four differently colored fluorescent reversible dye terminators. The terminators are incorporated according to sequence complementarity in each strand in a clonal cluster. After incorporation, excess reagents are washed away, the clusters are optically interrogated, and the fluorescence is recorded. With successive chemical steps, the reversible dye terminators are unblocked, the fluorescent labels are cleaved and washed away, and the next sequencing cycle is performed. This iterative, sequencing-by-synthesis process sometimes requires approximately 2.5 days to generate read lengths of 36 bases. With 50 x 10^6 clusters per flow cell, the overall sequence output can be greater than 1 billion base pairs (Gb) per analytical run.

Another nucleic acid sequencing technology that may be used with a method described herein is 454 sequencing (Roche). 454 sequencing uses a large-scale parallel pyrosequencing system

capable of sequencing about 400-600 megabases of DNA per run. The process typically involves two steps. In the first step, sample nucleic acid (e.g. DNA) is sometimes fractionated into smaller fragments (300-800 base pairs) and polished (made blunt at each end). Short adaptors are then ligated onto the ends of the fragments. These adaptors provide priming sequences for both amplification and sequencing of the sample-library fragments. One adaptor (Adaptor B) contains a 5'-biotin tag for immobilization of the DNA library onto streptavidin-coated beads. After nick repair, the non-biotinylated strand is released and used as a single-stranded template DNA (sstDNA) library. The sstDNA library is assessed for its quality and the optimal amount (DNA copies per bead) needed for emPCR is determined by titration. The sstDNA library is immobilized onto beads. The beads containing a library fragment carry a single sstDNA molecule. The bead-bound library is emulsified with the amplification reagents in a water-in-oil mixture. Each bead is captured within its own microreactor where PCR amplification occurs. This results in bead-immobilized, clonally amplified DNA fragments.

In the second step of 454 sequencing, single-stranded template DNA library beads are added to an incubation mix containing DNA polymerase and are layered with beads containing sulfurylase and luciferase onto a device containing pico-liter sized wells. Pyrosequencing is performed on each DNA fragment in parallel. Addition of one or more nucleotides generates a light signal that is recorded by a CCD camera in a sequencing instrument. The signal strength is proportional to the number of nucleotides incorporated. Pyrosequencing exploits the release of pyrophosphate (PPi) upon nucleotide addition. PPi is converted to ATP by ATP sulfurylase in the presence of adenosine 5′ phosphosulfate. Luciferase uses ATP to convert luciferin to oxyluciferin, and this reaction generates light that is discerned and analyzed (see, for example, Margulies, M. et al. Nature 437:376-380 (2005)).

Another nucleic acid sequencing technology that may be used in a method provided herein is Applied Biosystems' SOLiD™ technology. In SOLiD™ sequencing-by-ligation, a library of nucleic acid fragments is prepared from the sample and is used to prepare clonal bead populations. With this method, one species of nucleic acid fragment will be present on the surface of each bead (e.g. magnetic bead). Sample nucleic acid (e.g. genomic DNA) is sheared into fragments, and adaptors are subsequently attached to the 5′ and 3′ ends of the fragments to generate a fragment library. The adapters are typically universal adapter sequences so that the starting sequence of every fragment is both known and identical. Emulsion PCR takes place in microreactors containing all the necessary reagents for PCR. The resulting PCR products attached to the beads are then

covalently bound to a glass slide. Primers then hybridize to the adapter sequence within the library template. A set of four fluorescently labeled di-base probes compete for ligation to the sequencing primer. Specificity of the di-base probe is achieved by interrogating every 1st and 2nd base in each ligation reaction. Multiple cycles of ligation, detection and cleavage are performed with the number of cycles determining the eventual read length. Following a series of ligation cycles, the extension product is removed and the template is reset with a primer complementary to the n-1 position for a second round of ligation cycles. Often, five rounds of primer reset are completed for each sequence tag. Through the primer reset process, each base is interrogated in two independent ligation reactions by two different primers. For example, the base at read position 5 is assayed by primer number 2 in ligation cycle 2 and by primer number 3 in ligation cycle 1.

Another nucleic acid sequencing technology that may be used in a method described herein is the Helicos True Single Molecule Sequencing (tSMS). In the tSMS technique, a polyA sequence is added to the 3′ end of each nucleic acid (e.g. DNA) strand from the sample. Each strand is labeled by the addition of a fluorescently labeled adenosine nucleotide. The DNA strands are then hybridized to a flow cell, which contains millions of oligo-T capture sites that are immobilized to the flow cell surface. The templates can be at a density of about 100 million templates/cm^2. The flow cell is then loaded into a sequencing apparatus and a laser illuminates the surface of the flow cell, revealing the position of each template. A CCD camera can map the position of the templates on the flow cell surface. The template fluorescent label is then cleaved and washed away. The sequencing reaction begins by introducing a DNA polymerase and a fluorescently labeled nucleotide. The oligo-T nucleic acid serves as a primer. The polymerase incorporates the labeled nucleotides to the primer in a template directed manner. The polymerase and unincorporated nucleotides are removed. The templates that have directed incorporation of the fluorescently labeled nucleotide are detected by imaging the flow cell surface. After imaging, a cleavage step removes the fluorescent label, and the process is repeated with other fluorescently labeled nucleotides until the desired read length is achieved. Sequence information is collected with each nucleotide addition step (see, for example, Harris T. D. et al., Science 320:106-109 (2008)).

Another nucleic acid sequencing technology that may be used in a method provided herein is the single molecule, real-time (SMRT™) sequencing technology of Pacific Biosciences. With this method, each of the four DNA bases is attached to one of four different fluorescent dyes. These dyes are phospholinked. A single DNA polymerase is immobilized with a single molecule of template single stranded DNA at the bottom of a zero-mode waveguide (ZMW). A ZMW is a

confinement structure which enables observation of incorporation of a single nucleotide by DNA polymerase against the background of fluorescent nucleotides that rapidly diffuse in an out of the ZMW (in microseconds). It takes several milliseconds to incorporate a nucleotide into a growing strand. During this time, the fluorescent label is excited and produces a fluorescent signal, and the fluorescent tag is cleaved off. Detection of the corresponding fluorescence of the dye indicates which base was incorporated. The process is then repeated.

Another nucleic acid sequencing technology that may be used in a method described herein is ION TORRENT (Life Technologies) single molecule sequencing which pairs semiconductor technology with a simple sequencing chemistry to directly translate chemically encoded information (A, C, G, T) into digital information (0, 1) on a semiconductor chip. ION TORRENT uses a high-density array of micro-machined wells to perform nucleic acid sequencing in a massively parallel way. Each well holds a different DNA molecule. Beneath the wells is an ion-sensitive layer and beneath that an ion sensor. Typically, when a nucleotide is incorporated into a strand of DNA by a polymerase, a hydrogen ion is released as a byproduct. If a nucleotide, for example a C, is added to a DNA template and is then incorporated into a strand of DNA, a hydrogen ion will be released. The charge from that ion will change the pH of the solution, which can be detected by an ion sensor. A sequencer can call the base, going directly from chemical information to digital information. The sequencer then sequentially floods the chip with one nucleotide after another. If the next nucleotide that floods the chip is not a match, no voltage change will be recorded and no base will be called. If there are two identical bases on the DNA strand, the voltage will be double, and the chip will record two identical bases called. Because this is direct detection (i.e. detection without scanning, cameras or light), each nucleotide incorporation is recorded in seconds.

Another nucleic acid sequencing technology that may be used in a method described herein is the chemical-sensitive field effect transistor (CHEMFET) array. In one example of this sequencing technique, DNA molecules are placed into reaction chambers, and the template molecules can be hybridized to a sequencing primer bound to a polymerase. Incorporation of one or more triphosphates into a new nucleic acid strand at the 3′ end of the sequencing primer can be detected by a change in current by a CHEMFET sensor. An array can have multiple CHEMFET sensors. In another example, single nucleic acids are attached to beads, and the nucleic acids can be amplified on the bead, and the individual beads can be transferred to individual reaction chambers on a CHEMFET array, with each chamber having a CHEMFET sensor, and the nucleic acids can be sequenced (see, for example, U.S. Patent Application Publication No. 2009/0026082).

Another nucleic acid sequencing technology that may be used in a method described herein is electron microscopy. In one example of this sequencing technique, individual nucleic acid (e.g. DNA) molecules are labeled using metallic labels that are distinguishable using an electron microscope. These molecules are then stretched on a flat surface and imaged using an electron microscope to measure sequences (see, for example, Moudrianakis E. N. and Beer M. Proc Natl Acad Sci USA. 1965 March; 53:564-71). In some cases, transmission electron microscopy (TEM) is used (e.g. Halcyon Molecular's TEM method). This method, termed Individual Molecule Placement Rapid Nano Transfer (IMPRNT), includes utilizing single atom resolution transmission electron microscope imaging of high-molecular weight (e.g. about 150 kb or greater) DNA selectively labeled with heavy atom markers and arranging these molecules on ultra-thin films in ultra-dense (3nm strand-to-strand) parallel arrays with consistent base-to-base spacing. The electron microscope is used to image the molecules on the films to determine the position of the heavy atom markers and to extract base sequence information from the DNA (see, for example, International Patent Application No. WO 2009/046445).

Other sequencing methods that may be used to conduct methods herein include digital PCR and sequencing by hybridization. Digital polymerase chain reaction (digital PCR or dPCR) can be used to directly identify and quantify nucleic acids in a sample. Digital PCR can be performed in an emulsion, in some embodiments. For example, individual nucleic acids are separated, e.g., in a microfluidic chamber device, and each nucleic acid is individually amplified by PCR. Nucleic acids can be separated such that there is no more than one nucleic acid per well. In some embodiments, different probes can be used to distinguish various alleles (e.g. fetal alleles and maternal alleles). Alleles can be enumerated to determine copy number. In sequencing by hybridization, the method involves contacting a plurality of polynucleotide sequences with a plurality of polynucleotide probes, where each of the plurality of polynucleotide probes can be optionally tethered to a substrate. The substrate can be a flat surface with an array of known nucleotide sequences, in some embodiments. The pattern of hybridization to the array can be used to determine the polynucleotide sequences present in the sample. In some embodiments, each probe is tethered to a bead, e.g., a magnetic bead or the like. Hybridization to the beads can be identified and used to identify the plurality of polynucleotide sequences within the sample.

In some embodiments, nanopore sequencing can be used in a method described herein. Nanopore sequencing is a single-molecule sequencing technology whereby a single nucleic acid molecule (e.g. DNA) is sequenced directly as it passes through a nanopore. A nanopore is a small

hole or channel, of the order of 1 nanometer in diameter. Certain transmembrane cellular proteins can act as nanopores (e.g. alpha-hemolysin). In some cases, nanopores can be synthesized (e.g. using a silicon platform). Immersion of a nanopore in a conducting fluid and application of a potential across it results in a slight electrical current due to conduction of ions through the nanopore. The amount of current which flows is sensitive to the size of the nanopore. As a DNA molecule passes through a nanopore, each nucleotide on the DNA molecule obstructs the nanopore to a different degree and generates characteristic changes to the current. The amount of current which can pass through the nanopore at any given moment therefore varies depending on whether the nanopore is blocked by an A, a C, a G, a T, or in some cases, methyl-C. The change in the current through the nanopore as the DNA molecule passes through the nanopore represents a direct reading of the DNA sequence. In some cases a nanopore can be used to identify individual DNA bases as they pass through the nanopore in the correct order (see, for example, Soni GV and Meller A. Clin Chem 53: 1996-2001 (2007); International Patent Application No. WO2010/004265).

There are a number of ways that nanopores can be used to sequence nucleic acid molecules. In some embodiments, an exonuclease enzyme, such as a deoxyribonuclease, is used. In this case, the exonuclease enzyme is used to sequentially detach nucleotides from a nucleic acid (e.g. DNA) molecule. The nucleotides are then detected and discriminated by the nanopore in order of their release, thus reading the sequence of the original strand. For such an embodiment, the exonuclease enzyme can be attached to the nanopore such that a proportion of the nucleotides released from the DNA molecule is capable of entering and interacting with the channel of the nanopore. The exonuclease can be attached to the nanopore structure at a site in close proximity to the part of the nanopore that forms the opening of the channel. In some cases, the exonuclease enzyme can be attached to the nanopore structure such that its nucleotide exit trajectory site is orientated towards the part of the nanopore that forms part of the opening.

In some embodiments, nanopore sequencing of nucleic acids involves the use of an enzyme that pushes or pulls the nucleic acid (e.g. DNA) molecule through the pore. In this case, the ionic current fluctuates as a nucleotide in the DNA molecule passes through the pore. The fluctuations in the current are indicative of the DNA sequence. For such an embodiment, the enzyme can be attached to the nanopore structure such that it is capable of pushing or pulling the target nucleic acid through the channel of a nanopore without interfering with the flow of ionic current through the pore. The enzyme can be attached to the nanopore structure at a site in close proximity to the part of the structure that forms part of the opening. The enzyme can be attached to the subunit, for

example, such that its active site is orientated towards the part of the structure that forms part of the opening.

In some embodiments, nanopore sequencing of nucleic acids involves detection of polymerase bi-products in close proximity to a nanopore detector. In this case, nucleoside phosphates (nucleotides) are labeled so that a phosphate labeled species is released upon the addition of a polymerase to the nucleotide strand and the phosphate labeled species is detected by the pore. Typically, the phosphate species contains a specific label for each nucleotide. As nucleotides are sequentially added to the nucleic acid strand, the bi-products of the base addition are detected. The order that the phosphate labeled species are detected can be used to determine the sequence of the nucleic acid strand.

The length of the sequence read is often associated with the particular sequencing technology. High-throughput methods, for example, provide sequence reads that can vary in size from tens to hundreds of base pairs (bp). Nanopore sequencing, for example, can provide sequence reads that can vary in size from tens to hundreds to thousands of base pairs. In some embodiments, the sequence reads are of a mean, median or average length of about 15 bp to 900 bp long (e.g. about 20 bp, about 25 bp, about 30 bp, about 35 bp, about 40 bp, about 45 bp, about 50 bp, about 55 bp, about 60 bp, about 65 bp, about 70 bp, about 75 bp, about 80 bp, about 85 bp, about 90 bp, about 95 bp, about 100 bp, about 110 bp, about 120 bp, about 130, about 140 bp, about 150 bp, about 200 bp, about 250 bp, about 300 bp, about 350 bp, about 400 bp, about 450 bp, or about 500 bp. In some embodiments, the sequence reads are of a mean, median or average length of about 1000 bp or more.

In some embodiments, chromosome-specific sequencing is performed. In some embodiments, chromosome-specific sequencing is performed utilizing DANSR (digital analysis of selected regions). Digital analysis of selected regions enables simultaneous quantification of hundreds of loci by cfDNA-dependent catenation of two locus-specific oligonucleotides via an intervening 'bridge' oligo to form a PCR template. In some embodiments, chromosome-specific sequencing is performed by generating a library enriched in chromosome-specific sequences. In some embodiments, sequence reads are obtained only for a selected set of chromosomes. In some embodiments, sequence reads are obtained only for chromosomes 21, 18 and 13.

In some embodiments, nucleic acids may include a fluorescent signal or sequence tag information. Quantification of the signal or tag may be used in a variety of techniques such as, for example, flow cytometry, quantitative polymerase chain reaction (qPCR), gel electrophoresis, gene-chip analysis, microarray, mass spectrometry, cytofluorimetric analysis, fluorescence microscopy, confocal laser scanning microscopy, laser scanning cytometry, affinity chromatography, manual batch mode separation, electric field suspension, sequencing, and combination thereof.

Partial nucleotide sequence reads

In some embodiments, nucleotide sequence reads obtained from a sample are partial nucleotide sequence reads. As used herein, "partial nucleotide sequence reads" refers to sequence reads of any length with incomplete sequence information, also referred to as sequence ambiguity. Partial nucleotide sequence reads may lack information regarding nucleobase identity and/or nucleobase position or order. Partial nucleotide sequence reads generally do not include sequence reads in which the only incomplete sequence information (or in which less than all of the bases are sequenced or determined) is from inadvertent or unintentional sequencing errors. Such sequencing errors can be inherent to certain sequencing processes and include, for example, incorrect calls for nucleobase identity, and missing or extra nucleobases. Thus, for partial nucleotide sequence reads herein, certain information about the sequence is often deliberately excluded. That is, one deliberately obtains sequence information with respect to less than all of the nucleobases or which might otherwise be characterized as or be a sequencing error. In some embodiments, a partial nucleotide sequence read can span a portion of a nucleic acid fragment. In some embodiments, a partial nucleotide sequence read can span the entire length of a nucleic acid fragment.

A non-limiting example of a partial nucleotide sequence read is a sequence where the nucleotide species at less than all positions present in the nucleic acid are determined. Another non-limiting example is a sequence in which a subset of nucleobase species in the nucleic acid are determined. As used herein, "nucleobase species" refers to a type of nucleobase present in a sample nucleic acid. Non-modified nucleobase species typically include adenine (A), guanine (G), cytosine (C), thymine (T), methyl-cytosine (met-C), uracil (U, for RNA) or inosine (I). As used herein, "relative positional information" refers to the location of a nucleobase within a nucleotide sequence in relation to surrounding known nucleobases and/or nucleobase gaps. As used herein, "nucleobase gaps" refers to one or more nucleobase positions where the identity of the nucleobase

at each position is unknown. Such gaps exist between known nucleobases in the sequence read, and can be, independently, 1 to 100 sequential nucleobase positions (e.g., 5, 10, 20, 30, 40, 50, 60, 70, 80, or 90 sequential nucleobase positions). In some cases, one nucleotide species of a sequence read is decipherable while the remaining nucleotide species are indeterminate. For example, a partial nucleotide sequence read in which only the adenine nucleobases are identified may contain nucleobase gaps of varying length (e.g. A _ _ A _ A _ _ _ A). In the example sequence, the gaps comprise 2, 1, and 3 nucleobase positions. Such reads may sometimes be referred to as unary sequence reads (i.e., one nucleotide species is known at known positions and the other positions can be any one of three other nucleotides). In some cases, two or three nucleobase species of a sequence read are decipherable while the remaining nucleotide species are indeterminate (i.e., two or three nucleotide species are known at known positions and the other positions can be any one of three other nucleotide species).

Another non-limiting example of a partial nucleotide sequence read is a nucleic acid for which the composition of all or some nucleobase species is determined (i.e., base composition), but the order and relative positions of the nucleobases is not determined. For example, a nucleic acid may be digested e.g., by an endonuclease, into individual nucleobases prior to sequencing. In such cases, the number of nucleobases present in the nucleic acid can be determined for each nucleobase species (e.g., A, G, T, C, met-C, I, U) by an appropriate method (e.g., mass spectrometry; nanopore sequencing). For example, a nucleic acid may contain 30% A, 30% T, 20% G, and 20%C. Such partial nucleotide sequence reads can be mapped to one or more locations in a reference genome that comprise similar nucleobase compositions. In some cases, knowledge of some nucleotide dispersion within a given sequence read is combined with base composition data. In some cases, information pertaining to the variance, skewness and/or kurtosis of the nucleotide bases may provide further information pertaining to the uniqueness (e.g., mappability, identity) of a sequence.

Another non-limiting example of a partial nucleotide sequence read is a sequence where the nucleobase class (but not exact nucleobase identity) is known throughout all or part of the sequence read. As used herein, “nucleobase class” refers to a grouping of a nucleobase species subset present in the sample nucleic acid such as, for example, purines (Pu; e.g. A, G) and pyrimidines (Py; e.g. T, C). An example of a partial nucleotide sequence read comprising one or more nucleobase classes is: Pu-Pu-Py-Pu-Py-Py-Pu-Pu (i.e. purine-purine-pyrimidine-purine-pyrimidine-pyrimidine-purine-purine). This example sequence read would represent a nucleotide

sequence comprising the following nucleobase possibilities for each position: (A or G)-(A or G)-(T or C)-(A or G)-(T or C)-(T or C)-(A or G)-(A or G). Partial nucleotide sequence reads comprising one or more nucleobase classes can be contiguous reads of nucleobase classes or can contain nucleobase gaps, as described above. Such reads sometimes are referred to as binary sequence reads or binary partial reads (e.g., a first nucleotide class consisting of two possible bases is known at known positions and a second nucleotide class consisting of two possible bases is known at known positions, where the bases of the first nucleotide class are different than the bases of the second nucleotide class).

In some embodiments, partial nucleotide sequence reads contain relative positional information for one or more nucleobase species. In some embodiments, the partial nucleotide sequence reads can contain relative positional information for one or more, but not all, of adenine (A), guanine (G), cytosine (C), thymine (T), methyl-cytosine (met-C), or inosine (I). In some cases, the partial nucleotide sequence reads contain relative positional information for adenine. In some cases, the partial nucleotide sequence reads contain relative positional information for guanine. In some cases, the partial nucleotide sequence reads contain relative positional information for cytosine. In some cases, the partial nucleotide sequence reads contain relative positional information for thymine. In some cases, the partial nucleotide sequence reads contain relative positional information for methyl-cytosine.

In some embodiments, partial nucleotide sequence reads contain relative positional information for two or more nucleobase species. In some embodiments, the partial nucleotide sequence reads contain relative positional information for two or more nucleobase species which can include adenine (A), guanine (G), cytosine (C), thymine (T) and/or methyl-cytosine (met-C). In some embodiments, the partial nucleotide sequence reads contain relative positional information for two nucleobase species which can include adenine (A), guanine (G), cytosine (C), thymine (T) and/or methyl-cytosine (met-C). In some embodiments, the partial nucleotide sequence reads contain relative positional information for three nucleobase species which can include adenine (A), guanine (G), cytosine (C), thymine (T) and/or methyl-cytosine (met-C). In some embodiments, the partial nucleotide sequence reads contain relative positional information for four nucleobase species which can include adenine (A), guanine (G), cytosine (C), thymine (T) and/or methyl-cytosine (met-C). In some cases, partial nucleotide sequence reads are ternary partial reads (e.g., a first nucleotide species is known at known positions, a second nucleotide species is known at other

known positions and the other positions are any one of two nucleotide species other than the first nucleotide species and the second nucleotide species.)

Nucleobase classes

In some embodiments, partial nucleotide sequence reads contain one or more nucleobase classes. Such nucleobase classes contain a subset of nucleobases present in the sample nucleic acid. The subset of nucleobases can be any designation or combination of nucleobases known in the art. For example, a nucleobase class can be a designation of purine nucleobases (e.g. adenine, guanine) or pyrimidine nucleobases (e.g. thymine, cytosine, uracil). Thus a partial nucleotide sequence read may contain information about the purine and/or pyrimidine content, and often includes relative positional information for each purine and/or pyrimidine class. In some cases, nanopore sequencing can be used to identify the relative positions of purines and/or pyrimidines in a sample nucleic acid to generate partial nucleotide sequence reads. Other examples of nucleobase classes include, without limitation, groups of adjacent and consecutive nucleobases (e.g., nucleobase dimers, trimers, multimers), and nucleobase pair species in a duplex nucleic acid. In some embodiments, nucleobase classes can be homodimers, heterodimers, homotrimers, heterotrimers, homomultimers, or heteromultimers. For example, a nucleobase class can be a homodimer (i.e. two identical adjacent nucleobases) such as, for example, AA, TT, CC, GG; or a heterodimer (i.e. two different adjacent nucleobases), such as, for example AT, AG, AC, TA, TG, TC, CG, CA, CT, GT, GA, GC. Such homodimers and/or heterodimers, for example, can possess distinguishable characteristics that can be deciphered by any sequencing technology suitable to identify such nucleobase classes to generate partial nucleotide sequence reads. In some embodiments, the nucleobase class is one or more nucleobase pair species in a duplex nucleic acid. Such nucleobase pair species are typically the Watson and Crick nucleobase pairs found in double stranded nucleic acid (i.e. A-T and G-C pairs). In some cases, the identity of each nucleobase pair is determined for a length of duplex DNA, but the orientation (e.g., A-T vs. T-A) is not. Such pairs can each possess their own characteristics that can be deciphered with any sequencing technology suitable to identify such nucleobase classes and generate partial nucleotide sequence reads.

Labeled nucleobase species or class

In some embodiments, the nucleic acid to be sequenced can contain one or more nucleobase species and/or nucleobase classes which are in a form capable of interacting with an agent or station in an apparatus to produce a signal characteristic of that interaction. In such cases, the nucleobase species or class is considered as labeled. Some of the sequencing technologies in the art make use of labeled nucleobases to generate nucleotide sequence reads. In some embodiments, sequencing technologies in the art make use of one or more labeled nucleobase species or nucleobase classes to generate partial nucleotide sequence reads. Such labeling can be instrinsic or extrinsic, as described in further detail below.

If a native nucleobase species or class can undergo an interaction to produce a characteristic signal, then the nucleic acid is considered as intrinsically labeled. In such embodiments, it is not necessary that an extrinsic label be added to the nucleic acid. If a non-native molecule, however, is attached to a native nucleobase species or class to generate an interaction that produces the characteristic signal, then the nucleic acid is considered as extrinsically labeled. In some embodiments, the "label" can be, for example, light emitting, light absorbing, light diffracting, light scattering, energy accepting, energy absorbing, fluorescent, radioactive, or quenching. Non-limiting examples of "labels" are provided hereafter.

Many naturally occurring units of a nucleic acid are light emitting compounds or quenchers. For instance, nucleobases of native nucleic acid molecules have distinct absorption spectra, e.g., A, G, T, C, and U have absorption maximums at 259 nm, 252 nm, 267 nm, 271 nm, and 258 nm respectively. Modified nucleobases which include intrinsic labels also may be incorporated into nucleic acids. A nucleic acid molecule may include, for example, any of the following modified nucleobases which have characteristic energy emission patterns of a light emitting compound or a quenching compound: 2,4-dithiouracil, 2,4-Diselenouracil, hypoxanthine, mercaptopurine, 2-aminopurine, and selenopurine.

A nucleobase also may be considered as intrinsically labeled when a property of the nucleobase, other than a light emitting, quenching or radioactive property, provides information about the identity of the nucleobase without the addition of an extrinsic label, in certain embodiments. For example, the shape and charge of the nucleobase provides information about the nucleobase species which can result in a specific characteristic signal, such as a change in conductance

arising from the blockage of a conductance path by the nucleobase, which can, for example, be detected and deciphered using a nanopore sequencing technology.

In some embodiments, one or more nucleobase species or nucleobase classes can be extrinsically labeled. In some cases, a nucleobase species or nucleobase class is labeled with one or more detectable labels. In some cases, each nucleobase species or nucleobase class is labeled with a unique detectable label. In some embodiments, one or more nucleobase species or nucleobase classes are each labeled with a unique detectable label and one or more nucleobase species or nucleobase classes are unlabeled. Examples of detectable labels include, without limitation, sugars, peptides, proteins, antibodies, chemical compounds, conducting polymers, binding moieties such as biotin, mass tags, colorimetric agents, light emitting agents, chemiluminescent agents, light scattering agents, fluorescent tags, radioactive tags, charge tags (electrical or magnetic charge), volatile tags and hydrophobic tags, biomolecules (e.g., members of a binding pair antibody/antigen, antibody/antibody, antibody/antibody fragment, antibody/antibody receptor, antibody/protein A or protein G, hapten/anti-hapten, biotin/avidin, biotin/streptavidin, folic acid/folate binding protein, vitamin B12/intrinsic factor, chemical reactive group/complementary chemical reactive group (e.g., sulfhydryl/maleimide, sulfhydryl/haloacetyl derivative, amine/isotriocyanate, amine/succinimidyl ester, and amine/sulfonyl halides) and the like.

Hybridized probe species

Hybridizing one or more probes to a sample nucleic acid can facilitate determining relative positional information for nucleobase species and/or classes in partial nucleotide sequence reads. In some embodiments, one or more probe or oligonucleotide species can be hybridized to the sample nucleic acid. Such hybridization often occurs prior to sequencing, in some embodiments. As used herein, a probe or oligonucleotide “species” refers to a first probe or oligonucleotide having a nucleotide sequence that differs by one nucleotide base or more from the nucleotide sequence of a second probe or oligonucleotide when the nucleotide sequences of the first and second probes or oligonucleotides are aligned. Thus one probe or oligonucleotide species may differ from a second probe or oligonucleotide species by one or more nucleotides when the nucleotide sequences of the first and second probe or oligonucleotide are aligned with one another (e.g., about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more than 100 nucleotide differences).

In certain cases, the obtained partial nucleotide sequence reads can contain relative positional information for sequences complementary to the probe or oligonucleotide species. Probes can be of any length and sequence suitable for obtaining partial nucleotide sequence reads. In some cases, a probe is at least 2 to about 30 nucleotides in length. For example, a probe can be 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. Any number of probe species and any combination of probe species with various lengths and/or sequences can be used to generate partial sequence reads. In some embodiments, random probes are used. In some embodiments, probes with specific sequences identical and/or complementary to certain genome sections are used. In certain embodiments where nanopore sequencing is used to generate partial nucleotide sequence reads, a probe or oligonucleotide species may contain a signal-generating moiety that hybridizes to a sample nucleic acid and alters the passage of the nucleic acid through a nanopore, and/or generates a signal when released from the target nucleic acid when it passes through the nanopore.

Full sequence reads

In some embodiments, full nucleotide sequence reads are obtained in addition to or in lieu of the partial nucleotide sequence reads described above. Such "full" nucleotide sequence reads can be of any length and substantially contain no nucleobase gaps between identified nucleobases or a nucleobase class. Full nucleotide sequence reads can contain all nucleobases, all nucleobase classes, or a combination thereof. Thus, a full nucleotide sequence read generally is a run of continuous nucleobases where each nucleobase is identified (i.e. as A, G, C, T, etc.).

Sequencing Module

Sequencing and obtaining sequencing reads can be provided by a sequencing module or by an apparatus comprising a sequencing module. A "sequence receiving module" as used herein is the same as a "sequencing module". An apparatus comprising a sequencing module can be any apparatus that determines the sequence of a nucleic acid from a sequencing technology known in the art. In certain embodiments, an apparatus comprising a sequencing module performs a sequencing reaction known in the art. A sequencing module generally provides a nucleic acid sequence read according to data from a sequencing reaction (e.g., signals generated from a sequencing apparatus). In some embodiments, a sequencing module or an apparatus comprising a sequencing module is required to provide sequencing reads. In some embodiments a

sequencing module can receive, obtain, access or recover sequence reads from another sequencing module, computer peripheral, operator, server, hard drive, apparatus or from a suitable source. Sometimes a sequencing module can manipulate sequence reads. For example, a sequencing module can align, assemble, fragment, complement, reverse complement, error check, or error correct sequence reads. An apparatus comprising a sequencing module can comprise at least one processor. In some embodiments, sequencing reads are provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the sequencing module. In some embodiments, sequencing reads are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a sequencing module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). Sometimes a sequencing module gathers, assembles and/or receives data and/or information from another module, apparatus, peripheral, component or specialized component (e.g., a sequencer). In some embodiments, sequencing reads are provided by an apparatus comprising one or more of the following: one or more flow cells, a camera, a photo detector, a photo cell, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. Often a sequencing module receives, gathers and/or assembles sequence reads. Sometimes a sequencing module accepts and gathers input data and/or information from an operator of an apparatus. For example, sometimes an operator of an apparatus provides instructions, a constant, a threshold value, a formula or a predetermined value to a module. Sometimes a sequencing module can transform data and/or information that it receives into a contiguous nucleic acid sequence. In some embodiments, a nucleic acid sequence provided by a sequencing module is printed or displayed. In some embodiments, sequence reads are provided by a sequencing module and transferred from a sequencing module to an apparatus or an apparatus comprising any suitable peripheral, component or specialized component. In some embodiments, data and/or information are provided from a sequencing module to an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some cases, data and/or information related to sequence reads can be transferred from a sequencing module to any other suitable module. A sequencing module can transfer sequence reads to a mapping module or counting module, in some embodiments.

Mapping reads

Mapping nucleotide sequence reads (i.e., sequence information from a fragment whose physical genomic position is unknown) can be performed in a number of ways, and often comprises alignment of the obtained sequence reads with a matching sequence in a reference genome (e.g., Li et al., "Mapping short DNA sequencing reads and calling variants using mapping quality score," Genome Res., 2008 Aug 19.) In such alignments, sequence reads generally are aligned to a reference sequence and those that align are designated as being "mapped" or a "sequence tag." In some cases, a mapped sequence read is referred to as a “hit” or a “count”. In some embodiments, mapped sequence reads are grouped together according to various parameters and assigned to particular genomic sections, which are discussed in further detail below.

As used herein, the terms “aligned”, “alignment”, or “aligning” refer to two or more nucleic acid sequences that can be identified as a match (e.g., 100% identity) or partial match. Alignments can be done manually or by a computer algorithm, examples including the Efficient Local Alignment of Nucleotide Data (ELAND) computer program distributed as part of the Illumina Genomics Analysis pipeline. The alignment of a sequence read can be a 100% sequence match. In come cases, an alignment is less than a 100% sequence match (i.e., non-perfect match, partial match, partial alignment). In some embodiments an alignment is about a 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76% or 75% match. In some embodiments, an alignment comprises a mismatch. In some embodiments, an alignment comprises 1, 2, 3, 4 or 5 mismatches. Two or more sequences can be aligned using either strand. In some cases a nucleic acid sequence is aligned with the reverse complement of another nucleic acid sequence.

Various computational methods can be used to map each sequence read to a genomic section. Non-limiting examples of computer algorithms that can be used to align sequences include, without limitation, BLAST, BLITZ, FASTA, BOWTIE 1, BOWTIE 2, ELAND, MAQ, PROBEMATCH, SOAP or SEQMAP, or variations thereof or combinations thereof. In some embodiments, sequence reads can be aligned with sequences in a reference genome. In some embodiments, the sequence reads can be found and/or aligned with sequences in nucleic acid databases known in the art including, for example, GenBank, dbEST, dbSTS, EMBL (European Molecular Biology Laboratory) and DDBJ (DNA Databank of Japan). BLAST or similar tools can be used to search

the identified sequences against a sequence database. Search hits can then be used to sort the identified sequences into appropriate genomic sections (described hereafter), for example.

The term “sequence tag” is herein used interchangeably with the term “mapped sequence tag” to refer to a sequence read that has been specifically assigned i.e. mapped, to a larger sequence e.g. a reference genome, by alignment. Mapped sequence tags are uniquely mapped to a reference genome i.e. they are assigned to a single location to the reference genome. Tags that can be mapped to more than one location on a reference genome i.e. tags that do not map uniquely, are not included in the analysis. A "sequence tag" can be a nucleic acid (e.g. DNA) sequence (i.e. read) assigned specifically to a particular genomic section and/or chromosome (i.e. one of chromosomes 1-22, X or Y for a human subject). A sequence tag may be repetitive or non-repetitive within a single segment of the reference genome (e.g., a chromosome). In some embodiments, repetitive sequence tags are eliminated from further analysis (e.g. quantification). In some embodiments, a read may uniquely or non-uniquely map to portions in the reference genome. A read is considered to be “uniquely mapped” if it aligns with a single sequence in the reference genome. A read is considered to be “non-uniquely mapped” if it aligns with two or more sequences in the reference genome. In some embodiments, non-uniquely mapped reads are eliminated from further analysis (e.g. quantification). A certain, small degree of mismatch (0-1) may be allowed to account for single nucleotide polymorphisms that may exist between the reference genome and the reads from individual samples being mapped, in certain embodiments. In some embodiments, no degree of mismatch is allowed for a read to be mapped to a reference sequence.

As used herein, the term “reference genome” can refer to any particular known, sequenced or characterized genome, whether partial or complete, of any organism or virus which may be used to reference identified sequences from a subject. For example, a reference genome used for human subjects as well as many other organisms can be found at the National Center for Biotechnology Information at www.ncbi.nlm.nih.gov. A “genome” refers to the complete genetic information of an organism or virus, expressed in nucleic acid sequences. As used herein, a reference sequence or reference genome often is an assembled or partially assembled genomic sequence from an individual or multiple individuals. In some embodiments, a reference genome is an assembled or partially assembled genomic sequence from one or more human individuals. In some embodiments, a reference genome comprises sequences assigned to chromosomes.

In certain embodiments, where a sample nucleic acid is from a pregnant female, a reference sequence sometimes is not from the fetus, the mother of the fetus or the father of the fetus, and is referred to herein as an "external reference." A maternal reference may be prepared and used in some embodiments. When a reference from the pregnant female is prepared ("maternal reference sequence") based on an external reference, reads from DNA of the pregnant female that contains substantially no fetal DNA often are mapped to the external reference sequence and assembled. In certain embodiments the external reference is from DNA of an individual having substantially the same ethnicity as the pregnant female. A maternal reference sequence may not completely cover the maternal genomic DNA (e.g., it may cover about 50%, 60%, 70%, 80%, 90% or more of the maternal genomic DNA), and the maternal reference may not perfectly match the maternal genomic DNA sequence (e.g., the maternal reference sequence may include multiple mismatches).

In some cases, mappability is assessed for a genomic region (e.g., genomic section, genomic portion, bin). Mappability is the ability to unambiguously align a nucleotide sequence read to a portion of a reference genome, typically up to a specified number of mismatches, including, for example, 0, 1, 2 or more mismatches. For a given genomic region, the expected mappability can be estimated using a sliding-window approach of a preset read length and averaging the resulting read-level mappability values. Genomic regions comprising stretches of unique nucleotide sequence sometimes have a high mappability value.

Mapping partial sequence reads

In some embodiments, partial nucleotide sequence reads are mapped to a reference genome using methods described above. In such cases, the information contained in the partial nucleotide sequence read is sufficient to map the read to one or more locations in a reference genome. In some cases, the information contained in the partial nucleotide sequence read is sufficient to map the read to one specific location in a reference genome. Such a location in a reference genome is often part or all of a genome section as described above. The information contained in the partial nucleotide sequence read can be a certain number of nucleobase or nucleobase class identities, or can be nucleobase or nucleobase class identities over a specific length of read, as discussed in further detail below.

In some embodiments, partial nucleotide sequence reads contain a number of discrete position identities sufficient to map to a reference genome section. For example, certain genomic

sequences have a distinct pattern of nucleobases or nucleobase classes, such that a specific minimum number of one or more nucleobases must be known to uniquely match a partial nucleotide sequence read to such a genomic sequence. The minimum number of position identities for each nucleobase that is required for a positive and unique match can be calculated, often varies from sequence to sequence, and can vary among nucleobases for a given sequence. The minimum number of position identities also varies, for example, depending on whether 1, 2, 3, or 4 of the nucleobase species or a certain number of nucleobase classes are identified in the partial nucleotide sequence read.

In some embodiments, a partial nucleotide sequence read is of sufficient length to map to a reference genome section. As used herein, the length of a partial nucleotide sequence read refers to the length of sequence inclusive of identified nucleobases or nucleobase classes and unidentified nucleobase positions (i.e. positions located in one or more nucleotide sequence gaps). For example, certain genomic sequences have a distinct pattern of nucleobases or nucleobase classes, such that a specific length of sequence must be known to uniquely match a partial nucleotide sequence read to such a genomic sequence. The minimum length for each partial nucleotide sequence read that is required for a positive and unique match can be calculated and often varies from sequence to sequence and can vary depending on which of the nucleobase species or nucleobase classes are identified. The minimum length of sequence read also varies, for example, depending on whether 1, 2, 3, or 4 of the nucleobase species are identified in the read. In some embodiments, the partial nucleotide sequence read length is at least about 10 nucleobases (e.g., nucleobase positions) to about 150 nucleobases (e.g., nucleobase positions). In some embodiments, the partial nucleotide sequence read length is at least about 15 nucleobases to about 40 nucleobases. For example, a partial nucleotide sequence read length can be at least about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38 or 39 nucleobases (e.g., nucleobase positions). In some embodiments, the partial nucleotide sequence read length is at least about 50 nucleobases to about 60 nucleobases. In some embodiments, the partial nucleotide sequence read length is at least about 70 nucleobases to about 80 nucleobases. In some embodiments, the partial nucleotide sequence read length is at least about 100 nucleobases to about 110 nucleobases. In some embodiments, the partial nucleotide sequence read length is at least about 140 nucleobases to about 150 nucleobases. In some embodiments, the partial nucleotide sequence read length is about 20 nucleobases. In some embodiments, the partial nucleotide sequence read length is about 30 nucleobases. In some embodiments, the partial nucleotide sequence read length is about 36 nucleobases. In

some embodiments, the partial nucleotide sequence read length is about 54 nucleobases. In some embodiments, the partial nucleotide sequence read length is about 72 nucleobases. In some embodiments, the partial nucleotide sequence read length is about 108 nucleobases. In some embodiments, the partial nucleotide sequence read length is about 144 nucleobases.

In some embodiments, the minimum number of identified nucleobase species or nucleobase classes, or the minimum length of partial nucleotide sequence read, required for a positive and unique match to a reference genome can vary depending on the size of the reference genome. For example, a partial nucleotide sequence read may be required to contain more information for obtaining a positive and unique match to a full genome (e.g. human genome) versus obtaining a positive and unique match to a partial genome (i.e. a chromosome or portion thereof).

In some embodiments, the reference genome is an assembly of partial sequence information, sometimes referred to herein as a nucleotide barcode reference or nucleotide signature reference. Such barcodes or signatures can be representations of a complete reference genome, or portion thereof, where the complete nucleotide sequence or sequences are known. In some cases, the barcodes or signatures, and the corresponding sequences that they represent, are stored in a database. In embodiments where partial nucleotide sequence reads are mapped, the pattern of identified nucleobases or nucleobase classes in each partial nucleotide sequence read can be matched to a particular barcode or signature reference. Since the barcode or signature reference often represents a known sequence in a reference genome, the partial sequence reads can thus be mapped to a particular locus or genome section in a reference genome.

Mapping Module

Sequence reads can be mapped by a mapping module or by an apparatus comprising a mapping module, which mapping module generally maps reads to a reference genome or segment thereof. A mapping module can map sequencing reads by a suitable method known in the art. In some embodiments, a mapping module or an apparatus comprising a mapping module is required to provide mapped sequence reads. An apparatus comprising a mapping module can comprise at least one processor. In some embodiments, mapped sequencing reads are provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the mapping module. In some embodiments, sequencing reads are mapped by an apparatus that

includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a mapping module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). An apparatus may comprise a mapping module and a sequencing module. In some embodiments, sequence reads are mapped by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A mapping module can receive sequence reads from a sequencing module, in some embodiments. Mapped sequencing reads can be transferred from a mapping module to a counting module or a normalization module, in some embodiments.

Genomic sections

In some embodiments, mapped sequence reads (i.e. sequence tags) are grouped together according to various parameters and assigned to particular genomic sections. Often, the individual mapped sequence reads can be used to identify an amount of a genomic section present in a sample. In some embodiments, the amount of a genomic section can be indicative of the amount of a larger sequence (e.g. a chromosome) in the sample. The term "genomic section" can also be referred to herein as a "sequence window", "section", "bin", "locus", "region", "partition" or "portion". In some embodiments, a genomic section is an entire chromosome, segment of a chromosome, segment of a reference genome, multiple chromosome portions, multiple chromosomes, portions from multiple chromosomes, and/or combinations thereof. Sometimes a genomic section is predefined based on specific parameters. Sometimes a genomic section is arbitrarily defined based on partitioning of a genome (e.g., partitioned by size, segments, contiguous regions, contiguous regions of an arbitrarily defined size, and the like). In some cases, a genomic section is delineated based on one or more parameters which include, for example, length or a particular feature or features of the sequence. Genomic sections can be selected, filtered and/or removed from consideration using any suitable criteria know in the art or described herein. In some embodiments, a genomic section is based on a particular length of genomic sequence. In some embodiments, a method can include analysis of multiple mapped sequence reads to a plurality of genomic sections. The genomic sections can be approximately the same length or the genomic sections can be different lengths. Sometimes genomic sections are of about equal length. In some cases genomic sections of different lengths are adjusted or weighted. In some embodiments, a genomic section is about 10 kilobases (kb) to about 100 kb, about 20 kb to about 80 kb, about 30 kb to about 70 kb, about 40 kb to about 60 kb, and sometimes about 50 kb. In

some embodiments, a genomic section is about 10 kb to about 20 kb. A genomic section is not limited to contiguous runs of sequence. Thus, genomic sections can be made up of contiguous and/or non-contiguous sequences. A genomic section is not limited to a single chromosome. In some embodiments, a genomic section includes all or part of one chromosome or all or part of two or more chromosomes. In some cases, genomic sections may span one, two, or more entire chromosomes. In addition, the genomic sections may span joint or disjointed portions of multiple chromosomes.

In some embodiments, genomic sections can be particular chromosome segments in a chromosome of interest, such as, for example, chromosomes where a genetic variation is assessed (e.g. an aneuploidy of chromosomes 13, 18 and/or 21 or a sex chromosome). A genomic section can also be a pathogenic genome (e.g. bacterial, fungal or viral) or fragment thereof. Genomic sections can be genes, gene fragments, regulatory sequences, introns, exons, and the like.

In some embodiments, a genome (e.g. human genome) is partitioned into genomic sections based on the information content of the regions. The resulting genomic regions may contain sequences for multiple chromosomes and/or may contain sequences for portions of multiple chromosomes. In some cases, the partitioning may eliminate similar locations across the genome and only keep unique regions. The eliminated regions may be within a single chromosome or may span multiple chromosomes. The resulting genome is thus trimmed down and optimized for faster alignment, often allowing for focus on uniquely identifiable sequences. In some cases, the partitioning may down weight similar regions. The process for down weighting a genomic section is discussed in further detail below. In some embodiments, the partitioning of the genome into regions transcending chromosomes may be based on information gain produced in the context of classification. For example, the information content may be quantified using the p-value profile measuring the significance of particular genomic locations for distinguishing between groups of confirmed normal and abnormal subjects (e.g. euploid and trisomy subjects, respectively). In some embodiments, the partitioning of the genome into regions transcending chromosomes may be based on any other criterion, such as, for example, speed/convenience while aligning tags, high or low GC content, uniformity of GC content, other measures of sequence content (e.g. fraction of individual nucleotides, fraction of pyrimidines or purines, fraction of natural vs. non-natural nucleic acids, fraction of methylated nucleotides, and CpG content), methylation state, duplex melting

temperature, amenability to sequencing or PCR, uncertainty value assigned to individual bins, and/or a targeted search for particular features.

Sequence tag density

"Sequence tag density" refers to the normalized value of sequence tags or reads for a defined genomic section where the sequence tag density is used for comparing different samples and for subsequent analysis. The value of the sequence tag density often is normalized within a sample. In some embodiments, normalization can be performed by counting the number of tags falling within each genomic section; obtaining a median value of the total sequence tag count for each chromosome; obtaining a median value of all of the autosomal values; and using this value as a normalization constant to account for the differences in total number of sequence tags obtained for different samples. A sequence tag density sometimes is about 1 for a disomic chromosome. Sequence tag densities can vary according to sequencing artifacts, most notably G/C bias, which can be corrected by use of an external standard or internal reference (e.g., derived from substantially all of the sequence tags (genomic sequences), which may be, for example, a single chromosome or a calculated value from all autosomes, in some embodiments). Thus, dosage imbalance of a chromosome or chromosomal regions can be inferred from the percentage representation of the locus among other mappable sequenced tags of the specimen. Dosage imbalance of a particular chromosome or chromosomal regions therefore can be quantitatively determined and be normalized. Methods for sequence tag density normalization and quantification are discussed in further detail below.

In some embodiments, a proportion of all of the sequence reads are from a chromosome involved in an aneuploidy (e.g., chromosome 13, chromosome 18, chromosome 21), and other sequence reads are from other chromosomes. By taking into account the relative size of the chromosome involved in the aneuploidy (e.g., "target chromosome": chromosome 21) compared to other chromosomes, one could obtain a normalized frequency, within a reference range, of target chromosome-specific sequences, in some embodiments. If the fetus has an aneuploidy in a target chromosome, then the normalized frequency of the target chromosome-derived sequences is statistically greater than the normalized frequency of non-target chromosome-derived sequences, thus allowing the detection of the aneuploidy. The degree of change in the normalized frequency will be dependent on the fractional concentration of fetal nucleic acids in the analyzed sample, in some embodiments.

Counts

Sequence reads that are mapped or partitioned based on a selected feature or variable can be quantified to determine the number of reads that are mapped to a genomic section (e.g., bin, partition, genomic portion, portion of a reference genome, portion of a chromosome and the like), in some embodiments. Sometimes the quantity of sequence reads that are mapped to a genomic section are termed counts (e.g., a count). Often a count is associated with a genomic section. Sometimes counts for two or more genomic sections (e.g., a set of genomic sections) are mathematically manipulated (e.g., averaged, added, normalized, the like or a combination thereof). In some embodiments a count is determined from some or all of the sequence reads mapped to (i.e., associated with) a genomic section. In certain embodiments, a count is determined from a pre-defined subset of mapped sequence reads. Pre-defined subsets of mapped sequence reads can be defined or selected utilizing any suitable feature or variable. In some embodiments, pre-defined subsets of mapped sequence reads can include from 1 to n sequence reads, where n represents a number equal to the sum of all sequence reads generated from a test subject or reference subject sample.

Sometimes a count is derived from sequence reads that are processed or manipulated by a suitable method, operation or mathematical process known in the art. Sometimes a count is derived from sequence reads associated with a genomic section where some or all of the sequence reads are weighted, removed, filtered, normalized, adjusted, averaged, derived as a mean, added, or subtracted or processed by a combination thereof. In some embodiments, a count is derived from raw sequence reads and or filtered sequence reads. A count (e.g., counts) can be determined by a suitable method, operation or mathematical process. Sometimes a count value is determined by a mathematical process. Sometimes a count value is an average, mean or sum of sequence reads mapped to a genomic section. Often a count is a mean number of counts. In some embodiments, a count is associated with an uncertainty value. Counts can be processed (e.g., normalized) by a method known in the art and/or as described herein (e.g., bin-wise normalization, normalization by GC content, linear and nonlinear least squares regression, GC LOESS, LOWESS, PERUN, RM, GCRM, cQn and/or combinations thereof).

Counts (e.g., raw, filtered and/or normalized counts) can be processed and normalized to one or more elevations. Elevations and profiles are described in greater detail hereafter. Sometimes counts can be processed and/or normalized to a reference elevation. Reference elevations are

addressed later herein. Counts processed according to an elevation (e.g., processed counts) can be associated with an uncertainty value (e.g., a calculated variance, an error, standard deviation, p-value, mean absolute deviation, etc.). An uncertainty value typically defines a range above and below an elevation. A value for deviation can be used in place of an uncertainty value, and non-limiting examples of measures of deviation include standard deviation, average absolute deviation, median absolute deviation, standard score (e.g., Z-score, Z-value, normal score, standardized variable) and the like.

Counts are often obtained from a nucleic acid sample from a pregnant female bearing a fetus. Counts of nucleic acid sequence reads mapped to a genomic section often are counts representative of both the fetus and the mother of the fetus (e.g., a pregnant female subject). Sometimes some of the counts mapped to a genomic section are from a fetal genome and some of the counts mapped to the same genomic section are from the maternal genome.

Counting Module

Counts can be provided by a counting module or by an apparatus comprising a counting module. A counting module can determine, assemble, and/or display counts according to a counting method known in the art. A counting module generally determines or assembles counts according to counting methodology known in the art. In some embodiments, a counting module or an apparatus comprising a counting module is required to provide counts. An apparatus comprising a counting module can comprise at least one processor. In some embodiments, counts are provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the counting module. In some embodiments, reads are counted by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a counting module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, reads are counted by an apparatus comprising one or more of the following: a sequencing module, a mapping module, one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A counting module can receive data and/or information from a sequencing module and/or a mapping module, transform the data and/or information and provide counts (e.g., counts mapped to genomic sections). A counting module can receive mapped sequence reads from a mapping module. A counting

module can receive normalized mapped sequence reads from a mapping module or from a normalization module. A counting module can transfer data and/or information related to counts (e.g., counts, assembled counts and/or displays of counts) to any other suitable apparatus, peripheral, or module. Sometimes data and/or information related to counts are transferred from a counting module to a normalization module, a plotting module, a categorization module and/or an outcome module.

Data processing

Mapped sequence reads that have been counted are referred to herein as raw data, since the data represents unmanipulated counts (e.g., raw counts). In some embodiments, sequence read data in a data set can be processed further (e.g., mathematically and/or statistically manipulated) and/or displayed to facilitate providing an outcome. In some cases, processed counts can be referred to as a derivative of counts, and may include for example, normalized counts, levels, elevations, profiles, and the like. In certain embodiments, data sets, including larger data sets, may benefit from pre-processing to facilitate further analysis. Pre-processing of data sets sometimes involves removal of redundant and/or uninformative genomic sections or bins (e.g., bins with uninformative data, redundant mapped reads, genomic sections or bins with zero median counts, over represented or under represented sequences). Without being limited by theory, data processing and/or preprocessing may (i) remove noisy data, (ii) remove uninformative data, (iii) remove redundant data, (iv) reduce the complexity of larger data sets, and/or (v) facilitate transformation of the data from one form into one or more other forms. The terms “pre-processing” and “processing” when utilized with respect to data or data sets are collectively referred to herein as “processing”. Processing can render data more amenable to further analysis, and can generate an outcome in some embodiments.

The term “noisy data” as used herein refers to (a) data that has a significant variance between data points when analyzed or plotted, (b) data that has a significant standard deviation (e.g., greater than 3 standard deviations), (c) data that has a significant standard error of the mean, the like, and combinations of the foregoing. Noisy data sometimes occurs due to the quantity and/or quality of starting material (e.g., nucleic acid sample), and sometimes occurs as part of processes for preparing or replicating DNA used to generate sequence reads. In certain embodiments, noise results from certain sequences being over represented when prepared using PCR-based methods.

Methods described herein can reduce or eliminate the contribution of noisy data, and therefore reduce the effect of noisy data on the provided outcome.

The terms “uninformative data”, “uninformative bins”, and “uninformative genomic sections” as used herein refer to genomic sections, or data derived therefrom, having a numerical value that is significantly different from a predetermined threshold value or falls outside a predetermined cutoff range of values. The terms “threshold” and “threshold value” herein refer to any number that is calculated using a qualifying data set and serves as a limit of diagnosis of a genetic variation (e.g. a copy number variation, an aneuploidy, a chromosomal aberration, and the like). Sometimes a threshold is exceeded by results obtained by methods described herein and a subject is diagnosed with a genetic variation (e.g. trisomy 21). A threshold value or range of values often is calculated by mathematically and/or statistically manipulating sequence read data (e.g., from a reference and/or subject), in some embodiments, and in certain embodiments, sequence read data manipulated to generate a threshold value or range of values is sequence read data (e.g., from a reference and/or subject). In some embodiments, an uncertainty value is determined. An uncertainty value generally is a measure of variance or error and can be any suitable measure of variance or error. An uncertainty value can be a standard deviation, standard error, calculated variance, p-value, or mean absolute deviation (MAD), in some embodiments. In some embodiments an uncertainty value can be calculated according to a formula in Example 6.

Any suitable procedure can be utilized for processing data sets described herein. Non-limiting examples of procedures suitable for use for processing data sets include filtering, normalizing, weighting, monitoring peak heights, monitoring peak areas, monitoring peak edges, determining area ratios, mathematical processing of data, statistical processing of data, application of statistical algorithms, analysis with fixed variables, analysis with optimized variables, plotting data to identify patterns or trends for additional processing, the like and combinations of the foregoing. In some embodiments, data sets are processed based on various features (e.g., GC content, redundant mapped reads, centromere regions, telomere regions, the like and combinations thereof) and/or variables (e.g., fetal gender, maternal age, maternal ploidy, percent contribution of fetal nucleic acid, the like or combinations thereof). In certain embodiments, processing data sets as described herein can reduce the complexity and/or dimensionality of large and/or complex data sets. A non-limiting example of a complex data set includes sequence read data generated from one or more test subjects and a plurality of reference subjects of different ages and ethnic backgrounds. In

some embodiments, data sets can include from thousands to millions of sequence reads for each test and/or reference subject.

Data processing can be performed in any number of steps, in certain embodiments. For example, data may be processed using only a single processing procedure in some embodiments, and in certain embodiments data may be processed using 1 or more, 5 or more, 10 or more or 20 or more processing steps (e.g., 1 or more processing steps, 2 or more processing steps, 3 or more processing steps, 4 or more processing steps, 5 or more processing steps, 6 or more processing steps, 7 or more processing steps, 8 or more processing steps, 9 or more processing steps, 10 or more processing steps, 11 or more processing steps, 12 or more processing steps, 13 or more processing steps, 14 or more processing steps, 15 or more processing steps, 16 or more processing steps, 17 or more processing steps, 18 or more processing steps, 19 or more processing steps, or 20 or more processing steps). In some embodiments, processing steps may be the same step repeated two or more times (e.g., filtering two or more times, normalizing two or more times), and in certain embodiments, processing steps may be two or more different processing steps (e.g., filtering, normalizing; normalizing, monitoring peak heights and edges; filtering, normalizing, normalizing to a reference, statistical manipulation to determine p-values, and the like), carried out simultaneously or sequentially. In some embodiments, any suitable number and/or combination of the same or different processing steps can be utilized to process sequence read data to facilitate providing an outcome. In certain embodiments, processing data sets by the criteria described herein may reduce the complexity and/or dimensionality of a data set.

In some embodiments, one or more processing steps can comprise one or more filtering steps. The term “filtering” as used herein refers to removing genomic sections or bins from consideration. Bins can be selected for removal based on any suitable criteria, including but not limited to redundant data (e.g., redundant or overlapping mapped reads), non-informative data (e.g., bins with zero median counts), bins with over represented or under represented sequences, noisy data, the like, or combinations of the foregoing. A filtering process often involves removing one or more bins from consideration and subtracting the counts in the one or more bins selected for removal from the counted or summed counts for the bins, chromosome or chromosomes, or genome under consideration. In some embodiments, bins can be removed successively (e.g., one at a time to allow evaluation of the effect of removal of each individual bin), and in certain embodiments all bins marked for removal can be removed at the same time. In some embodiments, genomic sections characterized by a variance above or below a certain level are removed, which sometimes is

referred to herein as filtering "noisy" genomic sections. In certain embodiments, a filtering process comprises obtaining data points from a data set that deviate from the mean profile elevation of a genomic section, a chromosome, or segment of a chromosome by a predetermined multiple of the profile variance, and in certain embodiments, a filtering process comprises removing data points from a data set that do not deviate from the mean profile elevation of a genomic section, a chromosome or segment of a chromosome by a predetermined multiple of the profile variance. In some embodiments, a filtering process is utilized to reduce the number of candidate genomic sections analyzed for the presence or absence of a genetic variation. Reducing the number of candidate genomic sections analyzed for the presence or absence of a genetic variation (e.g., micro-deletion, micro-duplication) often reduces the complexity and/or dimensionality of a data set, and sometimes increases the speed of searching for and/or identifying genetic variations and/or genetic aberrations by two or more orders of magnitude.

In some embodiments, one or more processing steps can comprise one or more normalization steps. Normalization can be performed by a suitable method known in the art. Sometimes normalization comprises adjusting values measured on different scales to a notionally common scale. Sometimes normalization comprises a sophisticated mathematical adjustment to bring probability distributions of adjusted values into alignment. In some cases normalization comprises aligning distributions to a normal distribution. Sometimes normalization comprises mathematical adjustments that allow comparison of corresponding normalized values for different datasets in a way that eliminates the effects of certain gross influences (e.g., error and anomalies). Sometimes normalization comprises scaling. Normalization sometimes comprises division of one or more data sets by a predetermined variable or formula. Non-limiting examples of normalization methods include bin-wise normalization, normalization by GC content, linear and nonlinear least squares regression, LOESS, GC LOESS, LOWESS (locally weighted scatterplot smoothing), PERUN, repeat masking (RM), GC-normalization and repeat masking (GCRM), cQn and/or combinations thereof. In some embodiments, the determination of a presence or absence of a genetic variation (e.g., an aneuploidy) utilizes a normalization method (e.g., bin-wise normalization, normalization by GC content, linear and nonlinear least squares regression, LOESS, GC LOESS, LOWESS (locally weighted scatterplot smoothing), PERUN, repeat masking (RM), GC-normalization and repeat masking (GCRM), cQn, a normalization method known in the art and/or a combination thereof).

For example, LOESS is a regression modeling method known in the art that combines multiple regression models in a k-nearest-neighbor-based meta-model. LOESS is sometimes referred to as

a locally weighted polynomial regression. GC LOESS, in some embodiments, applies an LOESS model to the relation between fragment count (e.g., sequence reads, counts) and GC composition for genomic sections. Plotting a smooth curve through a set of data points using LOESS is sometimes called an LOESS curve, particularly when each smoothed value is given by a weighted quadratic least squares regression over the span of values of the y-axis scattergram criterion variable. For each point in a data set, the LOESS method fits a low-degree polynomial to a subset of the data, with explanatory variable values near the point whose response is being estimated. The polynomial is fitted using weighted least squares, giving more weight to points near the point whose response is being estimated and less weight to points further away. The value of the regression function for a point is then obtained by evaluating the local polynomial using the explanatory variable values for that data point. The LOESS fit is sometimes considered complete after regression function values have been computed for each of the data points. Many of the details of this method, such as the degree of the polynomial model and the weights, are flexible.

Any suitable number of normalizations can be used. In some embodiments, data sets can be normalized 1 or more, 5 or more, 10 or more or even 20 or more times. Data sets can be normalized to values (e.g., normalizing value) representative of any suitable feature or variable (e.g., sample data, reference data, or both). Non-limiting examples of types of data normalizations that can be used include normalizing raw count data for one or more selected test or reference genomic sections to the total number of counts mapped to the chromosome or the entire genome on which the selected genomic section or sections are mapped; normalizing raw count data for one or more selected genomic sections to a median reference count for one or more genomic sections or the chromosome on which a selected genomic section or segments is mapped; normalizing raw count data to previously normalized data or derivatives thereof; and normalizing previously normalized data to one or more other predetermined normalization variables. Normalizing a data set sometimes has the effect of isolating statistical error, depending on the feature or property selected as the predetermined normalization variable. Normalizing a data set sometimes also allows comparison of data characteristics of data having different scales, by bringing the data to a common scale (e.g., predetermined normalization variable). In some embodiments, one or more normalizations to a statistically derived value can be utilized to minimize data differences and diminish the importance of outlying data. Normalizing genomic sections, or bins, with respect to a normalizing value sometimes is referred to as “bin-wise normalization”.

In certain embodiments, a processing step comprising normalization includes normalizing to a static window, and in some embodiments, a processing step comprising normalization includes normalizing to a moving or sliding window. The term "window" as used herein refers to one or more genomic sections chosen for analysis, and sometimes used as a reference for comparison (e.g., used for normalization and/or other mathematical or statistical manipulation). The term "normalizing to a static window" as used herein refers to a normalization process using one or more genomic sections selected for comparison between a test subject and reference subject data set. In some embodiments the selected genomic sections are utilized to generate a profile. A static window generally includes a predetermined set of genomic sections that do not change during manipulations and/or analysis. The terms "normalizing to a moving window" and "normalizing to a sliding window" as used herein refer to normalizations performed to genomic sections localized to the genomic region (e.g., immediate genetic surrounding, adjacent genomic section or sections, and the like) of a selected test genomic section, where one or more selected test genomic sections are normalized to genomic sections immediately surrounding the selected test genomic section. In certain embodiments, the selected genomic sections are utilized to generate a profile. A sliding or moving window normalization often includes repeatedly moving or sliding to an adjacent test genomic section, and normalizing the newly selected test genomic section to genomic sections immediately surrounding or adjacent to the newly selected test genomic section, where adjacent windows have one or more genomic sections in common. In certain embodiments, a plurality of selected test genomic sections and/or chromosomes can be analyzed by a sliding window process.

In some embodiments, normalizing to a sliding or moving window can generate one or more values, where each value represents normalization to a different set of reference genomic sections selected from different regions of a genome (e.g., chromosome). In certain embodiments, the one or more values generated are cumulative sums (e.g., a numerical estimate of the integral of the normalized count profile over the selected genomic section, domain (e.g., part of chromosome), or chromosome). The values generated by the sliding or moving window process can be used to generate a profile and facilitate arriving at an outcome. In some embodiments, cumulative sums of one or more genomic sections can be displayed as a function of genomic position. Moving or sliding window analysis sometimes is used to analyze a genome for the presence or absence of micro-deletions and/or micro-insertions. In certain embodiments, displaying cumulative sums of one or more genomic sections is used to identify the presence or absence of regions of genetic variation (e.g., micro-deletions, micro-duplications). In some embodiments, moving or sliding

window analysis is used to identify genomic regions containing micro-deletions and in certain embodiments, moving or sliding window analysis is used to identify genomic regions containing micro-duplications.

A particularly useful normalization methodology for reducing error associated with nucleic acid indicators is referred to herein as Parameterized Error Removal and Unbiased Normalization (PERUN). PERUN methodology can be applied to a variety of nucleic acid indicators (e.g., nucleic acid sequence reads) for the purpose of reducing effects of error that confound predictions based on such indicators.

For example, PERUN methodology can be applied to nucleic acid sequence reads from a sample and reduce the effects of error that can impair nucleic acid elevation determinations (e.g., genomic section elevation determinations). Such an application is useful for using nucleic acid sequence reads to assess the presence or absence of a genetic variation in a subject manifested as a varying elevation of a nucleotide sequence (e.g., genomic section). Non-limiting examples of variations in genomic sections are chromosome aneuploidies (e.g., trisomy 21, trisomy 18, trisomy 13) and presence or absence of a sex chromosome (e.g., XX in females versus XY in males). A trisomy of an autosome (e.g., a chromosome other than a sex chromosome) can be referred to as an affected autosome. Other non-limiting examples of variations in genomic section elevations include microdeletions, microinsertions, duplications and mosaicism.

In certain applications, PERUN methodology can reduce experimental bias by normalizing nucleic acid indicators for particular genomic groups, the latter of which are referred to as bins. Bins include a suitable collection of nucleic acid indicators, a non-limiting example of which includes a length of contiguous nucleotides, which is referred to herein as a genomic section or portion of a reference genome. Bins can include other nucleic acid indicators as described herein. In such applications, PERUN methodology generally normalizes nucleic acid indicators at particular bins across a number of samples in three dimensions. A detailed description of particular PERUN applications is described in Example 4 and Example 5 herein.

In certain embodiments, PERUN methodology includes calculating a genomic section elevation for each bin from a fitted relation between (i) experimental bias for a bin of a reference genome to which sequence reads are mapped and (ii) counts of sequence reads mapped to the bin. Experimental bias for each of the bins can be determined across multiple samples according to a

fitted relation for each sample between (i) the counts of sequence reads mapped to each of the bins, and (ii) a mapping feature fore each of the bins. This fitted relation for each sample can be assembled for multiple samples in three dimensions. The assembly can be ordered according to the experimental bias in certain embodiments (e.g., FIG. 82, Example 4), although PERUN methodology may be practiced without ordering the assembly according to the experimental bias.

A relation can be generated by a method known in the art. A relation in two dimensions can be generated for each sample in certain embodiments, and a variable probative of error, or possibly probative of error, can be selected for one or more of the dimensions. A relation can be generated, for example, using graphing software known in the art that plots a graph using values of two or more variables provided by a user. A relation can be fitted using a method known in the art (e.g., graphing software). Certain relations can be fitted by linear regression, and the linear regression can generate a slope value and intercept value. Certain relations sometimes are not linear and can be fitted by a non-linear function, such as a parabolic, hyperbolic or exponential function, for example.

In PERUN methodology, one or more of the fitted relations may be linear. For an analysis of cell-free circulating nucleic acid from pregnant females, where the experimental bias is GC bias and the mapping feature is GC content, the fitted relation for a sample between the (i) the counts of sequence reads mapped to each bin, and (ii) GC content for each of the bins, can be linear. For the latter fitted relation, the slope pertains to GC bias, and a GC bias coefficient can be determined for each bin when the fitted relations are assembled across multiple samples. In such embodiments, the fitted relation for multiple samples and a bin between (i) GC bias coefficient for the bin, and (ii) counts of sequence reads mapped to bin, also can be linear. An intercept and slope can be obtained from the latter fitted relation. In such applications, the slope addresses sample-specific bias based on GC-content and the intercept addresses a bin-specific attenuation pattern common to all samples. PERUN methodology can significantly reduce such sample-specific bias and bin-specific attenuation when calculating genomic section elevations for providing an outcome (e.g., presence or absence of genetic variation; determination of fetal sex).

Thus, application of PERUN methodology to sequence reads across multiple samples in parallel can significantly reduce error caused by (i) sample-specific experimental bias (e.g., GC bias) and (ii) bin-specific attenuation common to samples. Other methods in which each of these two sources of error are addressed separately or serially often are not able to reduce these as

effectively as PERUN methodology. Without being limited by theory, it is expected that PERUN methodology reduces error more effectively in part because its generally additive processes do not magnify spread as much as generally multiplicative processes utilized in other normalization approaches (e.g., GC-LOESS).

Additional normalization and statistical techniques may be utilized in combination with PERUN methodology. An additional process can be applied before, after and/or during employment of PERUN methodology. Non-limiting examples of processes that can be used in combination with PERUN methodology are described hereafter.

In some embodiments, a secondary normalization or adjustment of a genomic section elevation for GC content can be utilized in conjunction with PERUN methodology. A suitable GC content adjustment or normalization procedure can be utilized (e.g., GC-LOESS, GCRM). In certain embodiments, a particular sample can be identified for application of an additional GC normalization process. For example, application of PERUN methodology can determine GC bias for each sample, and a sample associated with a GC bias above a certain threshold can be selected for an additional GC normalization process. In such embodiments, a predetermined threshold elevation can be used to select such samples for additional GC normalization.

In certain embodiments, a bin filtering or weighting process can be utilized in conjunction with PERUN methodology. A suitable bin filtering or weighting process can be utilized and non-limiting examples are described herein. Examples 4 and 5 describe utilization of R-factor measures of error for bin filtering.

GC Bias Module

Determining GC bias (e.g., determining GC bias for each of the portions of a reference genome (e.g., genomic sections)) can be provided by a GC bias module (e.g., by an apparatus comprising a GC bias module). In some embodiments, a GC bias module is required to provide a determination of GC bias. Sometimes a GC bias module provides a determination of GC bias from a fitted relationship (e.g., a fitted linear relationship) between counts of sequence reads mapped to each of the portions of a reference genome and GC content of each portion. An apparatus comprising a GC bias module can comprise at least one processor. In some embodiments, GC bias determinations (i.e., GC bias data) are provided by an apparatus that includes a processor

(e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the GC bias module. In some embodiments, GC bias data is provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a GC bias module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, GC bias data is provided by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A GC bias module can receive data and/or information from a suitable apparatus or module. Sometimes a GC bias module can receive data and/or information from a sequencing module, a normalization module, a weighting module, a mapping module or counting module. A GC bias module sometimes is part of a normalization module (e.g., PERUN normalization module). A GC bias module can receive sequencing reads from a sequencing module, mapped sequencing reads from a mapping module and/or counts from a counting module, in some embodiments. Often a GC bias module receives data and/or information from an apparatus or another module (e.g., a counting module), transforms the data and/or information and provides GC bias data and/or information (e.g., a determination of GC bias, a linear fitted relationship, and the like). GC bias data and/or information can be transferred from a GC bias module to a level module, filtering module, comparison module, a normalization module, a weighting module, a range setting module, an adjustment module, a categorization module, and/or an outcome module, in certain embodiments.

Level Module

Determining levels (e.g., elevations) and/or calculating genomic section levels (e.g., genomic section elevations) for portions of a reference genome can be provided by a level module (e.g., by an apparatus comprising a level module). In some embodiments, a level module is required to provide a level or a calculated genomic section level. Sometimes a level module provides a level from a fitted relationship (e.g., a fitted linear relationship) between a GC bias and counts of sequence reads mapped to each of the portions of a reference genome. Sometimes a level module calculates a genomic section level as part of PERUN. In some embodiments, a level module provides a genomic section level (i.e., L_i) according to equation $L_i = (m_i - G_i S)\, I^{-1}$ wherein G_i is the GC bias, m_i is measured counts mapped to each portion of a reference genome, i is a sample, and I is the intercept and S is the slope of the a fitted relationship (e.g., a fitted linear

relationship) between a GC bias and counts of sequence reads mapped to each of the portions of a reference genome. An apparatus comprising a level module can comprise at least one processor. In some embodiments, a level determination (i.e., level data) is provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the level module. In some embodiments, level data is provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a level module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, level data is provided by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A level module can receive data and/or information from a suitable apparatus or module. Sometimes a level module can receive data and/or information from a GC bias module, a sequencing module, a normalization module, a weighting module, a mapping module or counting module. A level module can receive sequencing reads from a sequencing module, mapped sequencing reads from a mapping module and/or counts from a counting module, in some embodiments. A level module sometimes is part of a normalization module (e.g., PERUN normalization module). Often a level module receives data and/or information from an apparatus or another module (e.g., a GC bias module), transforms the data and/or information and provides level data and/or information (e.g., a determination of level, a linear fitted relationship, and the like). Level data and/or information can be transferred from a level module to a comparison module, a normalization module, a weighting module, a range setting module, an adjustment module, a categorization module, a module in a normalization module and/or an outcome module, in certain embodiments.

Filtering Module

Filtering genomic sections can be provided by a filtering module (e.g., by an apparatus comprising a filtering module). In some embodiments, a filtering module is required to provide filtered genomic section data (e.g., filtered genomic sections) and/or to remove genomic sections from consideration. Sometimes a filtering module removes counts mapped to a genomic section from consideration. Sometimes a filtering module removes counts mapped to a genomic section from a determination of an elevation or a profile. A filtering module can filter data (e.g., counts, counts mapped to genomic sections, genomic sections, genomic sections elevations, normalized counts, raw counts, and the like) by one or more filtering procedures known in the art or described herein.

An apparatus comprising a filtering module can comprise at least one processor. In some embodiments, filtered data is provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the filtering module. In some embodiments, filtered data is provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a filtering module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, filtered data is provided by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A filtering module can receive data and/or information from a suitable apparatus or module. Sometimes a filtering module can receive data and/or information from a sequencing module, a normalization module, a weighting module, a mapping module or counting module. A filtering module can receive sequencing reads from a sequencing module, mapped sequencing reads from a mapping module and/or counts from a counting module, in some embodiments. Often a filtering module receives data and/or information from another apparatus or module, transforms the data and/or information and provides filtered data and/or information (e.g., filtered counts, filtered values, filtered genomic sections, and the like). Filtered data and/or information can be transferred from a filtering module to a comparison module, a normalization module, a weighting module, a range setting module, an adjustment module, a categorization module, and/or an outcome module, in certain embodiments.

Weighting Module

Weighting genomic sections can be provided by a weighting module (e.g., by an apparatus comprising a weighting module). In some embodiments, a weighting module is required to weight genomics sections and/or provide weighted genomic section values. A weighting module can weight genomic sections by one or more weighting procedures known in the art or described herein. An apparatus comprising a weighting module can comprise at least one processor. In some embodiments, weighted genomic sections are provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the weighting module. In some embodiments, weighted genomic sections are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a weighting module operates with one or more external processors (e.g., an internal

or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, weighted genomic sections are provided by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A weighting module can receive data and/or information from a suitable apparatus or module. Sometimes a weighting module can receive data and/or information from a sequencing module, a normalization module, a filtering module, a mapping module and/or a counting module. A weighting module can receive sequencing reads from a sequencing module, mapped sequencing reads from a mapping module and/or counts from a counting module, in some embodiments. In some embodiments a weighting module receives data and/or information from another apparatus or module, transforms the data and/or information and provides data and/or information (e.g., weighted genomic sections, weighted values, and the like). Weighted genomic section data and/or information can be transferred from a weighting module to a comparison module, a normalization module, a filtering module, a range setting module, an adjustment module, a categorization module, and/or an outcome module, in certain embodiments.

In some embodiments, a normalization technique that reduces error associated with insertions, duplications and/or deletions (e.g., maternal and/or fetal copy number variations), is utilized in conjunction with PERUN methodology.

Genomic section elevations calculated by PERUN methodology can be utilized directly for providing an outcome. In some embodiments, genomic section elevations can be utilized directly to provide an outcome for samples in which fetal fraction is about 2% to about 6% or greater (e.g., fetal fraction of about 4% or greater). Genomic section elevations calculated by PERUN methodology sometimes are further processed for the provision of an outcome. In some embodiments, calculated genomic section elevations are standardized. In certain embodiments, the sum, mean or median of calculated genomic section elevations for a test genomic section (e.g., chromosome 21) can be divided by the sum, mean or median of calculated genomic section elevations for genomic sections other than the test genomic section (e.g., autosomes other than chromosome 21), to generate an experimental genomic section elevation. An experimental genomic section elevation or a raw genomic section elevation can be used as part of a standardization analysis, such as calculation of a Z-score or Z-value. A Z-score can be generated for a sample by subtracting an expected genomic section elevation from an experimental genomic section elevation or raw genomic section elevation and the resulting value may be divided by a standard deviation for the samples. Resulting Z-scores can be distributed for different samples

and analyzed, or can be related to other variables, such as fetal fraction and others, and analyzed, to provide an outcome, in certain embodiments.

As noted herein, PERUN methodology is not limited to normalization according to GC bias and GC content per se, and can be used to reduce error associated with other sources of error. A non-limiting example of a source of non-GC content bias is mappability. When normalization parameters other than GC bias and content are addressed, one or more of the fitted relations may be non-linear (e.g., hyperbolic, exponential). Where experimental bias is determined from a non-linear relation, for example, an experimental bias curvature estimation may be analyzed in some embodiments.

PERUN methodology can be applied to a variety of nucleic acid indicators. Non-limiting examples of nucleic acid indicators are nucleic acid sequence reads and nucleic acid elevations at a particular location on a microarray. Non-limiting examples of sequence reads include those obtained from cell-free circulating DNA, cell-free circulating RNA, cellular DNA and cellular RNA. PERUN methodology can be applied to sequence reads mapped to suitable reference sequences, such as genomic reference DNA, cellular reference RNA (e.g., transcriptome), and portions thereof (e.g., part(s) of a genomic complement of DNA or RNA transcriptome, part(s) of a chromosome).

Thus, in certain embodiments, cellular nucleic acid (e.g., DNA or RNA) can serve as a nucleic acid indicator. Cellular nucleic acid reads mapped to reference genome portions can be normalized using PERUN methodology.

Cellular nucleic acid sometimes is an association with one or more proteins, and an agent that captures protein-associated nucleic acid can be utilized to enrich for the latter, in some embodiments. An agent in certain cases is an antibody or antibody fragment that specifically binds to a protein in association with cellular nucleic acid (e.g., an antibody that specifically binds to a chromatin protein (e.g., histone protein)). Processes in which an antibody or antibody fragment is used to enrich for cellular nucleic acid bound to a particular protein sometimes are referred to chromatin immunoprecipitation (ChIP) processes. ChIP-enriched nucleic acid is a nucleic acid in association with cellular protein, such as DNA or RNA for example. Reads of ChIP-enriched nucleic acid can be obtained using technology known in the art. Reads of ChIP-enriched nucleic acid can be mapped to one or more portions of a reference genome, and results can be normalized using PERUN methodology for providing an outcome.

Thus, provided in certain embodiments are methods for calculating with reduced bias genomic section elevations for a test sample, comprising: (a) obtaining counts of sequence reads mapped to bins of a reference genome, which sequence reads are reads of cellular nucleic acid from a test sample obtained by isolation of a protein to which the nucleic acid was associated; (b) determining experimental bias for each of the bins across multiple samples from a fitted relation between (i) the counts of the sequence reads mapped to each of the bins, and (ii) a mapping feature for each of the bins; and (c) calculating a genomic section elevation for each of the bins from a fitted relation between the experimental bias and the counts of the sequence reads mapped to each of the bins, thereby providing calculated genomic section elevations, whereby bias in the counts of the sequence reads mapped to each of the bins is reduced in the calculated genomic section elevations.

In certain embodiments, cellular RNA can serve as nucleic acid indicators. Cellular RNA reads can be mapped to reference RNA portions and normalized using PERUN methodology for providing an outcome. Known sequences for cellular RNA, referred to as a transcriptome, or a segment thereof, can be used as a reference to which RNA reads from a sample can be mapped. Reads of sample RNA can be obtained using technology known in the art. Results of RNA reads mapped to a reference can be normalized using PERUN methodology for providing an outcome.

Thus, provided in some embodiments are methods for calculating with reduced bias genomic section elevations for a test sample, comprising: (a) obtaining counts of sequence reads mapped to bins of reference RNA (e.g., reference transcriptome or segment(s) thereof), which sequence reads are reads of cellular RNA from a test sample; (b) determining experimental bias for each of the bins across multiple samples from a fitted relation between (i) the counts of the sequence reads mapped to each of the bins, and (ii) a mapping feature for each of the bins; and (c) calculating a genomic section elevation for each of the bins from a fitted relation between the experimental bias and the counts of the sequence reads mapped to each of the bins, thereby providing calculated genomic section elevations, whereby bias in the counts of the sequence reads mapped to each of the bins is reduced in the calculated genomic section elevations.

In some embodiments, microarray nucleic acid levels can serve as nucleic acid indicators. Nucleic acid levels across samples for a particular address, or hybridizing nucleic acid, on an array can be analyzed using PERUN methodology, thereby normalizing nucleic acid indicators provided by microarray analysis. In this manner, a particular address or hybridizing nucleic acid on a

microarray is analogous to a bin for mapped nucleic acid sequence reads, and PERUN methodology can be used to normalize microarray data to provide an improved outcome.

Thus, provided in certain embodiments are methods for reducing microarray nucleic acid level error for a test sample, comprising: (a) obtaining nucleic acid levels in a microarray to which test sample nucleic acid has been associated, which microarray includes an array of capture nucleic acids; (b) determining experimental bias for each of the capture nucleic acids across multiple samples from a fitted relation between (i) the test sample nucleic acid levels associated with each of the capture nucleic acids, and (ii) an association feature for each of the capture nucleic acids; and (c) calculating a test sample nucleic acid level for each of the capture nucleic acids from a fitted relation between the experimental bias and the levels of the test sample nucleic acid associated with each of the capture nucleic acids, thereby providing calculated levels, whereby bias in the levels of test sample nucleic acid associated with each of the capture nucleic acids is reduced in the calculated levels. The association feature mentioned above can be any feature correlated with hybridization of a test sample nucleic acid to a capture nucleic acid that gives rise to, or may give rise to, error in determining the level of test sample nucleic acid associated with a capture nucleic acid.

Normalization Module

Normalized data (e.g., normalized counts) can be provided by a normalization module (e.g., by an apparatus comprising a normalization module). In some embodiments, a normalization module is required to provide normalized data (e.g., normalized counts) obtained from sequencing reads. A normalization module can normalize data (e.g., counts, filtered counts, raw counts) by one or more normalization procedures known in the art. An apparatus comprising a normalization module can comprise at least one processor. In some embodiments, normalized data is provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the normalization module. In some embodiments, normalized data is provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a normalization module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, normalized data is provided by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g.,

an LED, LCT or CRT) and the like. A normalization module can receive data and/or information from a suitable apparatus or module. Sometimes a normalization module can receive data and/or information from a sequencing module, a normalization module, a mapping module or counting module. A normalization module can receive sequencing reads from a sequencing module, mapped sequencing reads from a mapping module and/or counts from a counting module, in some embodiments. Often a normalization module receives data and/or information from another apparatus or module, transforms the data and/or information and provides normalized data and/or information (e.g., normalized counts, normalized values, normalized reference values (NRVs), and the like). Normalized data and/or information can be transferred from a normalization module to a comparison module, a normalization module, a range setting module, an adjustment module, a categorization module, and/or an outcome module, in certain embodiments. Sometimes normalized counts (e.g., normalized mapped counts) are transferred to an expected representation module and/or to an experimental representation module from a normalization module.

In some embodiments, a processing step comprises a weighting. The terms “weighted”, “weighting” or “weight function” or grammatical derivatives or equivalents thereof, as used herein, refer to a mathematical manipulation of a portion or all of a data set sometimes utilized to alter the influence of certain data set features or variables with respect to other data set features or variables (e.g., increase or decrease the significance and/or contribution of data contained in one or more genomic sections or bins, based on the quality or usefulness of the data in the selected bin or bins). A weighting function can be used to increase the influence of data with a relatively small measurement variance, and/or to decrease the influence of data with a relatively large measurement variance, in some embodiments. For example, bins with under represented or low quality sequence data can be “down weighted” to minimize the influence on a data set, whereas selected bins can be “up weighted” to increase the influence on a data set. A non-limiting example of a weighting function is [1 / (standard deviation)^2]. A weighting step sometimes is performed in a manner substantially similar to a normalizing step. In some embodiments, a data set is divided by a predetermined variable (e.g., weighting variable). A predetermined variable (e.g., minimized target function, Phi) often is selected to weigh different parts of a data set differently (e.g., increase the influence of certain data types while decreasing the influence of other data types).

In certain embodiments, a processing step can comprise one or more mathematical and/or statistical manipulations. Any suitable mathematical and/or statistical manipulation, alone or in combination, may be used to analyze and/or manipulate a data set described herein. Any suitable

number of mathematical and/or statistical manipulations can be used. In some embodiments, a data set can be mathematically and/or statistically manipulated 1 or more, 5 or more, 10 or more or 20 or more times. Non-limiting examples of mathematical and statistical manipulations that can be used include addition, subtraction, multiplication, division, algebraic functions, least squares estimators, curve fitting, differential equations, rational polynomials, double polynomials, orthogonal polynomials, z-scores, p-values, chi values, phi values, analysis of peak elevations, determination of peak edge locations, calculation of peak area ratios, analysis of median chromosomal elevation, calculation of mean absolute deviation, sum of squared residuals, mean, standard deviation, standard error, the like or combinations thereof. A mathematical and/or statistical manipulation can be performed on all or a portion of sequence read data, or processed products thereof. Non-limiting examples of data set variables or features that can be statistically manipulated include raw counts, filtered counts, normalized counts, peak heights, peak widths, peak areas, peak edges, lateral tolerances, P-values, median elevations, mean elevations, count distribution within a genomic region, relative representation of nucleic acid species, the like or combinations thereof.

In some embodiments, a processing step can include the use of one or more statistical algorithms. Any suitable statistical algorithm, alone or in combination, may be used to analyze and/or manipulate a data set described herein. Any suitable number of statistical algorithms can be used. In some embodiments, a data set can be analyzed using 1 or more, 5 or more, 10 or more or 20 or more statistical algorithms. Non-limiting examples of statistical algorithms suitable for use with methods described herein include decision trees, counternulls, multiple comparisons, omnibus test, Behrens-Fisher problem, bootstrapping, Fisher's method for combining independent tests of significance, null hypothesis, type I error, type II error, exact test, one-sample Z test, two-sample Z test, one-sample t-test, paired t-test, two-sample pooled t-test having equal variances, two-sample unpooled t-test having unequal variances, one-proportion z-test, two-proportion z-test pooled, two-proportion z-test unpooled, one-sample chi-square test, two-sample F test for equality of variances, confidence interval, credible interval, significance, meta analysis, simple linear regression, robust linear regression, the like or combinations of the foregoing. Non-limiting examples of data set variables or features that can be analyzed using statistical algorithms include raw counts, filtered counts, normalized counts, peak heights, peak widths, peak edges, lateral tolerances, P-values, median elevations, mean elevations, count distribution within a genomic region, relative representation of nucleic acid species, the like or combinations thereof.

In certain embodiments, a data set can be analyzed by utilizing multiple (e.g., 2 or more) statistical algorithms (e.g., least squares regression, principle component analysis, linear discriminant analysis, quadratic discriminant analysis, bagging, neural networks, support vector machine models, random forests, classification tree models, K-nearest neighbors, logistic regression and/or loss smoothing) and/or mathematical and/or statistical manipulations (e.g., referred to herein as manipulations). The use of multiple manipulations can generate an N-dimensional space that can be used to provide an outcome, in some embodiments. In certain embodiments, analysis of a data set by utilizing multiple manipulations can reduce the complexity and/or dimensionality of the data set. For example, the use of multiple manipulations on a reference data set can generate an N-dimensional space (e.g., probability plot) that can be used to represent the presence or absence of a genetic variation, depending on the genetic status of the reference samples (e.g., positive or negative for a selected genetic variation). Analysis of test samples using a substantially similar set of manipulations can be used to generate an N-dimensional point for each of the test samples. The complexity and/or dimensionality of a test subject data set sometimes is reduced to a single value or N-dimensional point that can be readily compared to the N-dimensional space generated from the reference data. Test sample data that fall within the N-dimensional space populated by the reference subject data are indicative of a genetic status substantially similar to that of the reference subjects. Test sample data that fall outside of the N-dimensional space populated by the reference subject data are indicative of a genetic status substantially dissimilar to that of the reference subjects. In some embodiments, references are euploid or do not otherwise have a genetic variation or medical condition.

After data sets have been counted, optionally filtered and normalized, the processed data sets can be further manipulated by one or more filtering and/or normalizing procedures, in some embodiments. A data set that has been further manipulated by one or more filtering and/or normalizing procedures can be used to generate a profile, in certain embodiments. The one or more filtering and/or normalizing procedures sometimes can reduce data set complexity and/or dimensionality, in some embodiments. An outcome can be provided based on a data set of reduced complexity and/or dimensionality.

Non-limiting examples of genomic section filtering is provided herein in Example 4 with respect to PERUN methods. Genomic sections may be filtered based on, or based on part on, a measure of error. A measure of error comprising absolute values of deviation, such as an R-factor, can be used for genomic section removal or weighting in certain embodiments. An R-factor, in some

embodiments, is defined as the sum of the absolute deviations of the predicted count values from the actual measurements divided by the predicted count values from the actual measurements (e.g., Equation B herein). While a measure of error comprising absolute values of deviation may be used, a suitable measure of error may be alternatively employed. In certain embodiments, a measure of error not comprising absolute values of deviation, such as a dispersion based on squares, may be utilized. In some embodiments, genomic sections are filtered or weighted according to a measure of mappability (e.g., a mappability score; Example 5). A genomic section sometimes is filtered or weighted according to a relatively low number of sequence reads mapped to the genomic section (e.g., 0, 1, 2, 3, 4, 5 reads mapped to the genomic section). Genomic sections can be filtered or weighted according to the type of analysis being performed. For example, for chromosome 13, 18 and/or 21 aneuploidy analysis, sex chromosomes may be filtered, and only autosomes, or a subset of autosomes, may be analyzed.

In particular embodiments, the following filtering process may be employed. The same set of genomic sections (e.g., bins) within a given chromosome (e.g., chromosome 21) are selected and the number of reads in affected and unaffected samples are compared. The gap relates trisomy 21 and euploid samples and it involves a set of genomic sections covering most of chromosome 21. The set of genomic sections is the same between euploid and T21 samples. The distinction between a set of genomic sections and a single section is not crucial, as a genomic section can be defined. The same genomic region is compared in different patients. This process can be utilized for a trisomy analysis, such as for T13 or T18 in addition to, or instead of, T21.

After data sets have been counted, optionally filtered and normalized, the processed data sets can be manipulated by weighting, in some embodiments. One or more genomic sections can be selected for weighting to reduce the influence of data (e.g., noisy data, uninformative data) contained in the selected genomic sections, in certain embodiments, and in some embodiments, one or more genomic sections can be selected for weighting to enhance or augment the influence of data (e.g., data with small measured variance) contained in the selected genomic sections. In some embodiments, a data set is weighted utilizing a single weighting function that decreases the influence of data with large variances and increases the influence of data with small variances. A weighting function sometimes is used to reduce the influence of data with large variances and augment the influence of data with small variances (e.g., $[1/(\text{standard deviation})^2]$). In some embodiments, a profile plot of processed data further manipulated by weighting is generated to

facilitate classification and/or providing an outcome. An outcome can be provided based on a profile plot of weighted data

Filtering or weighting of genomic sections can be performed at one or more suitable points in an analysis. For example, genomic sections may be filtered or weighted before or after sequence reads are mapped to portions of a reference genome. Genomic sections may be filtered or weighted before or after an experimental bias for individual genome portions is determined in some embodiments. In certain embodiments, genomic sections may be filtered or weighted before or after genomic section elevations are calculated.

After data sets have been counted, optionally filtered, normalized, and optionally weighted, the processed data sets can be manipulated by one or more mathematical and/or statistical (e.g., statistical functions or statistical algorithm) manipulations, in some embodiments. In certain embodiments, processed data sets can be further manipulated by calculating Z-scores for one or more selected genomic sections, chromosomes, or portions of chromosomes. In some embodiments, processed data sets can be further manipulated by calculating P-values. Formulas for calculating Z-scores and P-values are presented in Example 1. In certain embodiments, mathematical and/or statistical manipulations include one or more assumptions pertaining to ploidy and/or fetal fraction. In some embodiments, a profile plot of processed data further manipulated by one or more statistical and/or mathematical manipulations is generated to facilitate classification and/or providing an outcome. An outcome can be provided based on a profile plot of statistically and/or mathematically manipulated data. An outcome provided based on a profile plot of statistically and/or mathematically manipulated data often includes one or more assumptions pertaining to ploidy and/or fetal fraction.

In certain embodiments, multiple manipulations are performed on processed data sets to generate an N-dimensional space and/or N-dimensional point, after data sets have been counted, optionally filtered and normalized. An outcome can be provided based on a profile plot of data sets analyzed in N-dimensions.

In some embodiments, data sets are processed utilizing one or more peak elevation analysis, peak width analysis, peak edge location analysis, peak lateral tolerances, the like, derivations thereof, or combinations of the foregoing, as part of or after data sets have processed and/or manipulated. In some embodiments, a profile plot of data processed utilizing one or more peak elevation analysis,

peak width analysis, peak edge location analysis, peak lateral tolerances, the like, derivations thereof, or combinations of the foregoing is generated to facilitate classification and/or providing an outcome. An outcome can be provided based on a profile plot of data that has been processed utilizing one or more peak elevation analysis, peak width analysis, peak edge location analysis, peak lateral tolerances, the like, derivations thereof, or combinations of the foregoing.

In some embodiments, the use of one or more reference samples known to be free of a genetic variation in question can be used to generate a reference median count profile, which may result in a predetermined value representative of the absence of the genetic variation, and often deviates from a predetermined value in areas corresponding to the genomic location in which the genetic variation is located in the test subject, if the test subject possessed the genetic variation. In test subjects at risk for, or suffering from a medical condition associated with a genetic variation, the numerical value for the selected genomic section or sections is expected to vary significantly from the predetermined value for non-affected genomic locations. In certain embodiments, the use of one or more reference samples known to carry the genetic variation in question can be used to generate a reference median count profile, which may result in a predetermined value representative of the presence of the genetic variation, and often deviates from a predetermined value in areas corresponding to the genomic location in which a test subject does not carry the genetic variation. In test subjects not at risk for, or suffering from a medical condition associated with a genetic variation, the numerical value for the selected genomic section or sections is expected to vary significantly from the predetermined value for affected genomic locations.

In some embodiments, analysis and processing of data can include the use of one or more assumptions. A suitable number or type of assumptions can be utilized to analyze or process a data set. Non-limiting examples of assumptions that can be used for data processing and/or analysis include maternal ploidy, fetal contribution, prevalence of certain sequences in a reference population, ethnic background, prevalence of a selected medical condition in related family members, parallelism between raw count profiles from different patients and/or runs after GC-normalization and repeat masking (e.g., GCRM), identical matches represent PCR artifacts (e.g., identical base position), assumptions inherent in a fetal quantifier assay (e.g., FQA), assumptions regarding twins (e.g., if 2 twins and only 1 is affected the effective fetal fraction is only 50% of the total measured fetal fraction (similarly for triplets, quadruplets and the like)), fetal cell free DNA (e.g., cfDNA) uniformly covers the entire genome, the like and combinations thereof.

In those instances where the quality and/or depth of mapped sequence reads does not permit an outcome prediction of the presence or absence of a genetic variation at a desired confidence level (e.g., 95% or higher confidence level), based on the normalized count profiles, one or more additional mathematical manipulation algorithms and/or statistical prediction algorithms, can be utilized to generate additional numerical values useful for data analysis and/or providing an outcome. The term "normalized count profile" as used herein refers to a profile generated using normalized counts. Examples of methods that can be used to generate normalized counts and normalized count profiles are described herein. As noted, mapped sequence reads that have been counted can be normalized with respect to test sample counts or reference sample counts. In some embodiments, a normalized count profile can be presented as a plot.

Profiles

In some embodiments, a processing step can comprise generating one or more profiles (e.g., profile plot) from various aspects of a data set or derivation thereof (e.g., product of one or more mathematical and/or statistical data processing steps known in the art and/or described herein). The term "profile" as used herein refers to a product of a mathematical and/or statistical manipulation of data that can facilitate identification of patterns and/or correlations in large quantities of data. A "profile" often includes values resulting from one or more manipulations of data or data sets, based on one or more criteria. A profile often includes multiple data points. Any suitable number of data points may be included in a profile depending on the nature and/or complexity of a data set. In certain embodiments, profiles may include 2 or more data points, 3 or more data points, 5 or more data points, 10 or more data points, 24 or more data points, 25 or more data points, 50 or more data points, 100 or more data points, 500 or more data points, 1000 or more data points, 5000 or more data points, 10,000 or more data points, or 100,000 or more data points.

In some embodiments, a profile is representative of the entirety of a data set, and in certain embodiments, a profile is representative of a portion or subset of a data set. That is, a profile sometimes includes or is generated from data points representative of data that has not been filtered to remove any data, and sometimes a profile includes or is generated from data points representative of data that has been filtered to remove unwanted data. In some embodiments, a data point in a profile represents the results of data manipulation for a genomic section. In certain embodiments, a data point in a profile includes results of data manipulation for groups of genomic

sections. In some embodiments, groups of genomic sections may be adjacent to one another, and in certain embodiments, groups of genomic sections may be from different parts of a chromosome or genome.

Data points in a profile derived from a data set can be representative of any suitable data categorization. Non-limiting examples of categories into which data can be grouped to generate profile data points include: genomic sections based on size, genomic sections based on sequence features (e.g., GC content, AT content, position on a chromosome (e.g., short arm, long arm, centromere, telomere), and the like), levels of expression, chromosome, the like or combinations thereof. In some embodiments, a profile may be generated from data points obtained from another profile (e.g., normalized data profile renormalized to a different normalizing value to generate a renormalized data profile). In certain embodiments, a profile generated from data points obtained from another profile reduces the number of data points and/or complexity of the data set. Reducing the number of data points and/or complexity of a data set often facilitates interpretation of data and/or facilitates providing an outcome.

A profile often is a collection of normalized or non-normalized counts for two or more genomic sections. A profile often includes at least one elevation, and often comprises two or more elevations (e.g., a profile often has multiple elevations). An elevation generally is for a set of genomic sections having about the same counts or normalized counts. Elevations are described in greater detail herein. In some cases, a profile comprises one or more genomic sections, which genomic sections can be weighted, removed, filtered, normalized, adjusted, averaged, derived as a mean, added, subtracted, processed or transformed by any combination thereof. A profile often comprises normalized counts mapped to genomic sections defining two or more elevations, where the counts are further normalized according to one of the elevations by a suitable method. Often counts of a profile (e.g., a profile elevation) are associated with an uncertainty value.

A profile comprising one or more elevations can include a first elevation and a second elevation. Sometimes a first elevation is different (e.g., significantly different) than a second elevation. In some embodiments a first elevation comprises a first set of genomic sections, a second elevation comprises a second set of genomic sections and the first set of genomic sections is not a subset of the second set of genomic sections. In some cases, a first set of genomic sections is different than a second set of genomic sections from which a first and second elevation are determined. Sometimes a profile can have multiple first elevations that are different (e.g., significantly different,

e.g., have a significantly different value) than a second elevation within the profile. Sometimes a profile comprises one or more first elevations that are significantly different than a second elevation within the profile and one or more of the first elevations are adjusted. Sometimes a profile comprises one or more first elevations that are significantly different than a second elevation within the profile, each of the one or more first elevations comprise a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation and one or more of the first elevations are adjusted. Sometimes a first elevation within a profile is removed from the profile or adjusted (e.g., padded). A profile can comprise multiple elevations that include one or more first elevations significantly different than one or more second elevations and often the majority of elevations in a profile are second elevations, which second elevations are about equal to one another. Sometimes greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90% or greater than 95% of the elevations in a profile are second elevations.

A profile sometimes is displayed as a plot. For example, one or more elevations representing counts (e.g., normalized counts) of genomic sections can be plotted and visualized. Non-limiting examples of profile plots that can be generated include raw count (e.g., raw count profile or raw profile), normalized count, bin-weighted, z-score, p-value, area ratio versus fitted ploidy, median elevation versus ratio between fitted and measured fetal fraction, principle components, the like, or combinations thereof. Profile plots allow visualization of the manipulated data, in some embodiments. In certain embodiments, a profile plot can be utilized to provide an outcome (e.g., area ratio versus fitted ploidy, median elevation versus ratio between fitted and measured fetal fraction, principle components). The terms “raw count profile plot” or “raw profile plot” as used herein refer to a plot of counts in each genomic section in a region normalized to total counts in a region (e.g., genome, genomic section, chromosome, chromosome bins or a segment of a chromosome). In some embodiments, a profile can be generated using a static window process, and in certain embodiments, a profile can be generated using a sliding window process.

A profile generated for a test subject sometimes is compared to a profile generated for one or more reference subjects, to facilitate interpretation of mathematical and/or statistical manipulations of a data set and/or to provide an outcome. In some embodiments, a profile is generated based on one or more starting assumptions (e.g., maternal contribution of nucleic acid (e.g., maternal fraction), fetal contribution of nucleic acid (e.g., fetal fraction), ploidy of reference sample, the like or combinations thereof). In certain embodiments, a test profile often centers around a

predetermined value representative of the absence of a genetic variation, and often deviates from a predetermined value in areas corresponding to the genomic location in which the genetic variation is located in the test subject, if the test subject possessed the genetic variation. In test subjects at risk for, or suffering from a medical condition associated with a genetic variation, the numerical value for a selected genomic section is expected to vary significantly from the predetermined value for non-affected genomic locations. Depending on starting assumptions (e.g., fixed ploidy or optimized ploidy, fixed fetal fraction or optimized fetal fraction or combinations thereof) the predetermined threshold or cutoff value or threshold range of values indicative of the presence or absence of a genetic variation can vary while still providing an outcome useful for determining the presence or absence of a genetic variation. In some embodiments, a profile is indicative of and/or representative of a phenotype.

By way of a non-limiting example, normalized sample and/or reference count profiles can be obtained from raw sequence read data by (a) calculating reference median counts for selected chromosomes, genomic sections or segments thereof from a set of references known not to carry a genetic variation, (b) removal of uninformative genomic sections from the reference sample raw counts (e.g., filtering); (c) normalizing the reference counts for all remaining bins to the total residual number of counts (e.g., sum of remaining counts after removal of uninformative bins) for the reference sample selected chromosome or selected genomic location, thereby generating a normalized reference subject profile; (d) removing the corresponding genomic sections from the test subject sample; and (e) normalizing the remaining test subject counts for one or more selected genomic locations to the sum of the residual reference median counts for the chromosome or chromosomes containing the selected genomic locations, thereby generating a normalized test subject profile. In certain embodiments, an additional normalizing step with respect to the entire genome, reduced by the filtered genomic sections in (b), can be included between (c) and (d). A data set profile can be generated by one or more manipulations of counted mapped sequence read data. Some embodiments include the following. Sequence reads are mapped and the number of sequence tags mapping to each genomic bin are determined (e.g., counted). A raw count profile is generated from the mapped sequence reads that are counted. An outcome is provided by comparing a raw count profile from a test subject to a reference median count profile for chromosomes, genomic sections or segments thereof from a set of reference subjects known not to possess a genetic variation, in certain embodiments.

In some embodiments, sequence read data is optionally filtered to remove noisy data or uninformative genomic sections. After filtering, the remaining counts typically are summed to generate a filtered data set. A filtered count profile is generated from a filtered data set, in certain embodiments.

After sequence read data have been counted and optionally filtered, data sets can be normalized to generate elevations or profiles. A data set can be normalized by normalizing one or more selected genomic sections to a suitable normalizing reference value. In some embodiments, a normalizing reference value is representative of the total counts for the chromosome or chromosomes from which genomic sections are selected. In certain embodiments, a normalizing reference value is representative of one or more corresponding genomic sections, portions of chromosomes or chromosomes from a reference data set prepared from a set of reference subjects known not to possess a genetic variation. In some embodiments, a normalizing reference value is representative of one or more corresponding genomic sections, portions of chromosomes or chromosomes from a test subject data set prepared from a test subject being analyzed for the presence or absence of a genetic variation. In certain embodiments, the normalizing process is performed utilizing a static window approach, and in some embodiments the normalizing process is performed utilizing a moving or sliding window approach. In certain embodiments, a profile comprising normalized counts is generated to facilitate classification and/or providing an outcome. An outcome can be provided based on a plot of a profile comprising normalized counts (e.g., using a plot of such a profile).

Elevations

In some embodiments, a value is ascribed to an elevation (e.g., a number). An elevation can be determined by a suitable method, operation or mathematical process (e.g., a processed elevation). The term “level” as used herein is synonymous with the term “elevation” as used herein. An elevation often is, or is derived from, counts (e.g., normalized counts) for a set of genomic sections. Sometimes an elevation of a genomic section is substantially equal to the total number of counts mapped to a genomic section (e.g., normalized counts). Often an elevation is determined from counts that are processed, transformed or manipulated by a suitable method, operation or mathematical process known in the art. Sometimes an elevation is derived from counts that are processed and non-limiting examples of processed counts include weighted, removed, filtered, normalized, adjusted, averaged, derived as a mean (e.g., mean elevation), added, subtracted,

transformed counts or combination thereof. Sometimes an elevation comprises counts that are normalized (e.g., normalized counts of genomic sections). An elevation can be for counts normalized by a suitable process, non-limiting examples of which include bin-wise normalization, normalization by GC content, linear and nonlinear least squares regression, GC LOESS, LOWESS, PERUN, RM, GCRM, cQn, the like and/or combinations thereof. An elevation can comprise normalized counts or relative amounts of counts. Sometimes an elevation is for counts or normalized counts of two or more genomic sections that are averaged and the elevation is referred to as an average elevation. Sometimes an elevation is for a set of genomic sections having a mean count or mean of normalized counts which is referred to as a mean elevation. Sometimes an elevation is derived for genomic sections that comprise raw and/or filtered counts. In some embodiments, an elevation is based on counts that are raw. Sometimes an elevation is associated with an uncertainty value. An elevation for a genomic section, or a "genomic section elevation," is synonymous with a "genomic section level" herein.

Normalized or non-normalized counts for two or more elevations (e.g., two or more elevations in a profile) can sometimes be mathematically manipulated (e.g., added, multiplied, averaged, normalized, the like or combination thereof) according to elevations. For example, normalized or non-normalized counts for two or more elevations can be normalized according to one, some or all of the elevations in a profile. Sometimes normalized or non-normalized counts of all elevations in a profile are normalized according to one elevation in the profile. Sometimes normalized or non-normalized counts of a fist elevation in a profile are normalized according to normalized or non-normalized counts of a second elevation in the profile.

Non-limiting examples of an elevation (e.g., a first elevation, a second elevation) are an elevation for a set of genomic sections comprising processed counts, an elevation for a set of genomic sections comprising a mean, median or average of counts, an elevation for a set of genomic sections comprising normalized counts, the like or any combination thereof. In some embodiments, a first elevation and a second elevation in a profile are derived from counts of genomic sections mapped to the same chromosome. In some embodiments, a first elevation and a second elevation in a profile are derived from counts of genomic sections mapped to different chromosomes.

In some embodiments an elevation is determined from normalized or non-normalized counts mapped to one or more genomic sections. In some embodiments, an elevation is determined from

normalized or non-normalized counts mapped to two or more genomic sections, where the normalized counts for each genomic section often are about the same. There can be variation in counts (e.g., normalized counts) in a set of genomic sections for an elevation. In a set of genomic sections for an elevation there can be one or more genomic sections having counts that are significantly different than in other genomic sections of the set (e.g., peaks and/or dips). Any suitable number of normalized or non-normalized counts associated with any suitable number of genomic sections can define an elevation.

Sometimes one or more elevations can be determined from normalized or non-normalized counts of all or some of the genomic sections of a genome. Often an elevation can be determined from all or some of the normalized or non-normalized counts of a chromosome, or segment thereof. Sometimes, two or more counts derived from two or more genomic sections (e.g., a set of genomic sections) determine an elevation. Sometimes two or more counts (e.g., counts from two or more genomic sections) determine an elevation. In some embodiments, counts from 2 to about 100,000 genomic sections determine an elevation. In some embodiments, counts from 2 to about 50,000, 2 to about 40,000, 2 to about 30,000, 2 to about 20,000, 2 to about 10,000, 2 to about 5000, 2 to about 2500, 2 to about 1250, 2 to about 1000, 2 to about 500, 2 to about 250, 2 to about 100 or 2 to about 60 genomic sections determine an elevation. In some embodiments counts from about 10 to about 50 genomic sections determine an elevation. In some embodiments counts from about 20 to about 40 or more genomic sections determine an elevation. In some embodiments, an elevation comprises counts from about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60 or more genomic sections. In some embodiments, an elevation corresponds to a set of genomic sections (e.g., a set of genomic sections of a reference genome, a set of genomic sections of a chromosome or a set of genomic sections of a segment of a chromosome).

In some embodiments, an elevation is determined for normalized or non-normalized counts of genomic sections that are contiguous. Sometimes genomic sections (e.g., a set of genomic sections) that are contiguous represent neighboring segments of a genome or neighboring segments of a chromosome or gene. For example, two or more contiguous genomic sections, when aligned by merging the genomic sections end to end, can represent a sequence assembly of a DNA sequence longer than each genomic section. For example two or more contiguous genomic sections can represent of an intact genome, chromosome, gene, intron, exon or segment

thereof. Sometimes an elevation is determined from a collection (e.g., a set) of contiguous genomic sections and/or non-contiguous genomic sections.

Significantly Different Elevations

In some embodiments, a profile of normalized counts comprises an elevation (e.g., a first elevation) significantly different than another elevation (e.g., a second elevation) within the profile. A first elevation may be higher or lower than a second elevation. In some embodiments, a first elevation is for a set of genomic sections comprising one or more reads comprising a copy number variation (e.g., a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation) and the second elevation is for a set of genomic sections comprising reads having substantially no copy number variation. In some embodiments, significantly different refers to an observable difference. Sometimes significantly different refers to statistically different or a statistically significant difference. A statistically significant difference is sometimes a statistical assessment of an observed difference. A statistically significant difference can be assessed by a suitable method in the art. Any suitable threshold or range can be used to determine that two elevations are significantly different. In some cases two elevations (e.g., mean elevations) that differ by about 0.01 percent or more (e.g., 0.01 percent of one or either of the elevation values) are significantly different. Sometimes two elevations (e.g., mean elevations) that differ by about 0.1 percent or more are significantly different. In some cases, two elevations (e.g., mean elevations) that differ by about 0.5 percent or more are significantly different. Sometimes two elevations (e.g., mean elevations) that differ by about 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or more than about 10% are significantly different. Sometimes two elevations (e.g., mean elevations) are significantly different and there is no overlap in either elevation and/or no overlap in a range defined by an uncertainty value calculated for one or both elevations. In some cases the uncertainty value is a standard deviation expressed as sigma. Sometimes two elevations (e.g., mean elevations) are significantly different and they differ by about 1 or more times the uncertainty value (e.g., 1 sigma). Sometimes two elevations (e.g., mean elevations) are significantly different and they differ by about 2 or more times the uncertainty value (e.g., 2 sigma), about 3 or more, about 4 or more, about 5 or more, about 6 or more, about 7 or more, about 8 or more, about 9 or more, or about 10 or more times the uncertainty value. Sometimes two elevations (e.g., mean elevations) are significantly different when they differ by about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, or 4.0 times the uncertainty value or more. In some

embodiments, the confidence level increases as the difference between two elevations increases. In some cases, the confidence level decreases as the difference between two elevations decreases and/or as the uncertainty value increases. For example, sometimes the confidence level increases with the ratio of the difference between elevations and the standard deviation (e.g., MADs).

In some embodiments, a first set of genomic sections often includes genomic sections that are different than (e.g., non-overlapping with) a second set of genomic sections. For example, sometimes a first elevation of normalized counts is significantly different than a second elevation of normalized counts in a profile, and the first elevation is for a first set of genomic sections, the second elevation is for a second set of genomic sections and the genomic sections do not overlap in the first set and second set of genomic sections. In some cases, a first set of genomic sections is not a subset of a second set of genomic sections from which a first elevation and second elevation are determined, respectively. Sometimes a first set of genomic sections is different and/or distinct from a second set of genomic sections from which a first elevation and second elevation are determined, respectively.

Sometimes a first set of genomic sections is a subset of a second set of genomic sections in a profile. For example, sometimes a second elevation of normalized counts for a second set of genomic sections in a profile comprises normalized counts of a first set of genomic sections for a first elevation in the profile and the first set of genomic sections is a subset of the second set of genomic sections in the profile. Sometimes an average, mean or median elevation is derived from a second elevation where the second elevation comprises a first elevation. Sometimes, a second elevation comprises a second set of genomic sections representing an entire chromosome and a first elevation comprises a first set of genomic sections where the first set is a subset of the second set of genomic sections and the first elevation represents a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation that is present in the chromosome.

In some embodiments, a value of a second elevation is closer to the mean, average or median value of a count profile for a chromosome, or segment thereof, than the first elevation. In some embodiments, a second elevation is a mean elevation of a chromosome, a portion of a chromosome or a segment thereof. In some embodiments, a first elevation is significantly different from a predominant elevation (e.g., a second elevation) representing a chromosome, or segment

thereof. A profile may include multiple first elevations that significantly differ from a second elevation, and each first elevation independently can be higher or lower than the second elevation. In some embodiments, a first elevation and a second elevation are derived from the same chromosome and the first elevation is higher or lower than the second elevation, and the second elevation is the predominant elevation of the chromosome. Sometimes, a first elevation and a second elevation are derived from the same chromosome, a first elevation is indicative of a copy number variation (e.g., a maternal and/or fetal copy number variation, deletion, insertion, duplication) and a second elevation is a mean elevation or predominant elevation of genomic sections for a chromosome, or segment thereof.

In some cases, a read in a second set of genomic sections for a second elevation substantially does not include a genetic variation (e.g., a copy number variation, a maternal and/or fetal copy number variation). Often, a second set of genomic sections for a second elevation includes some variability (e.g., variability in elevation, variability in counts for genomic sections). Sometimes, one or more genomic sections in a set of genomic sections for an elevation associated with substantially no copy number variation include one or more reads having a copy number variation present in a maternal and/or fetal genome. For example, sometimes a set of genomic sections include a copy number variation that is present in a small segment of a chromosome (e.g., less than 10 genomic sections) and the set of genomic sections is for an elevation associated with substantially no copy number variation. Thus a set of genomic sections that include substantially no copy number variation still can include a copy number variation that is present in less than about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 genomic sections of an elevation.

Sometimes a first elevation is for a first set of genomic sections and a second elevation is for a second set of genomic sections and the first set of genomic sections and second set of genomic sections are contiguous (e.g., adjacent with respect to the nucleic acid sequence of a chromosome or segment thereof). Sometimes the first set of genomic sections and second set of genomic sections are not contiguous.

Relatively short sequence reads from a mixture of fetal and maternal nucleic acid can be utilized to provide counts which can be transformed into an elevation and/or a profile. Counts, elevations and profiles can be depicted in electronic or tangible form and can be visualized. Counts mapped to genomic sections (e.g., represented as elevations and/or profiles) can provide a visual

representation of a fetal and/or a maternal genome, chromosome, or a portion or a segment of a chromosome that is present in a fetus and/or pregnant female.

Comparison Module

A first elevation can be identified as significantly different from a second elevation by a comparison module or by an apparatus comprising a comparison module. In some embodiments, a comparison module or an apparatus comprising a comparison module is required to provide a comparison between two elevations. An apparatus comprising a comparison module can comprise at least one processor. In some embodiments, elevations are determined to be significantly different by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the comparison module. In some embodiments, elevations are determined to be significantly different by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a comparison module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, elevations are determined to be significantly different by an apparatus comprising one or more of the following: one or more flow cells, a camera, fluid handling components, a printer, a display (e.g., an LED, LCT or CRT) and the like. A comparison module can receive data and/or information from a suitable module. A comparison module can receive data and/or information from a sequencing module, a mapping module, a counting module, or a normalization module. A comparison module can receive normalized data and/or information from a normalization module. Data and/or information derived from, or transformed by, a comparison module can be transferred from a comparison module to a range setting module, a plotting module, an adjustment module, a categorization module or an outcome module. A comparison between two or more elevations and/or an identification of an elevation as significantly different from another elevation can be transferred from (e.g., provided to) a comparison module to a categorization module, range setting module or adjustment module.

Reference Elevation and Normalized Reference Value

Sometimes a profile comprises a reference elevation (e.g., an elevation used as a reference). Often a profile of normalized counts provides a reference elevation from which expected elevations and expected ranges are determined (see discussion below on expected elevations and ranges).

A reference elevation often is for normalized counts of genomic sections comprising mapped reads from both a mother and a fetus. A reference elevation is often the sum of normalized counts of mapped reads from a fetus and a mother (e.g., a pregnant female). Sometimes a reference elevation is for genomic sections comprising mapped reads from a euploid mother and/or a euploid fetus. Sometimes a reference elevation is for genomic sections comprising mapped reads having a fetal genetic variation (e.g., an aneuploidy (e.g., a trisomy)), and/or reads having a maternal genetic variation (e.g., a copy number variation, insertion, deletion). Sometimes a reference elevation is for genomic sections that include substantially no maternal and/or fetal copy number variations. Sometimes a second elevation is used as a reference elevation. In some cases a profile comprises a first elevation of normalized counts and a second elevation of normalized counts, the first elevation is significantly different from the second elevation and the second elevation is the reference elevation. In some cases a profile comprises a first elevation of normalized counts for a first set of genomic sections, a second elevation of normalized counts for a second set of genomic sections, the first set of genomic sections includes mapped reads having a maternal and/or fetal copy number variation, the second set of genomic sections comprises mapped reads having substantially no maternal copy number variation and/or fetal copy number variation, and the second elevation is a reference elevation.

In some embodiments counts mapped to genomic sections for one or more elevations of a profile are normalized according to counts of a reference elevation. In some embodiments, normalizing counts of an elevation according to counts of a reference elevation comprise dividing counts of an elevation by counts of a reference elevation or a multiple or fraction thereof. Counts normalized according to counts of a reference elevation often have been normalized according to another process (e.g., PERUN) and counts of a reference elevation also often have been normalized (e.g., by PERUN). Sometimes the counts of an elevation are normalized according to counts of a reference elevation and the counts of the reference elevation are scalable to a suitable value either prior to or after normalizing. The process of scaling the counts of a reference elevation can comprise any suitable constant (i.e., number) and any suitable mathematical manipulation may be applied to the counts of a reference elevation.

A normalized reference value (NRV) is often determined according to the normalized counts of a reference elevation. Determining an NRV can comprise any suitable normalization process (e.g., mathematical manipulation) applied to the counts of a reference elevation where the same normalization process is used to normalize the counts of other elevations within the same profile.

Determining an NRV often comprises dividing a reference elevation by itself. Determining an NRV often comprises dividing a reference elevation by a multiple of itself. Determining an NRV often comprises dividing a reference elevation by the sum or difference of the reference elevation and a constant (e.g., any number).

An NRV is sometimes referred to as a null value. An NRV can be any suitable value. In some embodiments, an NRV is any value other than zero. Sometimes an NRV is a whole number. Sometimes an NRV is a positive integer. In some embodiments, an NRV is 1, 10, 100 or 1000. Often, an NRV is equal to 1. Sometimes an NRV is equal to zero. The counts of a reference elevation can be normalized to any suitable NRV. In some embodiments, the counts of a reference elevation are normalized to an NRV of zero. Often the counts of a reference elevation are normalized to an NRV of 1.

Expected Elevations

An expected elevation is sometimes a pre-defined elevation (e.g., a theoretical elevation, predicted elevation). An "expected elevation" is sometimes referred to herein as a "predetermined elevation value". In some embodiments, an expected elevation is a predicted value for an elevation of normalized counts for a set of genomic sections that include a copy number variation. In some cases, an expected elevation is determined for a set of genomic sections that include substantially no copy number variation. An expected elevation can be determined for a chromosome ploidy (e.g., 0, 1, 2 (i.e., diploid), 3 or 4 chromosomes) or a microploidy (homozygous or heterozygous deletion, duplication, insertion or absence thereof). Often an expected elevation is determined for a maternal microploidy (e.g., a maternal and/or fetal copy number variation).

An expected elevation for a genetic variation or a copy number variation can be determined by any suitable manner. Often an expected elevation is determined by a suitable mathematical manipulation of an elevation (e.g., counts mapped to a set of genomic sections for an elevation). Sometimes an expected elevation is determined by utilizing a constant sometimes referred to as an expected elevation constant. An expected elevation for a copy number variation is sometimes calculated by multiplying a reference elevation, normalized counts of a reference elevation or an NRV by an expected elevation constant, adding an expected elevation constant, subtracting an expected elevation constant, dividing by an expected elevation constant, or by a combination thereof. Often an expected elevation (e.g., an expected elevation of a maternal and/or fetal copy

number variation) determined for the same subject, sample or test group is determined according to the same reference elevation or NRV.

Often an expected elevation is determined by multiplying a reference elevation, normalized counts of a reference elevation or an NRV by an expected elevation constant where the reference elevation, normalized counts of a reference elevation or NRV is not equal to zero. Sometimes an expected elevation is determined by adding an expected elevation constant to reference elevation, normalized counts of a reference elevation or an NRV that is equal to zero. In some embodiments, an expected elevation, normalized counts of a reference elevation, NRV and expected elevation constant are scalable. The process of scaling can comprise any suitable constant (i.e., number) and any suitable mathematical manipulation where the same scaling process is applied to all values under consideration.

Expected Elevation Constant

An expected elevation constant can be determined by a suitable method. Sometimes an expected elevation constant is arbitrarily determined. Often an expected elevation constant is determined empirically. Sometimes an expected elevation constant is determined according to a mathematical manipulation. Sometimes an expected elevation constant is determined according to a reference (e.g., a reference genome, a reference sample, reference test data). In some embodiments, an expected elevation constant is predetermined for an elevation representative of the presence or absence of a genetic variation or copy number variation (e.g., a duplication, insertion or deletion). In some embodiments, an expected elevation constant is predetermined for an elevation representative of the presence or absence of a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation. An expected elevation constant for a copy number variation can be any suitable constant or set of constants.

In some embodiments, the expected elevation constant for a homozygous duplication (e.g., a homozygous duplication) can be from about 1.6 to about 2.4, from about 1.7 to about 2.3, from about 1.8 to about 2.2, or from about 1.9 to about 2.1. Sometimes the expected elevation constant for a homozygous duplication is about 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3 or about 2.4. Often the expected elevation constant for a homozygous duplication is about 1.90, 1.92, 1.94, 1.96, 1.98, 2.0, 2.02, 2.04, 2.06, 2.08 or about 2.10. Often the expected elevation constant for a homozygous duplication is about 2.

In some embodiments, the expected elevation constant for a heterozygous duplication (e.g., a homozygous duplication) is from about 1.2 to about 1.8, from about 1.3 to about 1.7, or from about 1.4 to about 1.6. Sometimes the expected elevation constant for a heterozygous duplication is about 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 or about 1.8. Often the expected elevation constant for a heterozygous duplication is about 1.40, 1.42, 1.44, 1.46, 1.48, 1.5, 1.52, 1.54, 1.56, 1.58 or about 1.60. In some embodiments, the expected elevation constant for a heterozygous duplication is about 1.5.

In some embodiments, the expected elevation constant for the absence of a copy number variation (e.g., the absence of a maternal copy number variation and/or fetal copy number variation) is from about 1.3 to about 0.7, from about 1.2 to about 0.8, or from about 1.1 to about 0.9. Sometimes the expected elevation constant for the absence of a copy number variation is about 1.3, 1.2, 1.1, 1.0, 0.9, 0.8 or about 0.7. Often the expected elevation constant for the absence of a copy number variation is about 1.09, 1.08, 1.06, 1.04, 1.02, 1.0, 0.98, 0.96, 0.94, or about 0.92. In some embodiments, the expected elevation constant for the absence of a copy number variation is about 1.

In some embodiments, the expected elevation constant for a heterozygous deletion (e.g., a maternal, fetal, or a maternal and a fetal heterozygous deletion) is from about 0.2 to about 0.8, from about 0.3 to about 0.7, or from about 0.4 to about 0.6. Sometimes the expected elevation constant for a heterozygous deletion is about 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 or about 0.8. Often the expected elevation constant for a heterozygous deletion is about 0.40, 0.42, 0.44, 0.46, 0.48, 0.5, 0.52, 0.54, 0.56, 0.58 or about 0.60. In some embodiments, the expected elevation constant for a heterozygous deletion is about 0.5.

In some embodiments, the expected elevation constant for a homozygous deletion (e.g., a homozygous deletion) can be from about -0.4 to about 0.4, from about -0.3 to about 0.3, from about -0.2 to about 0.2, or from about -0.1 to about 0.1. Sometimes the expected elevation constant for a homozygous deletion is about -0.4, -0.3, -0.2, -0.1, 0.0, 0.1, 0.2, 0.3 or about 0.4. Often the expected elevation constant for a homozygous deletion is about -0.1, -0.08, -0.06, -0.04, -0.02, 0.0, 0.02, 0.04, 0.06, 0.08 or about 0.10. Often the expected elevation constant for a homozygous deletion is about 0.

Expected Elevation Range

Sometimes the presence or absence of a genetic variation or copy number variation (e.g., a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation) is determined by an elevation that falls within or outside of an expected elevation range. An expected elevation range is often determined according to an expected elevation. Sometimes an expected elevation range is determined for an elevation comprising substantially no genetic variation or substantially no copy number variation. A suitable method can be used to determine an expected elevation range.

Sometimes, an expected elevation range is defined according to a suitable uncertainty value calculated for an elevation. Non-limiting examples of an uncertainty value are a standard deviation, standard error, calculated variance, p-value, and mean absolute deviation (MAD). Sometimes, an expected elevation range for a genetic variation or a copy number variation is determined, in part, by calculating the uncertainty value for an elevation (e.g., a first elevation, a second elevation, a first elevation and a second elevation). Sometimes an expected elevation range is defined according to an uncertainty value calculated for a profile (e.g., a profile of normalized counts for a chromosome or segment thereof). In some embodiments, an uncertainty value is calculated for an elevation comprising substantially no genetic variation or substantially no copy number variation. In some embodiments, an uncertainty value is calculated for a first elevation, a second elevation or a first elevation and a second elevation. In some embodiments an uncertainty value is determined for a first elevation, a second elevation or a second elevation comprising a first elevation.

An expected elevation range is sometimes calculated, in part, by multiplying, adding, subtracting, or dividing an uncertainty value by a constant (e.g., a predetermined constant) *n*. A suitable mathematical procedure or combination of procedures can be used. The constant *n* (e.g., predetermined constant *n*) is sometimes referred to as a confidence interval. A selected confidence interval is determined according to the constant *n* that is selected. The constant *n* (e.g., the predetermined constant *n*, the confidence interval) can be determined by a suitable manner. The constant *n* can be a number or fraction of a number greater than zero. The constant *n* can be a whole number. Often the constant *n* is a number less than 10. Sometimes the constant *n* is a number less than about 10, less than about 9, less than about 8, less than about 7, less than about 6, less than about 5, less than about 4, less than about 3, or less than about 2. Sometimes

the constant *n* is about 10, 9.5, 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2 or 1. The constant *n* can be determined empirically from data derived from subjects (a pregnant female and/or a fetus) with a known genetic disposition.

Often an uncertainty value and constant *n* defines a range (e.g.,, an uncertainty cutoff). For example, sometimes an uncertainty value is a standard deviation (e.g., +/- 5) and is multiplied by a constant *n* (e.g., a confidence interval) thereby defining a range or uncertainty cutoff (e.g., 5*n* to -5*n*).

In some embodiments, an expected elevation range for a genetic variation (e.g., a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and fetal copy number variation) is the sum of an expected elevation plus a constant *n* times the uncertainty (e.g., *n* x sigma (e.g., 6 sigma)). Sometimes the expected elevation range for a genetic variation or copy number variation designated by *k* can be defined by the formula:

Formula R: $(\text{Expected Elevation Range})_k = (\text{Expected Elevation})_k + n\sigma$

where σ is an uncertainty value, *n* is a constant (e.g., a predetermined constant) and the expected elevation range and expected elevation are for the genetic variation *k* (e.g., *k* = a heterozygous deletion, e.g., *k* = the absence of a genetic variation). For example, for an expected elevation equal to 1 (e.g., the absence of a copy number variation), an uncertainty value (i.e. σ) equal to +/- 0.05, and *n*=3, the expected elevation range is defined as 1.15 to 0.85. In some embodiments, the expected elevation range for a heterozygous duplication is determined as 1.65 to 1.35 when the expected elevation for a heterozygous duplication is 1.5, *n* = 3, and the uncertainty value σ is +/- 0.05. In some embodiments the expected elevation range for a heterozygous deletion is determined as 0.65 to 0.35 when the expected elevation for a heterozygous duplication is 0.5, *n* = 3, and the uncertainty value σ is +/- 0.05. In some embodiments the expected elevation range for a homozygous duplication is determined as 2.15 to 1.85 when the expected elevation for a heterozygous duplication is 2.0, *n* = 3 and the uncertainty value σ is +/- 0.05. In some embodiments the expected elevation range for a homozygous deletion is determined as 0.15 to -0.15 when the expected elevation for a heterozygous duplication is 0.0, *n* = 3 and the uncertainty value σ is +/- 0.05.

Sometimes an expected elevation range for a homozygous copy number variation (e.g., a maternal, fetal or maternal and fetal homozygous copy number variation) is determined, in part, according to an expected elevation range for a corresponding heterozygous copy number variation. For example, sometimes an expected elevation range for a homozygous duplication comprises all values greater than an upper limit of an expected elevation range for a heterozygous duplication. Sometimes an expected elevation range for a homozygous duplication comprises all values greater than or equal to an upper limit of an expected elevation range for a heterozygous duplication. Sometimes an expected elevation range for a homozygous duplication comprises all values greater than an upper limit of an expected elevation range for a heterozygous duplication and less than the upper limit defined by the formula R where σ is an uncertainty value and is a positive value, *n* is a constant and *k* is a homozygous duplication. Sometimes an expected elevation range for a homozygous duplication comprises all values greater than or equal to an upper limit of an expected elevation range for a heterozygous duplication and less than or equal to the upper limit defined by the formula R where σ is an uncertainty value, σ is a positive value, *n* is a constant and *k* is a homozygous duplication.

In some embodiments, an expected elevation range for a homozygous deletion comprises all values less than a lower limit of an expected elevation range for a heterozygous deletion. Sometimes an expected elevation range for a homozygous deletion comprises all values less than or equal to a lower limit of an expected elevation range for a heterozygous deletion. Sometimes an expected elevation range for a homozygous deletion comprises all values less than a lower limit of an expected elevation range for a heterozygous deletion and greater than the lower limit defined by the formula R where σ is an uncertainty value, σ is a negative value, *n* is a constant and *k* is a homozygous deletion. Sometimes an expected elevation range for a homozygous deletion comprises all values less than or equal to a lower limit of an expected elevation range for a heterozygous deletion and greater than or equal to the lower limit defined by the formula R where σ is an uncertainty value, σ is a negative value, *n* is a constant and *k* is a homozygous deletion.

An uncertainty value can be utilized to determine a threshold value. In some embodiments, a range (e.g., a threshold range) is obtained by calculating the uncertainty value determined from a raw, filtered and/or normalized counts. A range can be determined by multiplying the uncertainty value for an elevation (e.g. normalized counts of an elevation) by a predetermined constant (e.g., 1, 2, 3, 4, 5, 6, etc.) representing the multiple of uncertainty (e.g., number of standard deviations) chosen as a cutoff threshold (e.g., multiply by 3 for 3 standard deviations), whereby a range is

generated, in some embodiments. A range can be determined by adding and/or subtracting a value (e.g., a predetermined value, an uncertainty value, an uncertainty value multiplied by a predetermined constant) to and/or from an elevation whereby a range is generated, in some embodiments. For example, for an elevation equal to 1, a standard deviation of +/-0.2, where a predetermined constant is 3, the range can be calculated as (1 + 3(0.2)) to (1 + 3(-0.2)), or 1.6 to 0.4. A range sometimes can define an expected range or expected elevation range for a copy number variation. In certain embodiments, some or all of the genomic sections exceeding a threshold value, falling outside a range or falling inside a range of values, are removed as part of, prior to, or after a normalization process. In some embodiments, some or all of the genomic sections exceeding a calculated threshold value, falling outside a range or falling inside a range are weighted or adjusted as part of, or prior to the normalization or classification process. Examples of weighting are described herein. The terms “redundant data”, and “redundant mapped reads” as used herein refer to sample derived sequence reads that are identified as having already been assigned to a genomic location (e.g., base position) and/or counted for a genomic section.

In some embodiments an uncertainty value is determined according to the formula below:

$$Z = \frac{L_A - L_o}{\sqrt{\frac{\sigma_A^2}{N_A} + \frac{\sigma_o^2}{N_o}}}$$

Where Z represents the standardized deviation between two elevations, L is the mean (or median) elevation and sigma is the standard deviation (or MAD). The subscript O denotes a segment of a profile (e.g., a second elevation, a chromosome, an NRV, a “euploid level”, a level absent a copy number variation), and A denotes another segment of a profile (e.g., a first elevation, an elevation representing a copy number variation, an elevation representing an aneuploidy (e.g., a trisomy). The variable N_o represents the total number of genomic sections in the segment of the profile denoted by the subscript O. N_A represents the total number of genomic sections in the segment of the profile denoted by subscript A.

Categorizing a Copy Number Variation

An elevation (e.g., a first elevation) that significantly differs from another elevation (e.g., a second elevation) can often be categorized as a copy number variation (e.g., a maternal and/or fetal copy

number variation, a fetal copy number variation, a deletion, duplication, insertion) according to an expected elevation range. In some embodiments, the presence of a copy number variation is categorized when a first elevation is significantly different from a second elevation and the first elevation falls within the expected elevation range for a copy number variation. For example, a copy number variation (e.g., a maternal and/or fetal copy number variation, a fetal copy number variation) can be categorized when a first elevation is significantly different from a second elevation and the first elevation falls within the expected elevation range for a copy number variation. Sometimes a heterozygous duplication (e.g., a maternal or fetal, or maternal and fetal, heterozygous duplication) or heterozygous deletion (e.g., a maternal or fetal, or maternal and fetal, heterozygous deletion) is categorized when a first elevation is significantly different from a second elevation and the first elevation falls within the expected elevation range for a heterozygous duplication or heterozygous deletion, respectively. Sometimes a homozygous duplication or homozygous deletion is categorized when a first elevation is significantly different from a second elevation and the first elevation falls within the expected elevation range for a homozygous duplication or homozygous deletion, respectively.

Range Setting Module

Expected ranges (e.g., expected elevation ranges) for various copy number variations (e.g., duplications, insertions and/or deletions) or ranges for the absence of a copy number variation can be provided by a range setting module or by an apparatus comprising a range setting module. In some cases, expected elevations are provided by a range setting module or by an apparatus comprising a range setting module. In some embodiments, a range setting module or an apparatus comprising a range setting module is required to provide expected elevations and/or ranges. Sometimes a range setting module gathers, assembles and/or receives data and/or information from another module or apparatus. Sometimes a range setting module or an apparatus comprising a range setting module provides and/or transfers data and/or information to another module or apparatus. Sometimes a range setting module accepts and gathers data and/or information from a component or peripheral. Often a range setting module gathers and assembles elevations, reference elevations, uncertainty values, and/or constants. Sometimes a range setting module accepts and gathers input data and/or information from an operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to a module. An apparatus comprising a range setting module can comprise at least one processor. In some embodiments, expected elevations and expected

ranges are provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the range setting module. In some embodiments, expected ranges and elevations are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a range setting module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, expected ranges are provided by an apparatus comprising a suitable peripheral or component. A range setting module can receive normalized data from a normalization module or comparison data from a comparison module. Data and/or information derived from or transformed by a range setting module (e.g., set ranges, range limits, expected elevation ranges, thresholds, and/or threshold ranges) can be transferred from a range setting module to an adjustment module, an outcome module, a categorization module, plotting module or other suitable apparatus and/or module.

Categorization Module

A copy number variation (e.g., a maternal and/or fetal copy number variation, a fetal copy number variation, a duplication, insertion, deletion) can be categorized by a categorization module or by an apparatus comprising a categorization module. Sometimes a copy number variation (e.g., a maternal and/or fetal copy number variation) is categorized by a categorization module. Sometimes an elevation (e.g., a first elevation) determined to be significantly different from another elevation (e.g., a second elevation) is identified as representative of a copy number variation by a categorization module. Sometimes the absence of a copy number variation is determined by a categorization module. In some embodiments, a determination of a copy number variation can be determined by an apparatus comprising a categorization module. A categorization module can be specialized for categorizing a maternal and/or fetal copy number variation, a fetal copy number variation, a duplication, deletion or insertion or lack thereof or combination of the foregoing. For example, a categorization module that identifies a maternal deletion can be different than and/or distinct from a categorization module that identifies a fetal duplication. In some embodiments, a categorization module or an apparatus comprising a categorization module is required to identify a copy number variation or an outcome determinative of a copy number variation. An apparatus comprising a categorization module can comprise at least one processor. In some embodiments, a copy number variation or an outcome determinative of a copy number variation is categorized by an apparatus that includes a processor (e.g., one or more processors) which processor can

perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the categorization module. In some embodiments, a copy number variation or an outcome determinative of a copy number variation is categorized by an apparatus that may include multiple processors, such as processors coordinated and working in parallel. In some embodiments, a categorization module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). Sometimes a categorization module transfers or receives and/or gathers data and/or information to or from a component or peripheral. Often a categorization module receives, gathers and/or assembles counts, elevations, profiles, normalized data and/or information, reference elevations, expected elevations, expected ranges, uncertainty values, adjustments, adjusted elevations, plots, comparisons and/or constants. Sometimes a categorization module accepts and gathers input data and/or information from an operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to a module. In some embodiments, data and/or information are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, identification or categorization of a copy number variation or an outcome determinative of a copy number variation is provided by an apparatus comprising a suitable peripheral or component. Sometimes a categorization module gathers, assembles and/or receives data and/or information from another module or apparatus. A categorization module can receive normalized data from a normalization module, expected elevations and/or ranges from a range setting module, comparison data from a comparison module, plots from a plotting module, and/or adjustment data from an adjustment module. A categorization module can transform data and/or information that it receives into a determination of the presence or absence of a copy number variation. A categorization module can transform data and/or information that it receives into a determination that an elevation represents a genomic section comprising a copy number variation or a specific type of copy number variation (e.g., a maternal homozygous deletion). Data and/or information related to a copy number variation or an outcome determinative of a copy number variation can be transferred from a categorization module to a suitable apparatus and/or module. A copy number variation or an outcome determinative of a copy number variation categorized by methods described herein can be independently verified by further testing (e.g., by targeted sequencing of maternal and/or fetal nucleic acid).

Fetal Fraction Determination Based on Elevation

In some embodiments, a fetal fraction is determined according to an elevation categorized as representative of a maternal and/or fetal copy number variation. For example determining fetal fraction often comprises assessing an expected elevation for a maternal and/or fetal copy number variation utilized for the determination of fetal fraction. Sometimes a fetal fraction is determined for an elevation (e.g., a first elevation) categorized as representative of a copy number variation according to an expected elevation range determined for the same type of copy number variation. Often a fetal fraction is determined according to an observed elevation that falls within an expected elevation range and is thereby categorized as a maternal and/or fetal copy number variation. Sometimes a fetal fraction is determined when an observed elevation (e.g., a first elevation) categorized as a maternal and/or fetal copy number variation is different than the expected elevation determined for the same maternal and/or fetal copy number variation.

In some embodiments an elevation (e.g., a first elevation, an observed elevation), is significantly different than a second elevation, the first elevation is categorized as a maternal and/or fetal copy number variation, and a fetal fraction is determined according to the first elevation. Sometimes a first elevation is an observed and/or experimentally obtained elevation that is significantly different than a second elevation in a profile and a fetal fraction is determined according to the first elevation. Sometimes the first elevation is an average, mean or summed elevation and a fetal fraction is determined according to the first elevation. In some cases a first elevation and a second elevation are observed and/or experimentally obtained elevations and a fetal fraction is determined according to the first elevation. In some instances a first elevation comprises normalized counts for a first set of genomic sections and a second elevation comprises normalized counts for a second set of genomic sections and a fetal fraction is determined according to the first elevation. Sometimes a first set of genomic sections of a first elevation includes a copy number variation (e.g., the first elevation is representative of a copy number variation) and a fetal fraction is determined according to the first elevation. Sometimes the first set of genomic sections of a first elevation includes a homozygous or heterozygous maternal copy number variation and a fetal fraction is determined according to the first elevation. Sometimes a profile comprises a first elevation for a first set of genomic sections and a second elevation for a second set of genomic sections, the second set of genomic sections includes substantially no copy number variation (e.g., a maternal copy number variation, fetal copy number variation, or a maternal copy number

variation and a fetal copy number variation) and a fetal fraction is determined according to the first elevation.

In some embodiments an elevation (e.g., a first elevation, an observed elevation), is significantly different than a second elevation, the first elevation is categorized as for a maternal and/or fetal copy number variation, and a fetal fraction is determined according to the first elevation and/or an expected elevation of the copy number variation. Sometimes a first elevation is categorized as for a copy number variation according to an expected elevation for a copy number variation and a fetal fraction is determined according to a difference between the first elevation and the expected elevation. In some cases an elevation (e.g., a first elevation, an observed elevation) is categorized as a maternal and/or fetal copy number variation, and a fetal fraction is determined as twice the difference between the first elevation and expected elevation of the copy number variation. Sometimes an elevation (e.g., a first elevation, an observed elevation) is categorized as a maternal and/or fetal copy number variation, the first elevation is subtracted from the expected elevation thereby providing a difference, and a fetal fraction is determined as twice the difference. Sometimes an elevation (e.g., a first elevation, an observed elevation) is categorized as a maternal and/or fetal copy number variation, an expected elevation is subtracted from a first elevation thereby providing a difference, and the fetal fraction is determined as twice the difference.

Often a fetal fraction is provided as a percent. For example, a fetal fraction can be divided by 100 thereby providing a percent value. For example, for a first elevation representative of a maternal homozygous duplication and having an elevation of 155 and an expected elevation for a maternal homozygous duplication having an elevation of 150, a fetal fraction can be determined as 10% (e.g., (fetal fraction = 2 x (155 – 150)).

In some embodiments a fetal fraction is determined from two or more elevations within a profile that are categorized as copy number variations. For example, sometimes two or more elevations (e.g., two or more first elevations) in a profile are identified as significantly different than a reference elevation (e.g., a second elevation, an elevation that includes substantially no copy number variation), the two or more elevations are categorized as representative of a maternal and/or fetal copy number variation and a fetal fraction is determined from each of the two or more elevations. Sometimes a fetal fraction is determined from about 3 or more, about 4 or more, about 5 or more, about 6 or more, about 7 or more, about 8 or more, or about 9 or more fetal fraction determinations within a profile. Sometimes a fetal fraction is determined from about 10 or more,

about 20 or more, about 30 or more, about 40 or more, about 50 or more, about 60 or more, about 70 or more, about 80 or more, or about 90 or more fetal fraction determinations within a profile. Sometimes a fetal fraction is determined from about 100 or more, about 200 or more, about 300 or more, about 400 or more, about 500 or more, about 600 or more, about 700 or more, about 800 or more, about 900 or more, or about 1000 or more fetal fraction determinations within a profile. Sometimes a fetal fraction is determined from about 10 to about 1000, about 20 to about 900, about 30 to about 700, about 40 to about 600, about 50 to about 500, about 50 to about 400, about 50 to about 300, about 50 to about 200, or about 50 to about 100 fetal fraction determinations within a profile.

In some embodiments a fetal fraction is determined as the average or mean of multiple fetal fraction determinations within a profile. In some cases, a fetal fraction determined from multiple fetal fraction determinations is a mean (e.g., an average, a mean, a standard average, a median, or the like) of multiple fetal fraction determinations. Often a fetal fraction determined from multiple fetal fraction determinations is a mean value determined by a suitable method known in the art or described herein. Sometimes a mean value of a fetal fraction determination is a weighted mean. Sometimes a mean value of a fetal fraction determination is an unweighted mean. A mean, median or average fetal fraction determination (i.e., a mean, median or average fetal fraction determination value) generated from multiple fetal fraction determinations is sometimes associated with an uncertainty value (e.g., a variance, standard deviation, MAD, or the like). Before determining a mean, median or average fetal fraction value from multiple determinations, one or more deviant determinations are removed in some embodiments (described in greater detail herein).

Some fetal fraction determinations within a profile sometimes are not included in the overall determination of a fetal fraction (e.g., mean or average fetal fraction determination). Sometimes a fetal fraction determination is derived from a first elevation (e.g., a first elevation that is significantly different than a second elevation) in a profile and the first elevation is not indicative of a genetic variation. For example, some first elevations (e.g., spikes or dips) in a profile are generated from anomalies or unknown causes. Such values often generate fetal fraction determinations that differ significantly from other fetal fraction determinations obtained from true copy number variations. Sometimes fetal fraction determinations that differ significantly from other fetal fraction determinations in a profile are identified and removed from a fetal fraction determination. For example, some fetal fraction determinations obtained from anomalous spikes and dips are

identified by comparing them to other fetal fraction determinations within a profile and are excluded from the overall determination of fetal fraction.

Sometimes, an independent fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination is an identified, recognized and/or observable difference. In some cases, the term "differs significantly" can mean statistically different and/or a statistically significant difference. An "independent" fetal fraction determination can be a fetal fraction determined (e.g., in some cases a single determination) from a specific elevation categorized as a copy number variation. Any suitable threshold or range can be used to determine that a fetal fraction determination differs significantly from a mean, median or average fetal fraction determination. In some cases a fetal fraction determination differs significantly from a mean, median or average fetal fraction determination and the determination can be expressed as a percent deviation from the average or mean value. In some cases a fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination differs by about 10 percent or more. Sometimes a fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination differs by about 15 percent or more. Sometimes a fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination differs by about 15% to about 100% or more.

In some cases a fetal fraction determination differs significantly from a mean, median or average fetal fraction determination according to a multiple of an uncertainty value associated with the mean or average fetal fraction determination. Often an uncertainty value and constant *n* (e.g., a confidence interval) defines a range (e.g., an uncertainty cutoff). For example, sometimes an uncertainty value is a standard deviation for fetal fraction determinations (e.g., +/- 5) and is multiplied by a constant *n* (e.g., a confidence interval) thereby defining a range or uncertainty cutoff (e.g., 5*n* to -5*n,* sometimes referred to as 5 sigma). Sometimes an independent fetal fraction determination falls outside a range defined by the uncertainty cutoff and is considered significantly different from a mean, median or average fetal fraction determination. For example, for a mean value of 10 and an uncertainty cutoff of 3, an independent fetal fraction greater than 13 or less than 7 is significantly different. Sometimes a fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination differs by more than *n* times the uncertainty value (e.g., *n* x sigma) where *n* is about equal to or greater than 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. Sometimes a fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination differs by more than *n* times the uncertainty value (e.g., *n* x sigma)

where *n* is about equal to or greater than 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, or 4.0.

In some embodiments, an elevation is representative of a fetal and/or maternal microploidy. Sometimes an elevation (e.g., a first elevation, an observed elevation), is significantly different than a second elevation, the first elevation is categorized as a maternal and/or fetal copy number variation, and the first elevation and/or second elevation is representative of a fetal microploidy and/or a maternal microploidy. In some cases a first elevation is representative of a fetal microploidy, Sometimes a first elevation is representative of a maternal microploidy. Often a first elevation is representative of a fetal microploidy and a maternal microploidy. Sometimes an elevation (e.g., a first elevation, an observed elevation), is significantly different than a second elevation, the first elevation is categorized as a maternal and/or fetal copy number variation, the first elevation is representative of a fetal and/or maternal microploidy and a fetal fraction is determined according to the fetal and/or maternal microploidy. In some instances a first elevation is categorized as a maternal and/or fetal copy number variation, the first elevation is representative of a fetal microploidy and a fetal fraction is determined according to the fetal microploidy. Sometimes a first elevation is categorized as a maternal and/or fetal copy number variation, the first elevation is representative of a maternal microploidy and a fetal fraction is determined according to the maternal microploidy. Sometimes a first elevation is categorized as a maternal and/or fetal copy number variation, the first elevation is representative of a maternal and a fetal microploidy and a fetal fraction is determined according to the maternal and fetal microploidy.

In some embodiments, a determination of a fetal fraction comprises determining a fetal and/or maternal microploidy. Sometimes an elevation (e.g., a first elevation, an observed elevation), is significantly different than a second elevation, the first elevation is categorized as a maternal and/or fetal copy number variation, a fetal and/or maternal microploidy is determined according to the first elevation and/or second elevation and a fetal fraction is determined. Sometimes a first elevation is categorized as a maternal and/or fetal copy number variation, a fetal microploidy is determined according to the first elevation and/or second elevation and a fetal fraction is determined according to the fetal microploidy. In some cases a first elevation is categorized as a maternal and/or fetal copy number variation, a maternal microploidy is determined according to the first elevation and/or second elevation and a fetal fraction is determined according to the maternal microploidy. Sometimes a first elevation is categorized as a maternal and/or fetal copy number

variation, a maternal and fetal microploidy is determined according to the first elevation and/or second elevation and a fetal fraction is determined according to the maternal and fetal microploidy.

A fetal fraction often is determined when the microploidy of the mother is different from (e.g., not the same as) the microploidy of the fetus for a given elevation or for an elevation categorized as a copy number variation. Sometimes a fetal fraction is determined when the mother is homozygous for a duplication (e.g., a microploidy of 2) and the fetus is heterozygous for the same duplication (e.g., a microploidy of 1.5). Sometimes a fetal fraction is determined when the mother is heterozygous for a duplication (e.g., a microploidy of 1.5) and the fetus is homozygous for the same duplication (e.g., a microploidy of 2) or the duplication is absent in the fetus (e.g., a microploidy of 1). Sometimes a fetal fraction is determined when the mother is homozygous for a deletion (e.g., a microploidy of 0) and the fetus is heterozygous for the same deletion (e.g., a microploidy of 0.5). Sometimes a fetal fraction is determined when the mother is heterozygous for a deletion (e.g., a microploidy of 0.5) and the fetus is homozygous for the same deletion (e.g., a microploidy of 0) or the deletion is absent in the fetus (e.g., a microploidy of 1).

In some cases, a fetal fraction cannot be determined when the microploidy of the mother is the same (e.g., identified as the same) as the microploidy of the fetus for a given elevation identified as a copy number variation. For example, for a given elevation where both the mother and fetus carry the same number of copies of a copy number variation, a fetal fraction is not determined, in some embodiments. For example, a fetal fraction cannot be determined for an elevation categorized as a copy number variation when both the mother and fetus are homozygous for the same deletion or homozygous for the same duplication. In some cases, a fetal fraction cannot be determined for an elevation categorized as a copy number variation when both the mother and fetus are heterozygous for the same deletion or heterozygous for the same duplication. In embodiments where multiple fetal fraction determinations are made for a sample, determinations that significantly deviate from a mean, median or average value can result from a copy number variation for which maternal ploidy is equal to fetal ploidy, and such determinations can be removed from consideration.

In some embodiments the microploidy of a maternal copy number variation and fetal copy number variation is unknown. Sometimes, in cases when there is no determination of fetal and/or maternal microploidy for a copy number variation, a fetal fraction is generated and compared to a mean, median or average fetal fraction determination. A fetal fraction determination for a copy number

variation that differs significantly from a mean, median or average fetal fraction determination is sometimes because the microploidy of the mother and fetus are the same for the copy number variation. A fetal fraction determination that differs significantly from a mean, median or average fetal fraction determination is often excluded from an overall fetal fraction determination regardless of the source or cause of the difference. In some embodiments, the microploidy of the mother and/or fetus is determined and/or verified by a method known in the art (e.g., by targeted sequencing methods).

Elevation Adjustments

In some embodiments, one or more elevations are adjusted. A process for adjusting an elevation often is referred to as padding. In some embodiments, multiple elevations in a profile (e.g., a profile of a genome, a chromosome profile, a profile of a portion or segment of a chromosome) are adjusted. Sometimes, about 1 to about 10,000 or more elevations in a profile are adjusted. Sometimes about 1 to about a 1000, 1 to about 900, 1 to about 800, 1 to about 700, 1 to about 600, 1 to about 500, 1 to about 400, 1 to about 300, 1 to about 200, 1 to about 100, 1 to about 50, 1 to about 25, 1 to about 20, 1 to about 15, 1 to about 10, or 1 to about 5 elevations in a profile are adjusted. Sometimes one elevation is adjusted. In some embodiments, an elevation (e.g., a first elevation of a normalized count profile) that significantly differs from a second elevation is adjusted. Sometimes an elevation categorized as a copy number variation is adjusted. Sometimes an elevation (e.g., a first elevation of a normalized count profile) that significantly differs from a second elevation is categorized as a copy number variation (e.g., a copy number variation, e.g., a maternal copy number variation) and is adjusted. In some embodiments, an elevation (e.g., a first elevation) is within an expected elevation range for a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation and the elevation is adjusted. Sometimes, one or more elevations (e.g., elevations in a profile) are not adjusted. In some embodiments, an elevation (e.g., a first elevation) is outside an expected elevation range for a copy number variation and the elevation is not adjusted. Often, an elevation within an expected elevation range for the absence of a copy number variation is not adjusted. Any suitable number of adjustments can be made to one or more elevations in a profile. In some embodiments, one or more elevations are adjusted. Sometimes 2 or more, 3 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more and sometimes 10 or more elevations are adjusted.

In some embodiments, a value of a first elevation is adjusted according to a value of a second elevation. Sometimes a first elevation, identified as representative of a copy number variation, is adjusted to the value of a second elevation, where the second elevation is often associated with no copy number variation. In some cases, a value of a first elevation, identified as representative of a copy number variation, is adjusted so the value of the first elevation is about equal to a value of a second elevation.

An adjustment can comprise a suitable mathematical operation. Sometimes an adjustment comprises one or more mathematical operations. Sometimes an elevation is adjusted by normalizing, filtering, averaging, multiplying, dividing, adding or subtracting or combination thereof. Sometimes an elevation is adjusted by a predetermined value or a constant. Sometimes an elevation is adjusted by modifying the value of the elevation to the value of another elevation. For example, a first elevation may be adjusted by modifying its value to the value of a second elevation. A value in such cases may be a processed value (e.g., mean, normalized value and the like).

Sometimes an elevation is categorized as a copy number variation (e.g., a maternal copy number variation) and is adjusted according to a predetermined value referred to herein as a predetermined adjustment value (PAV). Often a PAV is determined for a specific copy number variation. Often a PAV determined for a specific copy number variation (e.g., homozygous duplication, homozygous deletion, heterozygous duplication, heterozygous deletion) is used to adjust an elevation categorized as a specific copy number variation (e.g., homozygous duplication, homozygous deletion, heterozygous duplication, heterozygous deletion). In some cases, an elevation is categorized as a copy number variation and is then adjusted according to a PAV specific to the type of copy number variation categorized. Sometimes an elevation (e.g., a first elevation) is categorized as a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation and is adjusted by adding or subtracting a PAV from the elevation. Often an elevation (e.g., a first elevation) is categorized as a maternal copy number variation and is adjusted by adding a PAV to the elevation. For example, an elevation categorized as a duplication (e.g., a maternal, fetal or maternal and fetal homozygous duplication) can be adjusted by adding a PAV determined for a specific duplication (e.g., a homozygous duplication) thereby providing an adjusted elevation. Often a PAV determined for a copy number duplication is a negative value. In some embodiments providing an adjustment to an elevation representative of a duplication by utilizing a PAV determined for a duplication results in a

reduction in the value of the elevation. In some embodiments, an elevation (e.g., a first elevation) that significantly differs from a second elevation is categorized as a copy number deletion (e.g., a homozygous deletion, heterozygous deletion, homozygous duplication, homozygous duplication) and the first elevation is adjusted by adding a PAV determined for a copy number deletion. Often a PAV determined for a copy number deletion is a positive value. In some embodiments providing an adjustment to an elevation representative of a deletion by utilizing a PAV determined for a deletion results in an increase in the value of the elevation.

A PAV can be any suitable value. Often a PAV is determined according to and is specific for a copy number variation (e.g., a categorized copy number variation). In some cases a PAV is determined according to an expected elevation for a copy number variation (e.g., a categorized copy number variation) and/or a PAV factor. A PAV sometimes is determined by multiplying an expected elevation by a PAV factor. For example, a PAV for a copy number variation can be determined by multiplying an expected elevation determined for a copy number variation (e.g., a heterozygous deletion) by a PAV factor determined for the same copy number variation (e.g., a heterozygous deletion). For example, PAV can be determined by the formula below:

PAV_k = (Expected Elevation)$_k$ **x** (PAV factor)$_k$

for the copy number variation k (e.g., k = a heterozygous deletion)

A PAV factor can be any suitable value. Sometimes a PAV factor for a homozygous duplication is between about -0.6 and about -0.4. Sometimes a PAV factor for a homozygous duplication is about -0.60, -0.59, -0.58, -0.57, -0.56, -0.55, -0.54, -0.53, -0.52, -0.51, -0.50, -0.49, -0.48, -0.47, -0.46, -0.45, -0.44, -0.43, -0.42, -0.41 and -0.40. Often a PAV factor for a homozygous duplication is about -0.5.

For example, for an NRV of about 1 and an expected elevation of a homozygous duplication equal to about 2, the PAV for the homozygous duplication is determined as about -1 according to the formula above. In this case, a first elevation categorized as a homozygous duplication is adjusted by adding about -1 to the value of the first elevation, for example.

Sometimes a PAV factor for a heterozygous duplication is between about -0.4 and about -0.2. Sometimes a PAV factor for a heterozygous duplication is about -0.40, -0.39, -0.38, -0.37, -0.36, -

0.35, -0.34, -0.33, -0.32, -0.31, -0.30, -0.29, -0.28, -0.27, -0.26, -0.25, -0.24, -0.23, -0.22, -0.21 and -0.20. Often a PAV factor for a heterozygous duplication is about -0.33.

For example, for an NRV of about 1 and an expected elevation of a heterozygous duplication equal to about 1.5, the PAV for the homozygous duplication is determined as about -0.495 according to the formula above. In this case, a first elevation categorized as a heterozygous duplication is adjusted by adding about -0.495 to the value of the first elevation, for example.

Sometimes a PAV factor for a heterozygous deletion is between about 0.4 and about 0.2. Sometimes a PAV factor for a heterozygous deletion is about 0.40, 0.39, 0.38, 0.37, 0.36, 0.35, 0.34, 0.33, 0.32, 0.31, 0.30, 0.29, 0.28, 0.27, 0.26, 0.25, 0.24, 0.23, 0.22, 0.21 and 0.20. Often a PAV factor for a heterozygous deletion is about 0.33.

For example, for an NRV of about 1 and an expected elevation of a heterozygous deletion equal to about 0.5, the PAV for the heterozygous deletion is determined as about 0.495 according to the formula above. In this case, a first elevation categorized as a heterozygous deletion is adjusted by adding about 0.495 to the value of the first elevation, for example.

Sometimes a PAV factor for a homozygous deletion is between about 0.6 and about 0.4. Sometimes a PAV factor for a homozygous deletion is about 0.60, 0.59, 0.58, 0.57, 0.56, 0.55, 0.54, 0.53, 0.52, 0.51, 0.50, 0.49, 0.48, 0.47, 0.46, 0.45, 0.44, 0.43, 0.42, 0.41 and 0.40. Often a PAV factor for a homozygous deletion is about 0.5.

For example, for an NRV of about 1 and an expected elevation of a homozygous deletion equal to about 0, the PAV for the homozygous deletion is determined as about 1 according to the formula above. In this case, a first elevation categorized as a homozygous deletion is adjusted by adding about 1 to the value of the first elevation, for example.

In some cases, a PAV is about equal to or equal to an expected elevation for a copy number variation (e.g., the expected elevation of a copy number variation).

In some embodiments, counts of an elevation are normalized prior to making an adjustment. In some cases, counts of some or all elevations in a profile are normalized prior to making an adjustment. For example, counts of an elevation can be normalized according to counts of a

reference elevation or an NRV. In some cases, counts of an elevation (e.g., a second elevation) are normalized according to counts of a reference elevation or an NRV and the counts of all other elevations (e.g., a first elevation) in a profile are normalized relative to the counts of the same reference elevation or NRV prior to making an adjustment.

In some embodiments, an elevation of a profile results from one or more adjustments. In some cases, an elevation of a profile is determined after one or more elevations in the profile are adjusted. In some embodiments, an elevation of a profile is re-calculated after one or more adjustments are made.

In some embodiments, a copy number variation (e.g., a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation) is determined (e.g., determined directly or indirectly) from an adjustment. For example, an elevation in a profile that was adjusted (e.g., an adjusted first elevation) can be identified as a maternal copy number variation. In some embodiments, the magnitude of the adjustment indicates the type of copy number variation (e.g., heterozygous deletion, homozygous duplication, and the like). In some cases, an adjusted elevation in a profile can be identified as representative of a copy number variation according to the value of a PAV for the copy number variation. For example, for a given profile, PAV is about -1 for a homozygous duplication, about -0.5 for a heterozygous duplication, about 0.5 for a heterozygous deletion and about 1 for a homozygous deletion. In the preceding example, an elevation adjusted by about -1 can be identified as a homozygous duplication, for example. In some embodiments, one or more copy number variations can be determined from a profile or an elevation comprising one or more adjustments.

In some cases, adjusted elevations within a profile are compared. Sometimes anomalies and errors are identified by comparing adjusted elevations. For example, often one or more adjusted elevations in a profile are compared and a particular elevation may be identified as an anomaly or error. Sometimes an anomaly or error is identified within one or more genomic sections making up an elevation. An anomaly or error may be identified within the same elevation (e.g., in a profile) or in one or more elevations that represent genomic sections that are adjacent, contiguous, adjoining or abutting. Sometimes one or more adjusted elevations are elevations of genomic sections that are adjacent, contiguous, adjoining or abutting where the one or more adjusted elevations are compared and an anomaly or error is identified. An anomaly or error can be a peak or dip in a profile or elevation where a cause of the peak or dip is known or unknown. In some cases

adjusted elevations are compared and an anomaly or error is identified where the anomaly or error is due to a stochastic, systematic, random or user error. Sometimes adjusted elevations are compared and an anomaly or error is removed from a profile. In some cases, adjusted elevations are compared and an anomaly or error is adjusted.

Adjustment Module

In some embodiments, adjustments (e.g., adjustments to elevations or profiles) are made by an adjustment module or by an apparatus comprising an adjustment module. In some embodiments, an adjustment module or an apparatus comprising an adjustment module is required to adjust an elevation. An apparatus comprising an adjustment module can comprise at least one processor. In some embodiments, an adjusted elevation is provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the adjustment module. In some embodiments, an elevation is adjusted by an apparatus that may include multiple processors, such as processors coordinated and working in parallel. In some embodiments, an adjustment module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). Sometimes an apparatus comprising an adjustment module gathers, assembles and/or receives data and/or information from another module or apparatus. Sometimes an apparatus comprising an adjustment module provides and/or transfers data and/or information to another module or apparatus.

Sometimes an adjustment module receives and gathers data and/or information from a component or peripheral. Often an adjustment module receives, gathers and/or assembles counts, elevations, profiles, reference elevations, expected elevations, expected elevation ranges, uncertainty values, adjustments and/or constants. Often an adjustment module receives gathers and/or assembles elevations (e.g., first elevations) that are categorized or determined to be copy number variations (e.g., a maternal copy number variation, fetal copy number variation, or a maternal copy number variation and a fetal copy number variation). Sometimes an adjustment module accepts and gathers input data and/or information from an operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to a module. In some embodiments, data and/or information are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, an elevation is adjusted by an apparatus comprising a suitable peripheral or

component. An apparatus comprising an adjustment module can receive normalized data from a normalization module, ranges from a range setting module, comparison data from a comparison module, elevations identified (e.g., identified as a copy number variation) from a categorization module, and/or adjustment data from another adjustment module. An adjustment module can receive data and/or information, transform the received data and/or information and provide adjustments. Data and/or information derived from, or transformed by, an adjustment module can be transferred from an adjustment module to a categorization module or to a suitable apparatus and/or module. An elevation adjusted by methods described herein can be independently verified and/or adjusted by further testing (e.g., by targeted sequencing of maternal and or fetal nucleic acid).

Plotting Module

In some embodiments a count, an elevation, and/or a profile is plotted (e.g., graphed). Sometimes a plot (e.g., a graph) comprises an adjustment. Sometimes a plot comprises an adjustment of a count, an elevation, and/or a profile. Sometimes a count, an elevation, and/or a profile is plotted and a count, elevation, and/or a profile comprises an adjustment. Often a count, an elevation, and/or a profile is plotted and a count, elevation, and/or a profile are compared. Sometimes a copy number variation (e.g., an aneuploidy, copy number variation) is identified and/or categorized from a plot of a count, an elevation, and/or a profile. Sometimes an outcome is determined from a plot of a count, an elevation, and/or a profile. In some embodiments, a plot (e.g., a graph) is made (e.g., generated) by a plotting module or an apparatus comprising a plotting module. In some embodiments, a plotting module or an apparatus comprising a plotting module is required to plot a count, an elevation or a profile. A plotting module may display a plot or send a plot to a display (e.g., a display module). An apparatus comprising a plotting module can comprise at least one processor. In some embodiments, a plot is provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the plotting module. In some embodiments, a plot is made by an apparatus that may include multiple processors, such as processors coordinated and working in parallel. In some embodiments, a plotting module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). Sometimes an apparatus comprising a plotting module gathers, assembles and/or receives data and/or information from another module or apparatus. Sometimes a plotting module receives and gathers data and/or information from a component or

peripheral. Often a plotting module receives, gathers, assembles and/or plots sequence reads, genomic sections, mapped reads, counts, elevations, profiles, reference elevations, expected elevations, expected elevation ranges, uncertainty values, comparisons, categorized elevations (e.g., elevations identified as copy number variations) and/or outcomes, adjustments and/or constants. Sometimes a plotting module accepts and gathers input data and/or information from an operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to a plotting module. In some embodiments, data and/or information are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, a count, an elevation and/or a profile is plotted by an apparatus comprising a suitable peripheral or component. An apparatus comprising a plotting module can receive normalized data from a normalization module, ranges from a range setting module, comparison data from a comparison module, categorization data from a categorization module, and/or adjustment data from an adjustment module. A plotting module can receive data and/or information, transform the data and/or information and provided plotted data. Sometimes an apparatus comprising a plotting module provides and/or transfers data and/or information to another module or apparatus. An apparatus comprising a plotting module can plot a count, an elevation and/or a profile and provide or transfer data and/or information related to the plotting to a suitable apparatus and/or module. Often a plotting module receives, gathers, assembles and/or plots elevations (e.g., profiles, first elevations) and transfers plotted data and/or information to and from an adjustment module and/or comparison module. Plotted data and/or information is sometimes transferred from a plotting module to a categorization module and/or a peripheral (e.g., a display or printer). In some embodiments, plots are categorized and/or determined to comprise a genetic variation (e.g., an aneuploidy) or a copy number variation (e.g., a maternal and/or fetal copy number variation). A count, an elevation and/or a profile plotted by methods described herein can be independently verified and/or adjusted by further testing (e.g., by targeted sequencing of maternal and or fetal nucleic acid).

Sometimes an outcome is determined according to one or more elevations. In some embodiments, a determination of the presence or absence of a genetic variation (e.g., a chromosome aneuploidy) is determined according to one or more adjusted elevations. Sometimes, a determination of the presence or absence of a genetic variation (e.g., a chromosome aneuploidy) is determined according to a profile comprising 1 to about 10,000 adjusted elevations. Often a determination of the presence or absence of a genetic variation (e.g., a chromosome

aneuploidy) is determined according to a profile comprising about 1 to about a 1000, 1 to about 900, 1 to about 800, 1 to about 700, 1 to about 600, 1 to about 500, 1 to about 400, 1 to about 300, 1 to about 200, 1 to about 100, 1 to about 50, 1 to about 25, 1 to about 20, 1 to about 15, 1 to about 10, or 1 to about 5 adjustments. Sometimes a determination of the presence or absence of a genetic variation (e.g., a chromosome aneuploidy) is determined according to a profile comprising about 1 adjustment (e.g., one adjusted elevation). Sometimes an outcome is determined according to one or more profiles (e.g., a profile of a chromosome or segment thereof) comprising one or more, 2 or more, 3 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more or sometimes 10 or more adjustments. Sometimes, a determination of the presence or absence of a genetic variation (e.g., a chromosome aneuploidy) is determined according to a profile where some elevations in a profile are not adjusted. Sometimes, a determination of the presence or absence of a genetic variation (e.g., a chromosome aneuploidy) is determined according to a profile where adjustments are not made.

In some embodiments, an adjustment of an elevation (e.g., a first elevation) in a profile reduces a false determination or false outcome. In some embodiments, an adjustment of an elevation (e.g., a first elevation) in a profile reduces the frequency and/or probability (e.g., statistical probability, likelihood) of a false determination or false outcome. A false determination or outcome can be a determination or outcome that is not accurate. A false determination or outcome can be a determination or outcome that is not reflective of the actual or true genetic make-up or the actual or true genetic disposition (e.g., the presence or absence of a genetic variation) of a subject (e.g., a pregnant female, a fetus and/or a combination thereof). Sometimes a false determination or outcome is a false negative determination. In some embodiments a negative determination or negative outcome is the absence of a genetic variation (e.g., aneuploidy, copy number variation). Sometimes a false determination or false outcome is a false positive determination or false positive outcome. In some embodiments a positive determination or positive outcome is the presence of a genetic variation (e.g., aneuploidy, copy number variation). In some embodiments, a determination or outcome is utilized in a diagnosis. In some embodiments, a determination or outcome is for a fetus.

Outcome

Methods described herein can provide a determination of the presence or absence of a genetic variation (e.g., fetal aneuploidy) for a sample, thereby providing an outcome (e.g., thereby

providing an outcome determinative of the presence or absence of a genetic variation (e.g., fetal aneuploidy)). A genetic variation often includes a gain, a loss and/or alteration (e.g., duplication, deletion, fusion, insertion, mutation, reorganization, substitution or aberrant methylation) of genetic information (e.g., chromosomes, segments of chromosomes, polymorphic regions, translocated regions, altered nucleotide sequence, the like or combinations of the foregoing) that results in a detectable change in the genome or genetic information of a test subject with respect to a reference. Presence or absence of a genetic variation can be determined by transforming, analyzing and/or manipulating sequence reads that have been mapped to genomic sections (e.g., genomic bins).

Methods described herein sometimes determine presence or absence of a fetal aneuploidy (e.g., full chromosome aneuploidy, partial chromosome aneuploidy or segmental chromosomal aberration (e.g., mosaicism, deletion and/or insertion)) for a test sample from a pregnant female bearing a fetus. Sometimes methods described herein detect euploidy or lack of euploidy (non-euploidy) for a sample from a pregnant female bearing a fetus. Methods described herein sometimes detect trisomy for one or more chromosomes (e.g., chromosome 13, chromosome 18, chromosome 21 or combination thereof) or segment thereof.

In some embodiments, presence or absence of a genetic variation (e.g., a fetal aneuploidy) is determined by a method described herein, by a method known in the art or by a combination thereof. Presence or absence of a genetic variation generally is determined from counts of sequence reads mapped to genomic sections of a reference genome. Counts of sequence reads utilized to determine presence or absence of a genetic variation sometimes are raw counts and/or filtered counts, and often are normalized counts. A suitable normalization process or processes can be used to generate normalized counts, non-limiting examples of which include bin-wise normalization, normalization by GC content, linear and nonlinear least squares regression, LOESS, GC LOESS, LOWESS, PERUN, RM, GCRM and combinations thereof. Normalized counts sometimes are expressed as one or more levels or elevations in a profile for a particular set or sets of genomic sections. Normalized counts sometimes are adjusted or padded prior to determining presence or absence of a genetic variation.

Presence or absence of a genetic variation (e.g., fetal aneuploidy) sometimes is determined without comparing counts for a set of genomic sections to a reference. Counts measured for a test sample and are in a test region (e.g., a set of genomic sections of interest) are referred to as "test

counts" herein. Test counts sometimes are processed counts, averaged or summed counts, a representation, normalized counts, or one or more levels or elevations, as described herein. Sometimes test counts are averaged or summed (e.g., an average, mean, median, mode or sum is calculated) for a set of genomic sections, and the averaged or summed counts are compared to a threshold or range. Test counts sometimes are expressed as a representation, which can be expressed as a ratio or percentage of counts for a first set of genomic sections to counts for a second set of genomic sections. Sometimes the first set of genomic sections is for one or more test chromosomes (e.g., chromosome 13, chromosome 18, chromosome 21, or combination thereof) and sometimes the second set of genomic sections is for the genome or a part of the genome (e.g., autosomes or autosomes and sex chromosomes). Sometimes a representation is compared to a threshold or range. Sometimes test counts are expressed as one or more levels or elevations for normalized counts over a set of genomic sections, and the one or more levels or elevations are compared to a threshold or range. Test counts (e.g., averaged or summed counts, representation, normalized counts, one or more levels or elevations) above or below a particular threshold, in a particular range or outside a particular range sometimes are determinative of the presence of a genetic variation or lack of euploidy (e.g., not euploidy). Test counts (e.g., averaged or summed counts, representation, normalized counts, one or more levels or elevations) below or above a particular threshold, in a particular range or outside a particular range sometimes are determinative of the absence of a genetic variation or euploidy.

Presence or absence of a genetic variation (e.g., fetal aneuploidy) sometimes is determined by comparing test counts (e.g., raw counts, filtered counts, averaged or summed counts, representation, normalized counts, one or more levels or elevations, for a set of genomic sections) to a reference. A reference can be a suitable determination of counts. Counts for a reference sometimes are raw counts, filtered counts, averaged or summed counts, representation, normalized counts, one or more levels or elevations, for a set of genomic sections. Reference counts often are counts for a euploid test region.

In certain embodiments, test counts sometimes are for a first set of genomic sections and a reference includes counts for a second set of genomic sections different than the first set of genomic sections. Reference counts sometimes are for a nucleic acid sample from the same pregnant female from which the test sample is obtained. Sometimes reference counts are for a nucleic acid sample from one or more pregnant females different than the female from which the test sample was obtained. In some embodiments, a first set of genomic sections is in chromosome

13, chromosome 18, chromosome 21, segment thereof or combination of the foregoing, and the second set of genomic sections is in another chromosome or chromosomes or segment thereof. In a non-limiting example, where a first set of genomic sections is in chromosome 21 or segment thereof, a second set of genomic sections often is in another chromosome (e.g., chromosome 1, chromosome 13, chromosome 14, chromosome 18, chromosome 19, segment thereof or combination of the foregoing). A reference often is located in a chromosome or segment thereof that is typically euploid. For example, chromosome 1 and chromosome 19 often are euploid in fetuses owing to a high rate of early fetal mortality associated with chromosome 1 and chromosome 19 aneuploidies. A measure of deviation between the test counts and the reference counts can be generated.

Sometimes a reference comprises counts for the same set of genomic sections as for the test counts, where the counts for the reference are from one or more reference samples (e.g., often multiple reference samples from multiple reference subjects). A reference sample often is from one or more pregnant females different than the female from which a test sample is obtained. A measure of deviation between the test counts and the reference counts can be generated.

A suitable measure of deviation between test counts and reference counts can be selected, non-limiting examples of which include standard deviation, average absolute deviation, median absolute deviation, maximum absolute deviation, standard score (e.g., z-value, z-score, normal score, standardized variable) and the like. In some embodiments, reference samples are euploid for a test region and deviation between the test counts and the reference counts is assessed. A deviation of less than three between test counts and reference counts (e.g., 3-sigma for standard deviation) often is indicative of a euploid test region (e.g., absence of a genetic variation). A deviation of greater than three between test counts and reference counts often is indicative of a non-euploid test region (e.g., presence of a genetic variation). Test counts significantly below reference counts, which reference counts are indicative of euploidy, sometimes are determinative of a monosomy. Test counts significantly above reference counts, which reference counts are indicative of euploidy, sometimes are determinative of a trisomy. A measure of deviation between test counts for a test sample and reference counts for multiple reference subjects can be plotted and visualized (e.g., z-score plot).

Any other suitable reference can be factored with test counts for determining presence or absence of a genetic variation (or determination of euploid or non-euploid) for a test region of a test sample.

For example, a fetal fraction determination can be factored with test counts to determine the presence or absence of a genetic variation. A suitable process for quantifying fetal fraction can be utilized, non-limiting examples of which include a mass spectrometric process, sequencing process or combination thereof.

Laboratory personnel (e.g., a laboratory manager) can analyze values (e.g., test counts, reference counts, level of deviation) underlying a determination of the presence or absence of a genetic variation (or determination of euploid or non-euploid for a test region). For calls pertaining to presence or absence of a genetic variation that are close or questionable, laboratory personnel can re-order the same test, and/or order a different test (e.g., karyotyping and/or amniocentesis in the case of fetal aneuploidy determinations), that makes use of the same or different sample nucleic acid from a test subject.

A genetic variation sometimes is associated with medical condition. An outcome determinative of a genetic variation is sometimes an outcome determinative of the presence or absence of a condition (e.g., a medical condition), disease, syndrome or abnormality, or includes, detection of a condition, disease, syndrome or abnormality (e.g., non-limiting examples listed in Table 1). In some cases a diagnosis comprises assessment of an outcome. An outcome determinative of the presence or absence of a condition (e.g., a medical condition), disease, syndrome or abnormality by methods described herein can sometimes be independently verified by further testing (e.g., by karyotyping and/or amniocentesis).

Analysis and processing of data can provide one or more outcomes. The term “outcome” as used herein can refer to a result of data processing that facilitates determining the presence or absence of a genetic variation (e.g., an aneuploidy, a copy number variation). Sometimes the term “outcome” as used herein refers to a conclusion that predicts and/or determines the presence or absence of a genetic variation (e.g., an aneuploidy, a copy number variation). Sometimes the term “outcome” as used herein refers to a conclusion that predicts and/or determines a risk or probability of the presence or absence of a genetic variation (e.g., an aneuploidy, a copy number variation) in a subject (e.g., a fetus). A diagnosis sometimes comprises use of an outcome. For example, a health practitioner may analyze an outcome and provide a diagnosis bases on, or based in part on, the outcome. In some embodiments, determination, detection or diagnosis of a condition, syndrome or abnormality (e.g., listed in Table 1) comprises use of an outcome determinative of the presence or absence of a genetic variation. In some embodiments, an

outcome based on counted mapped sequence reads or transformations thereof is determinative of the presence or absence of a genetic variation. In certain embodiments, an outcome generated utilizing one or more methods (e.g., data processing methods) described herein is determinative of the presence or absence of one or more conditions, syndromes or abnormalities listed in Table 1. Sometimes a diagnosis comprises a determination of a presence or absence of a condition, syndrome or abnormality. Often a diagnosis comprises a determination of a genetic variation as the nature and/or cause of a condition, syndrome or abnormality. Sometimes an outcome is not a diagnosis. An outcome often comprises one or more numerical values generated using a processing method described herein in the context of one or more considerations of probability. A consideration of risk or probability can include, but is not limited to: an uncertainty value, a measure of variability, confidence level, sensitivity, specificity, standard deviation, coefficient of variation (CV) and/or confidence level, Z-scores, Chi values, Phi values, ploidy values, fitted fetal fraction, area ratios, median elevation, the like or combinations thereof. A consideration of probability can facilitate determining whether a subject is at risk of having, or has, a genetic variation, and an outcome determinative of a presence or absence of a genetic disorder often includes such a consideration.

An outcome sometimes is a phenotype. An outcome sometimes is a phenotype with an associated level of confidence (e.g., an uncertainty value, e.g., a fetus is positive for trisomy 21 with a confidence level of 99%, a test subject is negative for a cancer associated with a genetic variation at a confidence level of 95%). Different methods of generating outcome values sometimes can produce different types of results. Generally, there are four types of possible scores or calls that can be made based on outcome values generated using methods described herein: true positive, false positive, true negative and false negative. The terms “score”, “scores”, “call” and “calls” as used herein refer to calculating the probability that a particular genetic variation is present or absent in a subject/sample. The value of a score may be used to determine, for example, a variation, difference, or ratio of mapped sequence reads that may correspond to a genetic variation. For example, calculating a positive score for a selected genetic variation or genomic section from a data set, with respect to a reference genome can lead to an identification of the presence or absence of a genetic variation, which genetic variation sometimes is associated with a medical condition (e.g., cancer, preeclampsia, trisomy, monosomy, and the like). In some embodiments, an outcome comprises an elevation, a profile and/or a plot (e.g., a profile plot). In those embodiments in which an outcome comprises a profile, a suitable profile or combination of profiles can be used for an outcome. Non-limiting examples of profiles that can be used for an

outcome include z-score profiles, p-value profiles, chi value profiles, phi value profiles, the like, and combinations thereof

An outcome generated for determining the presence or absence of a genetic variation sometimes includes a null result (e.g., a data point between two clusters, a numerical value with a standard deviation that encompasses values for both the presence and absence of a genetic variation, a data set with a profile plot that is not similar to profile plots for subjects having or free from the genetic variation being investigated). In some embodiments, an outcome indicative of a null result still is a determinative result, and the determination can include the need for additional information and/or a repeat of the data generation and/or analysis for determining the presence or absence of a genetic variation.

An outcome can be generated after performing one or more processing steps described herein, in some embodiments. In certain embodiments, an outcome is generated as a result of one of the processing steps described herein, and in some embodiments, an outcome can be generated after each statistical and/or mathematical manipulation of a data set is performed. An outcome pertaining to the determination of the presence or absence of a genetic variation can be expressed in a suitable form, which form comprises without limitation, a probability (e.g., odds ratio, p-value), likelihood, value in or out of a cluster, value over or under a threshold value, value within a range (e.g., a threshold range), value with a measure of variance or confidence, or risk factor, associated with the presence or absence of a genetic variation for a subject or sample. In certain embodiments, comparison between samples allows confirmation of sample identity (e.g., allows identification of repeated samples and/or samples that have been mixed up (e.g., mislabeled, combined, and the like)).

In some embodiments, an outcome comprises a value above or below a predetermined threshold or cutoff value (e.g., greater than 1, less than 1), and an uncertainty or confidence level associated with the value. Sometimes a predetermined threshold or cutoff value is an expected elevation or an expected elevation range. An outcome also can describe an assumption used in data processing. In certain embodiments, an outcome comprises a value that falls within or outside a predetermined range of values (e.g., a threshold range) and the associated uncertainty or confidence level for that value being inside or outside the range. In some embodiments, an outcome comprises a value that is equal to a predetermined value (e.g., equal to 1, equal to zero), or is equal to a value within a predetermined value range, and its associated uncertainty or

confidence level for that value being equal or within or outside a range. An outcome sometimes is graphically represented as a plot (e.g., profile plot).

As noted above, an outcome can be characterized as a true positive, true negative, false positive or false negative. The term "true positive" as used herein refers to a subject correctly diagnosed as having a genetic variation. The term "false positive" as used herein refers to a subject wrongly identified as having a genetic variation. The term "true negative" as used herein refers to a subject correctly identified as not having a genetic variation. The term "false negative" as used herein refers to a subject wrongly identified as not having a genetic variation. Two measures of performance for any given method can be calculated based on the ratios of these occurrences: (i) a sensitivity value, which generally is the fraction of predicted positives that are correctly identified as being positives; and (ii) a specificity value, which generally is the fraction of predicted negatives correctly identified as being negative. The term "sensitivity" as used herein refers to the number of true positives divided by the number of true positives plus the number of false negatives, where sensitivity (sens) may be within the range of $0 \leq sens \leq 1$. Ideally, the number of false negatives equal zero or close to zero, so that no subject is wrongly identified as not having at least one genetic variation when they indeed have at least one genetic variation. Conversely, an assessment often is made of the ability of a prediction algorithm to classify negatives correctly, a complementary measurement to sensitivity. The term "specificity" as used herein refers to the number of true negatives divided by the number of true negatives plus the number of false positives, where sensitivity (spec) may be within the range of $0 \leq spec \leq 1$. Ideally, the number of false positives equal zero or close to zero, so that no subject is wrongly identified as having at least one genetic variation when they do not have the genetic variation being assessed.

In certain embodiments, one or more of sensitivity, specificity and/or confidence level are expressed as a percentage. In some embodiments, the percentage, independently for each variable, is greater than about 90% (e.g., about 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%, or greater than 99% (e.g., about 99.5%, or greater, about 99.9% or greater, about 99.95% or greater, about 99.99% or greater)). Coefficient of variation (CV) in some embodiments is expressed as a percentage, and sometimes the percentage is about 10% or less (e.g., about 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1%, or less than 1% (e.g., about 0.5% or less, about 0.1% or less, about 0.05% or less, about 0.01% or less)). A probability (e.g., that a particular outcome is not due to chance) in certain embodiments is expressed as a Z-score, a p-value, or the results of a t-test. In some embodiments, a measured variance, confidence interval, sensitivity, specificity and the like (e.g.,

referred to collectively as confidence parameters) for an outcome can be generated using one or more data processing manipulations described herein. Specific examples of generating outcomes and associated confidence levels are described in the Example section.

A method that has sensitivity and specificity equaling one, or 100%, or near one (e.g., between about 90% to about 99%) sometimes is selected. In some embodiments, a method having a sensitivity equaling 1, or 100% is selected, and in certain embodiments, a method having a sensitivity near 1 is selected (e.g., a sensitivity of about 90%, a sensitivity of about 91%, a sensitivity of about 92%, a sensitivity of about 93%, a sensitivity of about 94%, a sensitivity of about 95%, a sensitivity of about 96%, a sensitivity of about 97%, a sensitivity of about 98%, or a sensitivity of about 99%). In some embodiments, a method having a specificity equaling 1, or 100% is selected, and in certain embodiments, a method having a specificity near 1 is selected (e.g., a specificity of about 90%, a specificity of about 91%, a specificity of about 92%, a specificity of about 93%, a specificity of about 94%, a specificity of about 95%, a specificity of about 96%, a specificity of about 97%, a specificity of about 98%, or a specificity of about 99%).

Outcome Module

The presence or absence of a genetic variation (an aneuploidy, a fetal aneuploidy, a copy number variation) can be identified by an outcome module or by an apparatus comprising an outcome module. Sometimes a genetic variation is identified by an outcome module. Often a determination of the presence or absence of an aneuploidy is identified by an outcome module. In some embodiments, an outcome determinative of a genetic variation (an aneuploidy, a copy number variation) can be identified by an outcome module or by an apparatus comprising an outcome module. An outcome module can be specialized for determining a specific genetic variation (e.g., a trisomy, a trisomy 21, a trisomy 18). For example, an outcome module that identifies a trisomy 21 can be different than and/or distinct from an outcome module that identifies a trisomy 18. In some embodiments, an outcome module or an apparatus comprising an outcome module is required to identify a genetic variation or an outcome determinative of a genetic variation (e.g., an aneuploidy, a copy number variation). An apparatus comprising an outcome module can comprise at least one processor. In some embodiments, a genetic variation or an outcome determinative of a genetic variation is provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the outcome module. In some embodiments, a

genetic variation or an outcome determinative of a genetic variation is identified by an apparatus that may include multiple processors, such as processors coordinated and working in parallel. In some embodiments, an outcome module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). Sometimes an apparatus comprising an outcome module gathers, assembles and/or receives data and/or information from another module or apparatus. Sometimes an apparatus comprising an outcome module provides and/or transfers data and/or information to another module or apparatus. Sometimes an outcome module transfers, receives or gathers data and/or information to or from a component or peripheral. Often an outcome module receives, gathers and/or assembles counts, elevations, profiles, normalized data and/or information, reference elevations, expected elevations, expected ranges, uncertainty values, adjustments, adjusted elevations, plots, categorized elevations, comparisons and/or constants. Sometimes an outcome module accepts and gathers input data and/or information from an operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to an outcome module. In some embodiments, data and/or information are provided by an apparatus that includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, identification of a genetic variation or an outcome determinative of a genetic variation is provided by an apparatus comprising a suitable peripheral or component. An apparatus comprising an outcome module can receive normalized data from a normalization module, expected elevations and/or ranges from a range setting module, comparison data from a comparison module, categorized elevations from a categorization module, plots from a plotting module, and/or adjustment data from an adjustment module. An outcome module can receive data and/or information, transform the data and/or information and provide an outcome. An outcome module can provide or transfer data and/or information related to a genetic variation or an outcome determinative of a genetic variation to a suitable apparatus and/or module. A genetic variation or an outcome determinative of a genetic variation identified by methods described herein can be independently verified by further testing (e.g., by targeted sequencing of maternal and/or fetal nucleic acid).

After one or more outcomes have been generated, an outcome often is used to provide a determination of the presence or absence of a genetic variation and/or associated medical condition. An outcome typically is provided to a health care professional (e.g., laboratory technician or manager; physician or assistant). Often an outcome is provided by an outcome module. Sometimes an outcome is provided by a plotting module. Sometimes an outcome is

provided on a peripheral or component of an apparatus. For example, sometimes an outcome is provided by a printer or display. In some embodiments, an outcome determinative of the presence or absence of a genetic variation is provided to a healthcare professional in the form of a report, and in certain embodiments the report comprises a display of an outcome value and an associated confidence parameter. Generally, an outcome can be displayed in a suitable format that facilitates determination of the presence or absence of a genetic variation and/or medical condition. Non-limiting examples of formats suitable for use for reporting and/or displaying data sets or reporting an outcome include digital data, a graph, a 2D graph, a 3D graph, and 4D graph, a picture, a pictograph, a chart, a bar graph, a pie graph, a diagram, a flow chart, a scatter plot, a map, a histogram, a density chart, a function graph, a circuit diagram, a block diagram, a bubble map, a constellation diagram, a contour diagram, a cartogram, spider chart, Venn diagram, nomogram, and the like, and combination of the foregoing. Various examples of outcome representations are shown in the drawings and are described in the Examples.

Generating an outcome can be viewed as a transformation of nucleic acid sequence read data, or the like, into a representation of a subject's cellular nucleic acid, in certain embodiments. For example, analyzing sequence reads of nucleic acid from a subject and generating a chromosome profile and/or outcome can be viewed as a transformation of relatively small sequence read fragments to a representation of relatively large chromosome structure. In some embodiments, an outcome results from a transformation of sequence reads from a subject (e.g., a pregnant female), into a representation of an existing structure (e.g., a genome, a chromosome or segment thereof) present in the subject (e.g., a maternal and/or fetal nucleic acid). In some embodiments, an outcome comprises a transformation of sequence reads from a first subject (e.g., a pregnant female), into a composite representation of structures (e.g., a genome, a chromosome or segment thereof), and a second transformation of the composite representation that yields a representation of a structure present in a first subject (e.g., a pregnant female) and/or a second subject (e.g., a fetus).

Use of Outcomes

A health care professional, or other qualified individual, receiving a report comprising one or more outcomes determinative of the presence or absence of a genetic variation can use the displayed data in the report to make a call regarding the status of the test subject or patient. The healthcare professional can make a recommendation based on the provided outcome, in some embodiments.

A health care professional or qualified individual can provide a test subject or patient with a call or score with regards to the presence or absence of the genetic variation based on the outcome value or values and associated confidence parameters provided in a report, in some embodiments. In certain embodiments, a score or call is made manually by a healthcare professional or qualified individual, using visual observation of the provided report. In certain embodiments, a score or call is made by an automated routine, sometimes embedded in software, and reviewed by a healthcare professional or qualified individual for accuracy prior to providing information to a test subject or patient. The term "receiving a report" as used herein refers to obtaining, by a communication means, a written and/or graphical representation comprising an outcome, which upon review allows a healthcare professional or other qualified individual to make a determination as to the presence or absence of a genetic variation in a test subject or patient. The report may be generated by a computer or by human data entry, and can be communicated using electronic means (e.g., over the internet, via computer, via fax, from one network location to another location at the same or different physical sites), or by a other method of sending or receiving data (e.g., mail service, courier service and the like). In some embodiments the outcome is transmitted to a health care professional in a suitable medium, including, without limitation, in verbal, document, or file form. The file may be, for example, but not limited to, an auditory file, a computer readable file, a paper file, a laboratory file or a medical record file.

The term "providing an outcome" and grammatical equivalents thereof, as used herein also can refer to a method for obtaining such information, including, without limitation, obtaining the information from a laboratory (e.g., a laboratory file). A laboratory file can be generated by a laboratory that carried out one or more assays or one or more data processing steps to determine the presence or absence of the medical condition. The laboratory may be in the same location or different location (e.g., in another country) as the personnel identifying the presence or absence of the medical condition from the laboratory file. For example, the laboratory file can be generated in one location and transmitted to another location in which the information therein will be transmitted to the pregnant female subject. The laboratory file may be in tangible form or electronic form (e.g., computer readable form), in certain embodiments.

In some embodiments, an outcome can be provided to a health care professional, physician or qualified individual from a laboratory and the health care professional, physician or qualified individual can make a diagnosis based on the outcome. In some embodiments, an outcome can be provided to a health care professional, physician or qualified individual from a laboratory and

the health care professional, physician or qualified individual can make a diagnosis based, in part, on the outcome along with additional data and/or information and other outcomes

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A healthcare professional or qualified individual, can provide a suitable recommendation based on the outcome or outcomes provided in the report. Non-limiting examples of recommendations that can be provided based on the provided outcome report includes, surgery, radiation therapy, chemotherapy, genetic counseling, after birth treatment solutions (e.g., life planning, long term assisted care, medicaments, symptomatic treatments), pregnancy termination, organ transplant, blood transfusion, the like or combinations of the foregoing. In some embodiments the recommendation is dependent on the outcome based classification provided (e.g., Down's syndrome, Turner syndrome, medical conditions associated with genetic variations in T13, medical conditions associated with genetic variations in T18).

Software can be used to perform one or more steps in the processes described herein, including but not limited to; counting, data processing, generating an outcome, and/or providing one or more recommendations based on generated outcomes, as described in greater detail hereafter.

Transformations

As noted above, data sometimes is transformed from one form into another form. The terms "transformed", "transformation", and grammatical derivations or equivalents thereof, as used herein refer to an alteration of data from a physical starting material (e.g., test subject and/or reference subject sample nucleic acid) into a digital representation of the physical starting material (e.g., sequence read data), and in some embodiments includes a further transformation into one or more numerical values or graphical representations of the digital representation that can be utilized to provide an outcome. In certain embodiments, the one or more numerical values and/or graphical representations of digitally represented data can be utilized to represent the appearance of a test subject's physical genome (e.g., virtually represent or visually represent the presence or absence of a genomic insertion, duplication or deletion; represent the presence or absence of a variation in the physical amount of a sequence associated with medical conditions). A virtual representation sometimes is further transformed into one or more numerical values or graphical representations of the digital representation of the starting material. These procedures can transform physical starting material into a numerical value or graphical representation, or a representation of the physical appearance of a test subject's genome.

In some embodiments, transformation of a data set facilitates providing an outcome by reducing data complexity and/or data dimensionality. Data set complexity sometimes is reduced during the process of transforming a physical starting material into a virtual representation of the starting material (e.g., sequence reads representative of physical starting material). A suitable feature or variable can be utilized to reduce data set complexity and/or dimensionality. Non-limiting examples of features that can be chosen for use as a target feature for data processing include GC content, fetal gender prediction, identification of chromosomal aneuploidy, identification of particular genes or proteins, identification of cancer, diseases, inherited genes/traits, chromosomal abnormalities, a biological category, a chemical category, a biochemical category, a category of genes or proteins, a gene ontology, a protein ontology, co-regulated genes, cell signaling genes, cell cycle genes, proteins pertaining to the foregoing genes, gene variants, protein variants, co-regulated genes, co-regulated proteins, amino acid sequence, nucleotide sequence, protein structure data and the like, and combinations of the foregoing. Non-limiting examples of data set complexity and/or dimensionality reduction include; reduction of a plurality of sequence reads to profile plots, reduction of a plurality of sequence reads to numerical values (e.g., normalized values, Z-scores, p-values); reduction of multiple analysis methods to probability plots or single points; principle component analysis of derived quantities; and the like or combinations thereof.

Genomic Section Normalization Systems, Apparatus and Computer Program Products

In certain aspects provided is a system comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads of circulating, cell-free sample nucleic acid from a test subject mapped to genomic sections of a reference genome; and which instructions executable by the one or more processors are configured to: (a) generate a sample normalized count profile by normalizing counts of the sequence reads for each of the genomic sections; and (b) determine the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both from the sample normalized count profile in (a).

Provided also in certain aspects is an apparatus comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads of circulating, cell-free sample nucleic acid from a test subject mapped to genomic sections of a reference genome; and which instructions executable by the one or more processors are configured to: (a) generate a sample normalized

count profile by normalizing counts of the sequence reads for each of the genomic sections; and (b) determine the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both from the sample normalized count profile in (a).

Also provided in certain aspects is a computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to: (a) access counts of sequence reads of circulating, cell-free sample nucleic acid from a test subject mapped to genomic sections of a reference genome; (b) generate a sample normalized count profile by normalizing counts of the sequence reads for each of the genomic sections; and (c) determine the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both from the sample normalized count profile in (b).

In some embodiments, the counts of the sequence reads for each of the genomic sections in a segment of the reference genome (e.g., the segment is a chromosome) individually are normalized according to the total counts of sequence reads in the genomic sections in the segment. Certain genomic sections in the segment sometimes are removed (e.g., filtered) and the remaining genomic sections in the segment are normalized.

In certain embodiments, the system, apparatus and/or computer program product comprises a: (i) a sequencing module configured to obtain nucleic acid sequence reads; (ii) a mapping module configured to map nucleic acid sequence reads to portions of a reference genome; (iii) a weighting module configured to weight genomic sections, (iv) a filtering module configured to filter genomic sections or counts mapped to a genomic section, (v) a counting module configured to provide counts of nucleic acid sequence reads mapped to portions of a reference genome; (vi) a normalization module configured to provide normalized counts; (vii) a comparison module configured to provide an identification of a first elevation that is significantly different than a second elevation; (viii) a range setting module configured to provide one or more expected level ranges; (ix) a categorization module configured to identify an elevation representative of a copy number variation; (x) an adjustment module configured to adjust a level identified as a copy number variation; (xi) a plotting module configured to graph and display a level and/or a profile; (xii) an outcome module configured to determine an outcome (e.g., outcome determinative of the presence or absence of a fetal aneuploidy); (xiii) a data display organization module configured to indicate the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both; (xiv) a logic processing module configured to perform one or more of map sequence reads, count

mapped sequence reads, normalize counts and generate an outcome; or (xv) combination of two or more of the foregoing.

In some embodiments the sequencing module and mapping module are configured to transfer sequence reads from the sequencing module to the mapping module. The mapping module and counting module sometimes are configured to transfer mapped sequence reads from the mapping module to the counting module. The counting module and filtering module sometimes are configured to transfer counts from the counting module to the filtering module. The counting module and weighting module sometimes are configured to transfer counts from the counting module to the weighting module. The mapping module and filtering module sometimes are configured to transfer mapped sequence reads from the mapping module to the filtering module. The mapping module and weighting module sometimes are configured to transfer mapped sequence reads from the mapping module to the weighting module. Sometimes the weighting module, filtering module and counting module are configured to transfer filtered and/or weighted genomic sections from the weighting module and filtering module to the counting module. The weighting module and normalization module sometimes are configured to transfer weighted genomic sections from the weighting module to the normalization module. The filtering module and normalization module sometimes are configured to transfer filtered genomic sections from the filtering module to the normalization module. In some embodiments, the normalization module and/or comparison module are configured to transfer normalized counts to the comparison module and/or range setting module. The comparison module, range setting module and/or categorization module independently are configured to transfer (i) an identification of a first elevation that is significantly different than a second elevation and/or (ii) an expected level range from the comparison module and/or range setting module to the categorization module, in some embodiments. In certain embodiments, the categorization module and the adjustment module are configured to transfer an elevation categorized as a copy number variation from the categorization module to the adjustment module. In some embodiments, the adjustment module, plotting module and the outcome module are configured to transfer one or more adjusted levels from the adjustment module to the plotting module or outcome module. The normalization module sometimes is configured to transfer mapped normalized sequence read counts to one or more of the comparison module, range setting module, categorization module, adjustment module, outcome module or plotting module.

Parameterized Error Removal and Unbiased Normalization Systems, Apparatus and Computer Program Products

Provided in certain aspects is a system comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a test sample; and which instructions executable by the one or more processors are configured to: (a) determine a guanine and cytosine (GC) bias for each of the portions of the reference genome for multiple samples from a fitted relation for each sample between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) GC content for each of the portions; and (b) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between (i) the GC bias and (ii) the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels, whereby bias in the counts of the sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels.

Also provided in some aspects is an apparatus comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a test sample; and which instructions executable by the one or more processors are configured to: (a) determine a guanine and cytosine (GC) bias for each of the portions of the reference genome for multiple samples from a fitted relation for each sample between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) GC content for each of the portions; and (b) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between (i) the GC bias and (ii) the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels, whereby bias in the counts of the sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels.

Also provided in certain aspects is a computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to: (a) access counts of sequence reads mapped to portions of a reference genome,

which sequence reads are reads of circulating cell-free nucleic acid from a test sample; (b) determine a guanine and cytosine (GC) bias for each of the portions of the reference genome for multiple samples from a fitted relation for each sample between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) GC content for each of the portions; and (c) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between (i) the GC bias and (ii) the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels, whereby bias in the counts of the sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels.

Provided in certain aspects is a system comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female bearing a fetus; and which instructions executable by the one or more processors are configured to: (a) determine a guanine and cytosine (GC) bias for each of the portions of the reference genome for multiple samples from a fitted relation for each sample between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) GC content for each of the portions; (b) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between the GC bias and the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels; and (c) identify the presence or absence of an aneuploidy for the fetus according to the calculated genomic section levels with a sensitivity of 95% or greater and a specificity of 95% or greater.

Also provided in certain aspects is an apparatus comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female bearing a fetus; and which instructions executable by the one or more processors are configured to: (a) determine a guanine and cytosine (GC) bias for each of the portions of the reference genome for multiple samples from a fitted relation for each sample between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) GC content for each of the portions; (b) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between the GC bias and the counts of the sequence reads mapped to each

of the portions of the reference genome, thereby providing calculated genomic section levels; and (c) identify the presence or absence of an aneuploidy for the fetus according to the calculated genomic section levels with a sensitivity of 95% or greater and a specificity of 95% or greater.

Provided also in certain aspects is a computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to: (a) access counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female bearing a fetus; (b) determine a guanine and cytosine (GC) bias for each of the portions of the reference genome for multiple samples from a fitted relation for each sample between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) GC content for each of the portions; (c) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between the GC bias and the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels; and (d) identify the presence or absence of an aneuploidy for the fetus according to the calculated genomic section levels with a sensitivity of 95% or greater and a specificity of 95% or greater.

Also provided in certain aspects is a system comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female bearing a fetus; and which instructions executable by the one or more processors are configured to: (a) determine experimental bias for each of the portions of the reference genome for multiple samples from a fitted relation between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) a mapping feature for each of the portions; and (b) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between the experimental bias and the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels, whereby bias in the counts of the sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels.

Provided also in certain aspects is an apparatus comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which

memory comprises counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female bearing a fetus; and which instructions executable by the one or more processors are configured to: (a) determine experimental bias for each of the portions of the reference genome for multiple samples from a fitted relation between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) a mapping feature for each of the portions; and (b) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between the experimental bias and the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels, whereby bias in the counts of the sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels.

Also provided in certain aspects is a computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to: (a) access counts of sequence reads mapped to portions of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a test sample; (b) determine experimental bias for each of the portions of the reference genome for multiple samples from a fitted relation between (i) the counts of the sequence reads mapped to each of the portions of the reference genome, and (ii) a mapping feature for each of the portions; and (c) calculate a genomic section level for each of the portions of the reference genome from a fitted relation between the experimental bias and the counts of the sequence reads mapped to each of the portions of the reference genome, thereby providing calculated genomic section levels, whereby bias in the counts of the sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels.

In certain embodiments, the system, apparatus and/or computer program product comprises a: (i) a sequencing module configured to obtain nucleic acid sequence reads; (ii) a mapping module configured to map nucleic acid sequence reads to portions of a reference genome; (iii) a weighting module configured to weight genomic sections; (iv) a filtering module configured to filter genomic sections or counts mapped to a genomic section; (v) a counting module configured to provide counts of nucleic acid sequence reads mapped to portions of a reference genome; (vi) a normalization module configured to provide normalized counts; (vii) a comparison module configured to provide an identification of a first elevation that is significantly different than a second elevation; (viii) a range setting module configured to provide one or more expected level ranges;

(ix) a categorization module configured to identify an elevation representative of a copy number variation; (x) an adjustment module configured to adjust a level identified as a copy number variation; (xi) a plotting module configured to graph and display a level and/or a profile; (xii) an outcome module configured to determine an outcome (e.g., outcome determinative of the presence or absence of a fetal aneuploidy); (xiii) a data display organization module configured to indicate the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both; (xiv) a logic processing module configured to perform one or more of map sequence reads, count mapped sequence reads, normalize counts and generate an outcome; or (xv) combination of two or more of the foregoing.

In some embodiments the sequencing module and mapping module are configured to transfer sequence reads from the sequencing module to the mapping module. The mapping module and counting module sometimes are configured to transfer mapped sequence reads from the mapping module to the counting module. The counting module and filtering module sometimes are configured to transfer counts from the counting module to the filtering module. The counting module and weighting module sometimes are configured to transfer counts from the counting module to the weighting module. The mapping module and filtering module sometimes are configured to transfer mapped sequence reads from the mapping module to the filtering module. The mapping module and weighting module sometimes are configured to transfer mapped sequence reads from the mapping module to the weighting module. Sometimes the weighting module, filtering module and counting module are configured to transfer filtered and/or weighted genomic sections from the weighting module and filtering module to the counting module. The weighting module and normalization module sometimes are configured to transfer weighted genomic sections from the weighting module to the normalization module. The filtering module and normalization module sometimes are configured to transfer filtered genomic sections from the filtering module to the normalization module. In some embodiments, the normalization module and/or comparison module are configured to transfer normalized counts to the comparison module and/or range setting module. The comparison module, range setting module and/or categorization module independently are configured to transfer (i) an identification of a first elevation that is significantly different than a second elevation and/or (ii) an expected level range from the comparison module and/or range setting module to the categorization module, in some embodiments. In certain embodiments, the categorization module and the adjustment module are configured to transfer an elevation categorized as a copy number variation from the categorization module to the adjustment module. In some embodiments, the adjustment module, plotting module

and the outcome module are configured to transfer one or more adjusted levels from the adjustment module to the plotting module or outcome module. The normalization module sometimes is configured to transfer mapped normalized sequence read counts to one or more of the comparison module, range setting module, categorization module, adjustment module, outcome module or plotting module.

Adjustment Systems, Apparatus and Computer Program Products

Provided in certain aspects is a system comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of nucleic acid sequence reads mapped to genomic sections of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female; and which instructions executable by the one or more processors are configured to: (a) normalize the counts mapped to the genomic sections of the reference genome, thereby providing a profile of normalized counts for the genomic sections; (b) identify a first elevation of the normalized counts significantly different than a second elevation of the normalized counts in the profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections; (c) determine an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the genome; (d) adjust the first elevation by a predetermined value when the first elevation is within one of the expected elevation ranges, thereby providing an adjustment of the first elevation; and (e) determine the presence or absence of a chromosome aneuploidy in the fetus according to the elevations of genomic sections comprising the adjustment of (d), whereby the outcome determinative of the presence or absence of the chromosome aneuploidy is generated from the nucleic acid sequence reads.

Also provided in some aspects is an apparatus comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of nucleic acid sequence reads mapped to genomic sections of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female; and which instructions executable by the one or more processors are configured to: (a) normalize the counts mapped to the genomic sections of the reference genome, thereby providing a profile of normalized counts for the genomic sections; (b) identify a first elevation of the normalized counts significantly different than a second elevation of the normalized counts in the

profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections; (c) determine an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the genome; (d) adjust the first elevation by a predetermined value when the first elevation is within one of the expected elevation ranges, thereby providing an adjustment of the first elevation; and (e) determine the presence or absence of a chromosome aneuploidy in the fetus according to the elevations of genomic sections comprising the adjustment of (d), whereby the outcome determinative of the presence or absence of the chromosome aneuploidy is generated from the nucleic acid sequence reads.

Provided also in certain aspects is a computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to: (a) access counts of nucleic acid sequence reads mapped to genomic sections of a reference genome, which sequence reads are reads of circulating cell-free nucleic acid from a pregnant female; (b) normalize the counts mapped to the genomic sections of the reference genome, thereby providing a profile of normalized counts for the genomic sections; (c) identify a first elevation of the normalized counts significantly different than a second elevation of the normalized counts in the profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections; (d) determine an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the genome; (e) adjust the first elevation by a predetermined value when the first elevation is within one of the expected elevation ranges, thereby providing an adjustment of the first elevation; and (f) determine the presence or absence of a chromosome aneuploidy in the fetus according to the elevations of genomic sections comprising the adjustment of (e), whereby the outcome determinative of the presence or absence of the chromosome aneuploidy is generated from the nucleic acid sequence reads.

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profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections; (c) determine an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the genome; and (d) identify a maternal and/or fetal copy number variation within the genomic section based on one of the expected elevation ranges, whereby the maternal and/or fetal copy number variation is identified from the nucleic acid sequence reads.

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whereby the maternal and/or fetal copy number variation is identified from the nucleic acid sequence reads.

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of (d), whereby the outcome determinative of the presence or absence of the chromosome aneuploidy is generated from the nucleic acid sequence reads.

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In certain embodiments, the system, apparatus and/or computer program product comprises a: (i) a sequencing module configured to obtain nucleic acid sequence reads; (ii) a mapping module configured to map nucleic acid sequence reads to portions of a reference genome; (iii) a weighting module configured to weight genomic sections; (iv) a filtering module configured to filter genomic sections or counts mapped to a genomic section; (v) a counting module configured to provide counts of nucleic acid sequence reads mapped to portions of a reference genome; (vi) a normalization module configured to provide normalized counts; (vii) a comparison module configured to provide an identification of a first elevation that is significantly different than a second elevation; (viii) a range setting module configured to provide one or more expected level ranges; (ix) a categorization module configured to identify an elevation representative of a copy number variation; (x) an adjustment module configured to adjust a level identified as a copy number variation; (xi) a plotting module configured to graph and display a level and/or a profile; (xii) an outcome module configured to determine an outcome (e.g., outcome determinative of the presence or absence of a fetal aneuploidy); (xiii) a data display organization module configured to indicate

the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both; (xiv) a logic processing module configured to perform one or more of map sequence reads, count mapped sequence reads, normalize counts and generate an outcome; or (xv) combination of two or more of the foregoing.

In some embodiments the sequencing module and mapping module are configured to transfer sequence reads from the sequencing module to the mapping module. The mapping module and counting module sometimes are configured to transfer mapped sequence reads from the mapping module to the counting module. The counting module and filtering module sometimes are configured to transfer counts from the counting module to the filtering module. The counting module and weighting module sometimes are configured to transfer counts from the counting module to the weighting module. The mapping module and filtering module sometimes are configured to transfer mapped sequence reads from the mapping module to the filtering module. The mapping module and weighting module sometimes are configured to transfer mapped sequence reads from the mapping module to the weighting module. Sometimes the weighting module, filtering module and counting module are configured to transfer filtered and/or weighted genomic sections from the weighting module and filtering module to the counting module. The weighting module and normalization module sometimes are configured to transfer weighted genomic sections from the weighting module to the normalization module. The filtering module and normalization module sometimes are configured to transfer filtered genomic sections from the filtering module to the normalization module. In some embodiments, the normalization module and/or comparison module are configured to transfer normalized counts to the comparison module and/or range setting module. The comparison module, range setting module and/or categorization module independently are configured to transfer (i) an identification of a first elevation that is significantly different than a second elevation and/or (ii) an expected level range from the comparison module and/or range setting module to the categorization module, in some embodiments. In certain embodiments, the categorization module and the adjustment module are configured to transfer an elevation categorized as a copy number variation from the categorization module to the adjustment module. In some embodiments, the adjustment module, plotting module and the outcome module are configured to transfer one or more adjusted levels from the adjustment module to the plotting module or outcome module. The normalization module sometimes is configured to transfer mapped normalized sequence read counts to one or more of the comparison module, range setting module, categorization module, adjustment module, outcome module or plotting module.

Machines, Software and Interfaces

Certain processes and methods described herein (e.g., quantifying, mapping, normalizing, range setting, adjusting, categorizing, counting and/or determining sequence reads, counts, elevations (e.g., elevations) and/or profiles) often cannot be performed without a computer, processor, software, module or other apparatus. Methods described herein typically are computer-implemented methods, and one or more portions of a method sometimes are performed by one or more processors. Embodiments pertaining to methods described in this document generally are applicable to the same or related processes implemented by instructions in systems, apparatus and computer program products described herein. In some embodiments, processes and methods described herein (e.g., quantifying, counting and/or determining sequence reads, counts, elevations and/or profiles) are performed by automated methods. In some embodiments, an automated method is embodied in software, modules, processors, peripherals and/or an apparatus comprising the like, that determine sequence reads, counts, mapping, mapped sequence tags, elevations, profiles, normalizations, comparisons, range setting, categorization, adjustments, plotting, outcomes, transformations and identifications. As used herein, software refers to computer readable program instructions that, when executed by a processor, perform computer operations, as described herein.

Sequence reads, counts, elevations, and profiles derived from a test subject (e.g., a patient, a pregnant female) and/or from a reference subject can be further analyzed and processed to determine the presence or absence of a genetic variation. Sequence reads, counts, elevations and/or profiles sometimes are referred to as “data” or “data sets”. In some embodiments, data or data sets can be characterized by one or more features or variables (e.g., sequence based [e.g., GC content, specific nucleotide sequence, the like], function specific [e.g., expressed genes, cancer genes, the like], location based [genome specific, chromosome specific, genomic section or bin specific], the like and combinations thereof). In certain embodiments, data or data sets can be organized into a matrix having two or more dimensions based on one or more features or variables. Data organized into matrices can be organized using any suitable features or variables. A non-limiting example of data in a matrix includes data that is organized by maternal age, maternal ploidy, and fetal contribution. In certain embodiments, data sets characterized by one or more features or variables sometimes are processed after counting.

Apparatuses, software and interfaces may be used to conduct methods described herein. Using apparatuses, software and interfaces, a user may enter, request, query or determine options for using particular information, programs or processes (e.g., mapping sequence reads, processing mapped data and/or providing an outcome), which can involve implementing statistical analysis algorithms, statistical significance algorithms, statistical algorithms, iterative steps, validation algorithms, and graphical representations, for example. In some embodiments, a data set may be entered by a user as input information, a user may download one or more data sets by a suitable hardware media (e.g., flash drive), and/or a user may send a data set from one system to another for subsequent processing and/or providing an outcome (e.g., send sequence read data from a sequencer to a computer system for sequence read mapping; send mapped sequence data to a computer system for processing and yielding an outcome and/or report).

A system typically comprises one or more apparatus. Each apparatus comprises one or more of memory, one or more processors, and instructions. Where a system includes two or more apparatus, some or all of the apparatus may be located at the same location, some or all of the apparatus may be located at different locations, all of the apparatus may be located at one location and/or all of the apparatus may be located at different locations. Where a system includes two or more apparatus, some or all of the apparatus may be located at the same location as a user, some or all of the apparatus may be located at a location different than a user, all of the apparatus may be located at the same location as the user, and/or all of the apparatus may be located at one or more locations different than the user.

A system sometimes comprises a computing apparatus and a sequencing apparatus, where the sequencing apparatus is configured to receive physical nucleic acid and generate sequence reads, and the computing apparatus is configured to process the reads from the sequencing apparatus. The computing apparatus sometimes is configured to determine the presence or absence of a genetic variation (e.g., copy number variation; fetal chromosome aneuploidy) from the sequence reads.

A user may, for example, place a query to software which then may acquire a data set via internet access, and in certain embodiments, a programmable processor may be prompted to acquire a suitable data set based on given parameters. A programmable processor also may prompt a user to select one or more data set options selected by the processor based on given parameters. A programmable processor may prompt a user to select one or more data set options selected by the

processor based on information found via the internet, other internal or external information, or the like. Options may be chosen for selecting one or more data feature selections, one or more statistical algorithms, one or more statistical analysis algorithms, one or more statistical significance algorithms, iterative steps, one or more validation algorithms, and one or more graphical representations of methods, apparatuses, or computer programs.

Systems addressed herein may comprise general components of computer systems, such as, for example, network servers, laptop systems, desktop systems, handheld systems, personal digital assistants, computing kiosks, and the like. A computer system may comprise one or more input means such as a keyboard, touch screen, mouse, voice recognition or other means to allow the user to enter data into the system. A system may further comprise one or more outputs, including, but not limited to, a display screen (e.g., CRT or LCD), speaker, FAX machine, printer (e.g., laser, ink jet, impact, black and white or color printer), or other output useful for providing visual, auditory and/or hardcopy output of information (e.g., outcome and/or report).

In a system, input and output means may be connected to a central processing unit which may comprise among other components, a microprocessor for executing program instructions and memory for storing program code and data. In some embodiments, processes may be implemented as a single user system located in a single geographical site. In certain embodiments, processes may be implemented as a multi-user system. In the case of a multi-user implementation, multiple central processing units may be connected by means of a network. The network may be local, encompassing a single department in one portion of a building, an entire building, span multiple buildings, span a region, span an entire country or be worldwide. The network may be private, being owned and controlled by a provider, or it may be implemented as an internet based service where the user accesses a web page to enter and retrieve information. Accordingly, in certain embodiments, a system includes one or more machines, which may be local or remote with respect to a user. More than one machine in one location or multiple locations may be accessed by a user, and data may be mapped and/or processed in series and/or in parallel. Thus, a suitable configuration and control may be utilized for mapping and/or processing data using multiple machines, such as in local network, remote network and/or "cloud" computing platforms.

A system can include a communications interface in some embodiments. A communications interface allows for transfer of software and data between a computer system and one or more

external devices. Non-limiting examples of communications interfaces include a modem, a network interface (such as an Ethernet card), a communications port, a PCMCIA slot and card, and the like. Software and data transferred via a communications interface generally are in the form of signals, which can be electronic, electromagnetic, optical and/or other signals capable of being received by a communications interface. Signals often are provided to a communications interface via a channel. A channel often carries signals and can be implemented using wire or cable, fiber optics, a phone line, a cellular phone link, an RF link and/or other communications channels. Thus, in an example, a communications interface may be used to receive signal information that can be detected by a signal detection module.

Data may be input by a suitable device and/or method, including, but not limited to, manual input devices or direct data entry devices (DDEs). Non-limiting examples of manual devices include keyboards, concept keyboards, touch sensitive screens, light pens, mouse, tracker balls, joysticks, graphic tablets, scanners, digital cameras, video digitizers and voice recognition devices. Non-limiting examples of DDEs include bar code readers, magnetic strip codes, smart cards, magnetic ink character recognition, optical character recognition, optical mark recognition, and turnaround documents.

In some embodiments, output from a sequencing apparatus may serve as data that can be input via an input device. In certain embodiments, mapped sequence reads may serve as data that can be input via an input device. In certain embodiments, simulated data is generated by an in silico process and the simulated data serves as data that can be input via an input device. The term "in silico" refers to research and experiments performed using a computer. In silico processes include, but are not limited to, mapping sequence reads and processing mapped sequence reads according to processes described herein.

A system may include software useful for performing a process described herein, and software can include one or more modules for performing such processes (e.g., sequencing module, logic processing module, data display organization module). The term "software" refers to computer readable program instructions that, when executed by a computer, perform computer operations. Instructions executable by the one or more processors sometimes are provided as executable code, that when executed, can cause one or more processors to implement a method described herein. A module described herein can exist as software, and instructions (e.g., processes, routines, subroutines) embodied in the software can be implemented or performed by a processor.

For example, a module (e.g., a software module) can be a part of a program that performs a particular process or task. The term “module” refers to a self-contained functional unit that can be used in a larger apparatus or software system. A module can comprise a set of instructions for carrying out a function of the module. A module can transform data and/or information. Data and/or information can be in a suitable form. For example, data and/or information can be digital or analogue. In some cases, data and/or information can be packets, bytes, characters, or bits. In some embodiments, data and/or information can be any gathered, assembled or usable data or information. Non-limiting examples of data and/or information include a suitable media, pictures, video, sound (e.g. frequencies, audible or non-audible), numbers, constants, a value, objects, time, functions, instructions, maps, references, sequences, reads, mapped reads, elevations, ranges, thresholds, signals, displays, representations, or transformations thereof. A module can accept or receive data and/or information, transform the data and/or information into a second form, and provide or transfer the second form to an apparatus, peripheral, component or another module. A module can perform one or more of the following non-limiting functions: mapping sequence reads, providing counts, assembling genomic sections, providing or determining an elevation, providing a count profile, normalizing (e.g., normalizing reads, normalizing counts, and the like), providing a normalized count profile or elevations of normalized counts, comparing two or more elevations, providing uncertainty values, providing or determining expected elevations and expected ranges(e.g., expected elevation ranges, threshold ranges and threshold elevations), providing adjustments to elevations (e.g., adjusting a first elevation, adjusting a second elevation, adjusting a profile of a chromosome or a segment thereof, and/or padding), providing identification (e.g., identifying a copy number variation, genetic variation or aneuploidy), categorizing, plotting, and/or determining an outcome, for example. A processor can, in some cases, carry out the instructions in a module. In some embodiments, one or more processors are required to carry out instructions in a module or group of modules. A module can provide data and/or information to another module, apparatus or source and can receive data and/or information from another module, apparatus or source.

A computer program product sometimes is embodied on a tangible computer-readable medium, and sometimes is tangibly embodied on a non-transitory computer-readable medium. A module sometimes is stored on a computer readable medium (e.g., disk, drive) or in memory (e.g., random access memory). A module and processor capable of implementing instructions from a module can be located in an apparatus or in different apparatus. A module and/or processor capable of implementing an instruction for a module can be located in the same location as a user (e.g., local

network) or in a different location from a user (e.g., remote network, cloud system). In embodiments in which a method is carried out in conjunction with two or more modules, the modules can be located in the same apparatus, one or more modules can be located in different apparatus in the same physical location, and one or more modules may be located in different apparatus in different physical locations.

An apparatus, in some embodiments, comprises at least one processor for carrying out the instructions in a module. Counts of sequence reads mapped to genomic sections of a reference genome sometimes are accessed by a processor that executes instructions configured to carry out a method described herein. Counts that are accessed by a processor can be within memory of a system, and the counts can be accessed and placed into the memory of the system after they are obtained. In some embodiments, an apparatus includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from a module. In some embodiments, an apparatus includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, an apparatus operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)). In some embodiments, an apparatus comprises a module. Sometimes an apparatus comprises one or more modules. An apparatus comprising a module often can receive and transfer one or more of data and/or information to and from other modules. In some cases, an apparatus comprises peripherals and/or components. Sometimes an apparatus can comprise one or more peripherals or components that can transfer data and/or information to and from other modules, peripherals and/or components. Sometimes an apparatus interacts with a peripheral and/or component that provides data and/or information. Sometimes peripherals and components assist an apparatus in carrying out a function or interact directly with a module. Non-limiting examples of peripherals and/or components include a suitable computer peripheral, I/O or storage method or device including but not limited to scanners, printers, displays (e.g., monitors, LED, LCT or CRTs), cameras, microphones, pads (e.g., ipads, tablets), touch screens, smart phones, mobile phones, USB I/O devices, USB mass storage devices, keyboards, a computer mouse, digital pens, modems, hard drives, jump drives, flash drives, a processor, a server, CDs, DVDs, graphic cards, specialized I/O devices (e.g., sequencers, photo cells, photo multiplier tubes, optical readers, sensors, etc.), one or more flow cells, fluid handling components, network interface controllers, ROM, RAM, wireless transfer methods and devices (Bluetooth, WiFi, and the like,), the world wide web (www), the internet, a computer and/or another module.

One or more of a sequencing module, logic processing module and data display organization module can be utilized in a method described herein. Sometimes a logic processing module, sequencing module or data display organization module, or an apparatus comprising one or more such modules, gather, assemble, receive, provide and/or transfer data and/or information to or from another module, apparatus, component, peripheral or operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to a logic processing module, sequencing module or data display organization module. A logic processing module, sequencing module or data display organization module can receive data and/or information from another module, non-limiting examples of which include a logic processing module, sequencing module, data display organization module, sequencing module, sequencing module, mapping module, counting module, normalization module, comparison module, range setting module, categorization module, adjustment module, plotting module, outcome module, data display organization module and/or logic processing module, the like or combination thereof. Data and/or information derived from or transformed by a logic processing module, sequencing module or data display organization module can be transferred from a logic processing module, sequencing module or data display organization module to a sequencing module, sequencing module, mapping module, counting module, normalization module, comparison module, range setting module, categorization module, adjustment module, plotting module, outcome module, data display organization module, logic processing module or other suitable apparatus and/or module. A sequencing module can receive data and/or information form a logic processing module and/or sequencing module and transfer data and/or information to a logic processing module and/or a mapping module, for example. Sometimes a logic processing module orchestrates, controls, limits, organizes, orders, distributes, partitions, transforms and/or regulates data and/or information or the transfer of data and/or information to and from one or more other modules, peripherals or devices. A data display organization module can receive data and/or information form a logic processing module and/or plotting module and transfer data and/or information to a logic processing module, plotting module, display, peripheral or device. An apparatus comprising a logic processing module, sequencing module or data display organization module can comprise at least one processor. In some embodiments, data and/or information are provided by an apparatus that includes a processor (e.g., one or more processors) which processor can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) from the logic processing module, sequencing module and/or data display organization module. In some embodiments, a logic processing module, sequencing module or

data display organization module operates with one or more external processors (e.g., an internal or external network, server, storage device and/or storage network (e.g., a cloud)).

Software often is provided on a program product containing program instructions recorded on a computer readable medium, including, but not limited to, magnetic media including floppy disks, hard disks, and magnetic tape; and optical media including CD-ROM discs, DVD discs, magneto-optical discs, flash drives, RAM, floppy discs, the like, and other such media on which the program instructions can be recorded. In online implementation, a server and web site maintained by an organization can be configured to provide software downloads to remote users, or remote users may access a remote system maintained by an organization to remotely access software. Software may obtain or receive input information. Software may include a module that specifically obtains or receives data (e.g., a data receiving module that receives sequence read data and/or mapped read data) and may include a module that specifically processes the data (e.g., a processing module that processes received data (e.g., filters, normalizes, provides an outcome and/or report). The terms "obtaining" and "receiving" input information refers to receiving data (e.g., sequence reads, mapped reads) by computer communication means from a local, or remote site, human data entry, or any other method of receiving data. The input information may be generated in the same location at which it is received, or it may be generated in a different location and transmitted to the receiving location. In some embodiments, input information is modified before it is processed (e.g., placed into a format amenable to processing (e.g., tabulated)).
In some embodiments, provided are computer program products, such as, for example, a computer program product comprising a computer usable medium having a computer readable program code embodied therein, the computer readable program code adapted to be executed to implement a method comprising: (a) obtaining sequence reads of sample nucleic acid from a test subject; (b) mapping the sequence reads obtained in (a) to a known genome, which known genome has been divided into genomic sections; (c) counting the mapped sequence reads within the genomic sections; (d) generating a sample normalized count profile by normalizing the counts for the genomic sections obtained in (c); and (e) determining the presence or absence of a genetic variation from the sample normalized count profile in (d).

Software can include one or more algorithms in certain embodiments. An algorithm may be used for processing data and/or providing an outcome or report according to a finite sequence of instructions. An algorithm often is a list of defined instructions for completing a task. Starting from an initial state, the instructions may describe a computation that proceeds through a defined series

of successive states, eventually terminating in a final ending state. The transition from one state to the next is not necessarily deterministic (e.g., some algorithms incorporate randomness). By way of example, and without limitation, an algorithm can be a search algorithm, sorting algorithm, merge algorithm, numerical algorithm, graph algorithm, string algorithm, modeling algorithm, computational genometric algorithm, combinatorial algorithm, machine learning algorithm, cryptography algorithm, data compression algorithm, parsing algorithm and the like. An algorithm can include one algorithm or two or more algorithms working in combination. An algorithm can be of any suitable complexity class and/or parameterized complexity. An algorithm can be used for calculation and/or data processing, and in some embodiments, can be used in a deterministic or probabilistic/predictive approach. An algorithm can be implemented in a computing environment by use of a suitable programming language, non-limiting examples of which are C, C++, Java, Perl, Python, Fortran, and the like. In some embodiments, an algorithm can be configured or modified to include margin of errors, statistical analysis, statistical significance, and/or comparison to other information or data sets (e.g., applicable when using a neural net or clustering algorithm).

In certain embodiments, several algorithms may be implemented for use in software. These algorithms can be trained with raw data in some embodiments. For each new raw data sample, the trained algorithms may produce a representative processed data set or outcome. A processed data set sometimes is of reduced complexity compared to the parent data set that was processed. Based on a processed set, the performance of a trained algorithm may be assessed based on sensitivity and specificity, in some embodiments. An algorithm with the highest sensitivity and/or specificity may be identified and utilized, in certain embodiments.

In certain embodiments, simulated (or simulation) data can aid data processing, for example, by training an algorithm or testing an algorithm. In some embodiments, simulated data includes hypothetical various samplings of different groupings of sequence reads. Simulated data may be based on what might be expected from a real population or may be skewed to test an algorithm and/or to assign a correct classification. Simulated data also is referred to herein as “virtual” data. Simulations can be performed by a computer program in certain embodiments. One possible step in using a simulated data set is to evaluate the confidence of an identified result, e.g., how well a random sampling matches or best represents the original data. One approach is to calculate a probability value (p-value), which estimates the probability of a random sample having better score than the selected samples. In some embodiments, an empirical model may be assessed, in which it is assumed that at least one sample matches a reference sample (with or without resolved

variations). In some embodiments, another distribution, such as a Poisson distribution for example, can be used to define the probability distribution.

A system may include one or more processors in certain embodiments. A processor can be connected to a communication bus. A computer system may include a main memory, often random access memory (RAM), and can also include a secondary memory. Memory in some embodiments comprises a non-transitory computer-readable storage medium. Secondary memory can include, for example, a hard disk drive and/or a removable storage drive, representing a floppy disk drive, a magnetic tape drive, an optical disk drive, memory card and the like. A removable storage drive often reads from and/or writes to a removable storage unit. Non-limiting examples of removable storage units include a floppy disk, magnetic tape, optical disk, and the like, which can be read by and written to by, for example, a removable storage drive. A removable storage unit can include a computer-usable storage medium having stored therein computer software and/or data.

A processor may implement software in a system. In some embodiments, a processor may be programmed to automatically perform a task described herein that a user could perform. Accordingly, a processor, or algorithm conducted by such a processor, can require little to no supervision or input from a user (e.g., software may be programmed to implement a function automatically). In some embodiments, the complexity of a process is so large that a single person or group of persons could not perform the process in a timeframe short enough for determining the presence or absence of a genetic variation.

In some embodiments, secondary memory may include other similar means for allowing computer programs or other instructions to be loaded into a computer system. For example, a system can include a removable storage unit and an interface device. Non-limiting examples of such systems include a program cartridge and cartridge interface (such as that found in video game devices), a removable memory chip (such as an EPROM, or PROM) and associated socket, and other removable storage units and interfaces that allow software and data to be transferred from the removable storage unit to a computer system.

One entity can generate counts of sequence reads, map the sequence reads to genomic sections, count the mapped reads, and utilize the counted mapped reads in a method, system, apparatus or computer program product described herein, in some embodiments. Counts of sequence reads

mapped to genomic sections sometimes are transferred by one entity to a second entity for use by the second entity in a method, system, apparatus or computer program product described herein, in certain embodiments.

In some embodiments, one entity generates sequence reads and a second entity maps those sequence reads to genomic sections in a reference genome in some embodiments. The second entity sometimes counts the mapped reads and utilizes the counted mapped reads in a method, system, apparatus or computer program product described herein. Sometimes the second entity transfers the mapped reads to a third entity, and the third entity counts the mapped reads and utilizes the mapped reads in a method, system, apparatus or computer program product described herein. Sometimes the second entity counts the mapped reads and transfers the counted mapped reads to a third entity, and the third entity utilizes the counted mapped reads in a method, system, apparatus or computer program product described herein. In embodiments involving a third entity, the third entity sometimes is the same as the first entity. That is, the first entity sometimes transfers sequence reads to a second entity, which second entity can map sequence reads to genomic sections in a reference genome and/or count the mapped reads, and the second entity can transfer the mapped and/or counted reads to a third entity. A third entity sometimes can utilize the mapped and/or counted reads in a method, system, apparatus or computer program product described herein, wherein the third entity sometimes is the same as the first entity, and sometimes the third entity is different from the first or second entity.

In some embodiments, one entity obtains blood from a pregnant female, optionally isolates nucleic acid from the blood (e.g., from the plasma or serum), and transfers the blood or nucleic acid to a second entity that generates sequence reads from the nucleic acid.

Certain System, Apparatus and Computer Program Product Embodiments

Multiple system, apparatus and computer program product embodiments are described herein. In certain aspects, provided is a system comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise: (i) multiple nucleobase gaps between identified nucleobases, or (ii) one or more nucleobase

classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii); and which instructions executable by the one or more processors are configured to: (a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and (b) detect the presence or absence of a genetic variation based on the normalized counts.

Provided also in certain aspects is an apparatus comprising one or more processors and memory, which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise: (i) multiple nucleobase gaps between identified nucleobases, or (ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii); and which instructions executable by the one or more processors are configured to: (a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and (b) detect the presence or absence of a genetic variation based on the normalized counts.

Also provide in certain aspects is a computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to: (a) access counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise: (i) multiple nucleobase gaps between identified nucleobases, or (ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or a combination of (i) and (ii); (b) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts; and (c) detect the presence or absence of a genetic variation based on the normalized counts.

In some embodiments, the system, apparatus and/or computer program product comprises a reference comparison module. A reference comparison module often is configured to compare the number of counts of partial nucleotide sequence reads mapped to genomic sections of a sample, or compare normalized counts, or derivative of the foregoing, to mapped counts of a reference, or portion thereof, thereby making a reference comparison. Counts for a reference segment and

counts for a test segment to which the reference counts are compared may be raw, filtered or normalized counts, or a combination of the foregoing. A reference sometimes is counts of sequence reads or partial nucleotide sequence reads mapped to a reference chromosome or segment thereof. Counts mapped for the reference sometimes are from the same sample or from a different sample with respect to the counts of partial nucleotide sequence reads or normalized counts to which the reference is compared. Sometimes, the reference is for a reference chromosome (e.g., chromosome 1, 14 and/or 19) or segment thereof, and counts for the reference are compared to counts of partial nucleotide sequence reads for a test chromosome (e.g., chromosome 13, 18 and/or 21) or segment thereof (a "test genomic segment"). A reference comparison module often is configured to access, receive, utilize, store, search for and/or align counts of partial nucleotide sequence reads (e.g., from a mapping module or normalization module). A reference comparison module often is configured to provide a suitable comparison between counts of partial nucleotide sequence reads for the test genomic segment and a reference, non-limiting examples of which comparison include a simple comparison (e.g., match or no match between counts of partial nucleotide sequence reads for the test genomic segment and counts for the reference), mathematical comparison (e.g., ratio, percentage), statistical comparison (e.g., multiple comparisons, multiple testing, standardization (e.g., Z-score analyses)), the like and combinations thereof. A suitable reference comparison value can be provided by a reference comparison module, non-limiting examples of which include presence or absence of a match between counts of partial nucleotide sequence reads for a test genomic segment and counts for a reference, a ratio, percentage, Z-score, a value coupled with a measure of variance or uncertainty (e.g., standard deviation, median absolute deviation, confidence interval), the like and combinations thereof. A reference comparison module sometimes is configured to transmit a comparison value to another module or apparatus, such as a an outcome module, display apparatus or printer apparatus, for example.

An apparatus or system comprising a module described herein (e.g., a reference comparison module) can comprise one or more processors. In some embodiments, an apparatus or system can include multiple processors, such as processors coordinated and working in parallel. A processor (e.g., one or more processors) in a system or apparatus can perform and/or implement one or more instructions (e.g., processes, routines and/or subroutines) in a module described herein. A module described herein sometimes is located in memory or associated with an apparatus or system. In some embodiments, a module described herein operates with one or more external processors (e.g., an internal or external network, server, storage device and/or

storage network (e.g., a cloud)). Sometimes a module described herein is configured to access, gather, assemble and/or receive data and/or information from another module, apparatus or system (e.g., component, peripheral). Sometimes a module described herein is configured to provide and/or transfer data and/or information to another module, apparatus or system (e.g., component, peripheral). Sometimes a module described herein is configured to access, accept, receive and/or gather input data and/or information from an operator of an apparatus or system (i.e., user). For example, sometimes a user provides a constant, a threshold value, a formula and/or a predetermined value to a module. A module described herein sometimes is configured to transform data and/or information it accesses, receives, gathers and/or assembles.

Modules described herein that are configured to generate, access, transfer and/or manipulate nucleotide sequence reads often are configured to generate, access, transfer and/or manipulate partial nucleotide sequence reads. In some embodiments, a sequencing apparatus is configured only to generate partial nucleotide sequence reads, and sometimes is configured to generate partial nucleotide sequence reads and full sequence reads (i.e., positions of each of four bases are known for reads).

In certain embodiments, a system, apparatus and/or computer program product comprises a: (i) a sequencing module configured to obtain nucleic acid sequence reads and/or partial nucleotide sequence reads; (ii) a mapping module configured to map nucleic acid sequence reads to portions of a reference genome; (iii) a counting module configured to provide counts of nucleic acid sequence reads mapped to portions of a reference genome; (iv) a normalization module configured to provide normalized counts; (v) a comparison module configured to provide an identification of a first elevation that is significantly different than a second elevation; (vi) a range setting module configured to provide one or more expected level ranges; (vii) a categorization module configured to identify an elevation representative of a copy number variation; (viii) an adjustment module configured to adjust a level identified as a copy number variation; (ix) a plotting module configured to graph and display a level and/or a profile; (x) an outcome module configured to determine the presence or absence of a genetic variation, or determine an outcome (e.g., outcome determinative of the presence or absence of a fetal aneuploidy); (xi) a data display organization module configured to indicate the presence or absence of a segmental chromosomal aberration or a fetal aneuploidy or both; (xii) a logic processing module configured to perform one or more of map sequence reads, count mapped sequence reads, normalize counts and generate an outcome; (xiii) a reference comparison module, or (xiv) combination of two or more of the foregoing.

In some embodiments a sequencing module and mapping module are configured to transfer sequence reads from the sequencing module to the mapping module. The mapping module and counting module sometimes are configured to transfer mapped sequence reads from the mapping module to the counting module. In some embodiments, the normalization module and/or comparison module are configured to transfer normalized counts to the comparison module and/or range setting module. The comparison module, range setting module and/or categorization module independently are configured to transfer (i) an identification of a first elevation that is significantly different than a second elevation and/or (ii) an expected level range from the comparison module and/or range setting module to the categorization module, in some embodiments. In certain embodiments, the categorization module and the adjustment module are configured to transfer an elevation categorized as a copy number variation from the categorization module to the adjustment module. In some embodiments, the adjustment module, plotting module and the outcome module are configured to transfer one or more adjusted levels from the adjustment module to the plotting module or outcome module. The normalization module sometimes is configured to transfer mapped normalized sequence read counts to one or more of the comparison module, range setting module, categorization module, adjustment module, outcome module or plotting module.

Genetic Variations and Medical Conditions

The presence or absence of a genetic variance can be determined using a method or apparatus described herein. In certain embodiments, the presence or absence of one or more genetic variations is determined according to an outcome provided by methods and apparatuses described herein. A genetic variation generally is a particular genetic phenotype present in certain individuals, and often a genetic variation is present in a statistically significant sub-population of individuals. In some embodiments, a genetic variation is a chromosome abnormality (e.g., aneuploidy), partial chromosome abnormality or mosaicism, each of which is described in greater detail herein. Non-limiting examples of genetic variations include one or more deletions (e.g., micro-deletions), duplications (e.g., micro-duplications), insertions, mutations, polymorphisms (e.g., single-nucleotide polymorphisms), fusions, repeats (e.g., short tandem repeats), distinct methylation sites, distinct methylation patterns, the like and combinations thereof. An insertion, repeat, deletion, duplication, mutation or polymorphism can be of any length, and in some embodiments, is about 1 base or base pair (bp) to about 250 megabases (Mb) in length. In some embodiments, an insertion, repeat, deletion, duplication, mutation or polymorphism is about 1 base

or base pair (bp) to about 1,000 kilobases (kb) in length (e.g., about 10 bp, 50 bp, 100 bp, 500 bp, 1kb, 5 kb, 10kb, 50 kb, 100 kb, 500 kb, or 1000 kb in length).

A genetic variation is sometime a deletion. Sometimes a deletion is a mutation (e.g., a genetic aberration) in which a part of a chromosome or a sequence of DNA is missing. A deletion is often the loss of genetic material. Any number of nucleotides can be deleted. A deletion can comprise the deletion of one or more entire chromosomes, a segment of a chromosome, an allele, a gene, an intron, an exon, any non-coding region, any coding region, a segment thereof or combination thereof. A deletion can comprise a microdeletion. A deletion can comprise the deletion of a single base.

A genetic variation is sometimes a genetic duplication. Sometimes a duplication is a mutation (e.g., a genetic aberration) in which a part of a chromosome or a sequence of DNA is copied and inserted back into the genome. Sometimes a genetic duplication (i.e. duplication) is any duplication of a region of DNA. In some embodiments, a duplication is a nucleic acid sequence that is repeated, often in tandem, within a genome or chromosome. In some embodiments, a duplication can comprise a copy of one or more entire chromosomes, a segment of a chromosome, an allele, a gene, an intron, an exon, any non-coding region, any coding region, segment thereof or combination thereof. A duplication can comprise a microduplication. A duplication sometimes comprises one or more copies of a duplicated nucleic acid. A duplication sometimes is characterized as a genetic region repeated one or more times (e.g., repeated 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 times). Duplications can range from small regions (thousands of base pairs) to whole chromosomes in some instances. Duplications frequently occur as the result of an error in homologous recombination or due to a retrotransposon event. Duplications have been associated with certain types of proliferative diseases. Duplications can be characterized using genomic microarrays or comparative genetic hybridization (CGH).

A genetic variation is sometimes an insertion. An insertion is sometimes the addition of one or more nucleotide base pairs into a nucleic acid sequence. An insertion is sometimes a microinsertion. Sometimes an insertion comprises the addition of a segment of a chromosome into a genome, chromosome, or segment thereof. Sometimes an insertion comprises the addition of an allele, a gene, an intron, an exon, any non-coding region, any coding region, segment thereof or combination thereof into a genome or segment thereof. Sometimes an insertion comprises the

addition (i.e., insertion) of nucleic acid of unknown origin into a genome, chromosome, or segment thereof. Sometimes an insertion comprises the addition (i.e. insertion) of a single base.

As used herein a "copy number variation" generally is a class or type of genetic variation or chromosomal aberration. A copy number variation can be a deletion (e.g. micro-deletion), duplication (e.g., a micro-duplication) or insertion (e.g., a micro-insertion). Often, the prefix "micro" as used herein sometimes is a segment of nucleic acid less than 5 Mb in length. A copy number variation can include one or more deletions (e.g. micro-deletion), duplications and/or insertions (e.g., a micro-duplication, micro-insertion) of a segment of a chromosome. In some cases a duplication comprises an insertion. Sometimes an insertion is a duplication. Sometimes an insertion is not a duplication. For example, often a duplication of a sequence in a genomic section increases the counts for a genomic section in which the duplication is found. Often a duplication of a sequence in a genomic section increases the elevation. Sometimes, a duplication present in genomic sections making up a first elevation increases the elevation relative to a second elevation where a duplication is absent. Sometimes an insertion increases the counts of a genomic section and a sequence representing the insertion is present (i.e., duplicated) at another location within the same genomic section. Sometimes an insertion does not significantly increase the counts of a genomic section or elevation and the sequence that is inserted is not a duplication of a sequence within the same genomic section. Sometimes an insertion is not detected or represented as a duplication and a duplicate sequence representing the insertion is not present in the same genomic section.

In some embodiments a copy number variation is a fetal copy number variation. Often, a fetal copy number variation is a copy number variation in the genome of a fetus. In some embodiments a copy number variation is a maternal copy number variation. Sometimes a maternal and/or fetal copy number variation is a copy number variation within the genome of a pregnant female (e.g., a female subject bearing a fetus), a female subject that gave birth or a female capable of bearing a fetus. A copy number variation can be a heterozygous copy number variation where the variation (e.g., a duplication or deletion) is present on one allele of a genome. A copy number variation can be a homozygous copy number variation where the variation is present on both alleles of a genome. In some embodiments a copy number variation is a heterozygous or homozygous fetal copy number variation. In some embodiments a copy number variation is a heterozygous or homozygous maternal and/or fetal copy number variation. A copy number variation sometimes is

present in a maternal genome and a fetal genome, a maternal genome and not a fetal genome, or a fetal genome and not a maternal genome.

"Ploidy" refers to the number of chromosomes present in a fetus or mother. Sometimes "Ploidy" is the same as "chromosome ploidy". In humans, for example, autosomal chromosomes are often present in pairs. For example, in the absence of a genetic variation, most humans have two of each autosomal chromosome (e.g., chromosomes 1-22). The presence of the normal complement of 2 autosomal chromosomes in a human is often referred to as euploid. "Microploidy" is similar in meaning to ploidy. "Microploidy" often refers to the ploidy of a segment of a chromosome. The term "microploidy" sometimes refers to the presence or absence of a copy number variation (e.g., a deletion, duplication and/or an insertion) within a chromosome (e.g., a homozygous or heterozygous deletion, duplication, or insertion, the like or absence thereof). "Ploidy" and "microploidy" sometimes are determined after normalization of counts of an elevation in a profile (e.g., after normalizing counts of an elevation to an NRV of 1). Thus, an elevation representing an autosomal chromosome pair (e.g., a euploid) is often normalized to an NRV of 1 and is referred to as a ploidy of 1. Similarly, an elevation within a segment of a chromosome representing the absence of a duplication, deletion or insertion is often normalized to an NRV of 1 and is referred to as a microploidy of 1. Ploidy and microploidy are often bin-specific (e.g., genomic section specific) and sample-specific. Ploidy is often defined as integral multiples of ½, with the values of 1, ½, 0, 3/2, and 2 representing euploidy (e.g., 2 chromosomes), 1 chromosome present (e.g., a chromosome deletion), no chromosome present, 3 chromosomes (e.g., a trisomy) and 4 chromosomes, respectively. Likewise, microploidy is often defined as integral multiples of ½, with the values of 1, ½, 0, 3/2, and 2 representing euploidy (e.g., no copy number variation), a heterozygous deletion, homozygous deletion, heterozygous duplication and homozygous duplication, respectively. Some examples of ploidy values for a fetus are provided in Table 2 for an NRV of 1.

Sometimes the microploidy of a fetus matches the microploidy of the mother of the fetus (i.e., the pregnant female subject). Sometimes the microploidy of a fetus matches the microploidy of the mother of the fetus and both the mother and fetus carry the same heterozygous copy number variation, homozygous copy number variation or both are euploid. Sometimes the microploidy of a fetus is different than the microploidy of the mother of the fetus. For example, sometimes the microploidy of a fetus is heterozygous for a copy number variation, the mother is homozygous for a

copy number variation and the microploidy of the fetus does not match (e.g., does not equal) the microploidy of the mother for the specified copy number variation.

A microploidy is often associated with an expected elevation. For example, sometimes an elevation (e.g., an elevation in a profile, sometimes an elevation that includes substantially no copy number variation) is normalized to an NRV of 1 and the microploidy of a homozygous duplication is 2, a heterozygous duplication is 1.5, a heterozygous deletion is 0.5 and a homozygous deletion is zero.

A genetic variation for which the presence or absence is identified for a subject is associated with a medical condition in certain embodiments. Thus, technology described herein can be used to identify the presence or absence of one or more genetic variations that are associated with a medical condition or medical state. Non-limiting examples of medical conditions include those associated with intellectual disability (e.g., Down Syndrome), aberrant cell-proliferation (e.g., cancer), presence of a micro-organism nucleic acid (e.g., virus, bacterium, fungus, yeast), and preeclampsia.

Non-limiting examples of genetic variations, medical conditions and states are described hereafter.

Fetal Gender

In some embodiments, the prediction of a fetal gender or gender related disorder (e.g., sex chromosome aneuploidy) can be determined by a method or apparatus described herein. Gender determination generally is based on a sex chromosome. In humans, there are two sex chromosomes, the X and Y chromosomes. The Y chromosome contains a gene, SRY, which triggers embryonic development as a male. The Y chromosomes of humans and other mammals also contain other genes needed for normal sperm production. Individuals with XX are female and XY are male and non-limiting variations, often referred to as sex chromosome aneuploidies, include X0, XYY, XXX and XXY. In some cases, males have two X chromosomes and one Y chromosome (XXY; Klinefelter's Syndrome), or one X chromosome and two Y chromosomes (XYY syndrome; Jacobs Syndrome), and some females have three X chromosomes (XXX; Triple X Syndrome) or a single X chromosome instead of two (X0; Turner Syndrome). In some cases, only a portion of cells in an individual are affected by a sex chromosome aneuploidy which may be

referred to as a mosaicism (e.g., Turner mosaicism). Other cases include those where SRY is damaged (leading to an XY female), or copied to the X (leading to an XX male).

In certain cases, it can be beneficial to determine the gender of a fetus in utero. For example, a patient (e.g., pregnant female) with a family history of one or more sex-linked disorders may wish to determine the gender of the fetus she is carrying to help assess the risk of the fetus inheriting such a disorder. Sex-linked disorders include, without limitation, X-linked and Y-linked disorders. X-linked disorders include X-linked recessive and X-linked dominant disorders. Examples of X-linked recessive disorders include, without limitation, immune disorders (e.g., chronic granulomatous disease (CYBB), Wiskott–Aldrich syndrome, X-linked severe combined immunodeficiency, X-linked agammaglobulinemia, hyper-IgM syndrome type 1, IPEX, X-linked lymphoproliferative disease, Properdin deficiency), hematologic disorders (e.g., Hemophilia A, Hemophilia B, X-linked sideroblastic anemia), endocrine disorders (e.g., androgen insensitivity syndrome/Kennedy disease, KAL1 Kallmann syndrome, X-linked adrenal hypoplasia congenital), metabolic disorders (e.g., ornithine transcarbamylase deficiency, oculocerebrorenal syndrome, adrenoleukodystrophy, glucose-6-phosphate dehydrogenase deficiency, pyruvate dehydrogenase deficiency, Danon disease/glycogen storage disease Type IIb, Fabry's disease, Hunter syndrome, Lesch–Nyhan syndrome, Menkes disease/occipital horn syndrome), nervous system disorders (e.g., Coffin–Lowry syndrome, MASA syndrome, X-linked alpha thalassemia mental retardation syndrome, Siderius X-linked mental retardation syndrome, color blindness, ocular albinism, Norrie disease, choroideremia, Charcot–Marie–Tooth disease (CMTX2-3), Pelizaeus–Merzbacher disease, SMAX2), skin and related tissue disorders (e.g., dyskeratosis congenital, hypohidrotic ectodermal dysplasia (EDA), X-linked ichthyosis, X-linked endothelial corneal dystrophy), neuromuscular disorders (e.g., Becker's muscular dystrophy/Duchenne, centronuclear myopathy (MTM1), Conradi–Hünermann syndrome, Emery–Dreifuss muscular dystrophy 1), urologic disorders (e.g., Alport syndrome, Dent's disease, X-linked nephrogenic diabetes insipidus), bone/tooth disorders (e.g., AMELX Amelogenesis imperfecta), and other disorders (e.g., Barth syndrome, McLeod syndrome, Smith-Fineman-Myers syndrome, Simpson–Golabi–Behmel syndrome, Mohr–Tranebjærg syndrome, Nasodigitoacoustic syndrome). Examples of X-linked dominant disorders include, without limitation, X-linked hypophosphatemia, Focal dermal hypoplasia, Fragile X syndrome, Aicardi syndrome, Incontinentia pigmenti, Rett syndrome, CHILD syndrome, Lujan–Fryns syndrome, and Orofaciodigital syndrome 1. Examples of Y-linked disorders include, without limitation, male infertility, retinits pigmentosa, and azoospermia.

Chromosome Abnormalities

In some embodiments, the presence or absence of a fetal chromosome abnormality can be determined by using a method or apparatus described herein. Chromosome abnormalities include, without limitation, a gain or loss of an entire chromosome or a region of a chromosome comprising one or more genes. Chromosome abnormalities include monosomies, trisomies, polysomies, loss of heterozygosity, deletions and/or duplications of one or more nucleotide sequences (e.g., one or more genes), including deletions and duplications caused by unbalanced translocations. The terms "aneuploidy" and "aneuploid" as used herein refer to an abnormal number of chromosomes in cells of an organism. As different organisms have widely varying chromosome complements, the term "aneuploidy" does not refer to a particular number of chromosomes, but rather to the situation in which the chromosome content within a given cell or cells of an organism is abnormal. In some embodiments, the term “aneuploidy” herein refers to an imbalance of genetic material caused by a loss or gain of a whole chromosome, or part of a chromosome. An “aneuploidy” can refer to one or more deletions and/or insertions of a segment of a chromosome.

The term "monosomy" as used herein refers to lack of one chromosome of the normal complement. Partial monosomy can occur in unbalanced translocations or deletions, in which only a segment of the chromosome is present in a single copy. Monosomy of sex chromosomes (45, X) causes Turner syndrome, for example.

The term "disomy" refers to the presence of two copies of a chromosome. For organisms such as humans that have two copies of each chromosome (those that are diploid or "euploid"), disomy is the normal condition. For organisms that normally have three or more copies of each chromosome (those that are triploid or above), disomy is an aneuploid chromosome state. In uniparental disomy, both copies of a chromosome come from the same parent (with no contribution from the other parent).

The term “euploid”, in some embodiments, refers a normal complement of chromosomes.

The term "trisomy" as used herein refers to the presence of three copies, instead of two copies, of a particular chromosome. The presence of an extra chromosome 21, which is found in human Down syndrome, is referred to as "Trisomy 21." Trisomy 18 and Trisomy 13 are two other human

autosomal trisomies. Trisomy of sex chromosomes can be seen in females (e.g., 47, XXX in Triple X Syndrome) or males (e.g., 47, XXY in Klinefelter's Syndrome; or 47, XYY in Jacobs Syndrome).

The terms "tetrasomy" and "pentasomy" as used herein refer to the presence of four or five copies of a chromosome, respectively. Although rarely seen with autosomes, sex chromosome tetrasomy and pentasomy have been reported in humans, including XXXX, XXXY, XXYY, XYYY, XXXXX, XXXXY, XXXYY, XXYYY and XYYYY.

Chromosome abnormalities can be caused by a variety of mechanisms. Mechanisms include, but are not limited to (i) nondisjunction occurring as the result of a weakened mitotic checkpoint, (ii) inactive mitotic checkpoints causing non-disjunction at multiple chromosomes, (iii) merotelic attachment occurring when one kinetochore is attached to both mitotic spindle poles, (iv) a multipolar spindle forming when more than two spindle poles form, (v) a monopolar spindle forming when only a single spindle pole forms, and (vi) a tetraploid intermediate occurring as an end result of the monopolar spindle mechanism.

The terms "partial monosomy" and "partial trisomy" as used herein refer to an imbalance of genetic material caused by loss or gain of part of a chromosome. A partial monosomy or partial trisomy can result from an unbalanced translocation, where an individual carries a derivative chromosome formed through the breakage and fusion of two different chromosomes. In this situation, the individual would have three copies of part of one chromosome (two normal copies and the segment that exists on the derivative chromosome) and only one copy of part of the other chromosome involved in the derivative chromosome.

The term "mosaicism" as used herein refers to aneuploidy in some cells, but not all cells, of an organism. Certain chromosome abnormalities can exist as mosaic and non-mosaic chromosome abnormalities. For example, certain trisomy 21 individuals have mosaic Down syndrome and some have non-mosaic Down syndrome. Different mechanisms can lead to mosaicism. For example, (i) an initial zygote may have three 21st chromosomes, which normally would result in simple trisomy 21, but during the course of cell division one or more cell lines lost one of the 21st chromosomes; and (ii) an initial zygote may have two 21st chromosomes, but during the course of cell division one of the 21st chromosomes were duplicated. Somatic mosaicism likely occurs through mechanisms distinct from those typically associated with genetic syndromes involving complete or mosaic aneuploidy. Somatic mosaicism has been identified in certain types of cancers and in neurons, for

example. In certain instances, trisomy 12 has been identified in chronic lymphocytic leukemia (CLL) and trisomy 8 has been identified in acute myeloid leukemia (AML). Also, genetic syndromes in which an individual is predisposed to breakage of chromosomes (chromosome instability syndromes) are frequently associated with increased risk for various types of cancer, thus highlighting the role of somatic aneuploidy in carcinogenesis. Methods and protocols described herein can identify presence or absence of non-mosaic and mosaic chromosome abnormalities.

Tables 1A and 1B present a non-limiting list of chromosome conditions, syndromes and/or abnormalities that can be potentially identified by methods and apparatus described herein. Table 1B is from the DECIPHER database as of October 6, 2011 (e.g., version 5.1, based on positions mapped to GRCh37; available at uniform resource locator (URL) dechipher.sanger.ac.uk).

Table 1A

Chromosome	Abnormality	Disease Association
X	XO	Turner's Syndrome
Y	XXY	Klinefelter syndrome
Y	XYY	Double Y syndrome
Y	XXX	Trisomy X syndrome
Y	XXXX	Four X syndrome
Y	Xp21 deletion	Duchenne's/Becker syndrome, congenital adrenal hypoplasia, chronic granulomatus disease
Y	Xp22 deletion	steroid sulfatase deficiency
Y	Xq26 deletion	X-linked lymphproliferative disease
1	1p (somatic) monosomy trisomy	neuroblastoma
2	monosomy trisomy 2q	growth retardation, developmental and mental delay, and minor physical abnormalities
3	monosomy trisomy (somatic)	Non-Hodgkin's lymphoma
4	monosomy trisomy (somatic)	Acute non lymphocytic leukemia (ANLL)
5	5p	Cri du chat; Lejeune syndrome
5	5q (somatic) monosomy trisomy	myelodysplastic syndrome
6	monosomy trisomy (somatic)	clear-cell sarcoma
7	7q11.23 deletion	William's syndrome
7	monosomy trisomy	monosomy 7 syndrome of childhood; somatic: renal cortical adenomas; myelodysplastic syndrome
8	8q24.1 deletion	Langer-Giedon syndrome
8	monosomy trisomy	myelodysplastic syndrome; Warkany syndrome; somatic: chronic myelogenous leukemia
9	monosomy 9p	Alfi's syndrome
9	monosomy 9p partial trisomy	Rethore syndrome

Chromosome	Abnormality	Disease Association
9	trisomy	complete trisomy 9 syndrome; mosaic trisomy 9 syndrome
10	Monosomy trisomy (somatic)	ALL or ANLL
11	11p-	Aniridia; Wilms tumor
11	11q-	Jacobson Syndrome
11	monosomy (somatic) trisomy	myeloid lineages affected (ANLL, MDS)
12	monosomy trisomy (somatic)	CLL, Juvenile granulosa cell tumor (JGCT)
13	13q-	13q-syndrome; Orbeli syndrome
13	13q14 deletion	retinoblastoma
13	monosomy trisomy	Patau's syndrome
14	monosomy trisomy (somatic)	myeloid disorders (MDS, ANLL, atypical CML)
15	15q11-q13 deletion monosomy	Prader-Willi, Angelman's syndrome
15	trisomy (somatic)	myeloid and lymphoid lineages affected, e.g., MDS, ANLL, ALL, CLL)
16	16q13.3 deletion	Rubenstein-Taybi
3	monosomy trisomy (somatic)	papillary renal cell carcinomas (malignant)
17	17p-(somatic)	17p syndrome in myeloid malignancies
17	17q11.2 deletion	Smith-Magenis
17	17q13.3	Miller-Dieker
17	monosomy trisomy (somatic)	renal cortical adenomas
17	17p11.2-12 trisomy	Charcot-Marie Tooth Syndrome type 1; HNPP
18	18p-	18p partial monosomy syndrome or Grouchy Lamy Thieffry syndrome
18	18q-	Grouchy Lamy Salmon Landry Syndrome
18	monosomy trisomy	Edwards Syndrome

Chromosome	Abnormality	Disease Association
19	monosomy trisomy	
20	20p-	trisomy 20p syndrome
20	20p11.2-12 deletion	Alagille
20	20q-	somatic: MDS, ANLL, polycythemia vera, chronic neutrophilic leukemia
20	monosomy trisomy (somatic)	papillary renal cell carcinomas (malignant)
21	monosomy trisomy	Down's syndrome
22	22q11.2 deletion	DiGeorge's syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, autosomal dominant Opitz G/BBB syndrome, Caylor cardiofacial syndrome
22	monosomy trisomy	complete trisomy 22 syndrome

Table 1B

Syndrome	Chromosome	Start	End	Interval (Mb)	Grade
12q14 microdeletion syndrome	12	65,071,919	68,645,525	3.57	
15q13.3 microdeletion syndrome	15	30,769,995	32,701,482	1.93	
15q24 recurrent microdeletion syndrome	15	74,377,174	76,162,277	1.79	
15q26 overgrowth syndrome	15	99,357,970	102,521,392	3.16	
16p11.2 microduplication syndrome	16	29,501,198	30,202,572	0.70	
16p11.2-p12.2 microdeletion syndrome	16	21,613,956	29,042,192	7.43	
16p13.11 recurrent microdeletion (neurocognitive disorder susceptibility locus)	16	15,504,454	16,284,248	0.78	

Syndrome	Chromosome	Start	End	Interval (Mb)	Grade
16p13.11 recurrent microduplication (neurocognitive disorder susceptibility locus)	16	15,504,454	16,284,248	0.78	
17q21.3 recurrent microdeletion syndrome	17	43,632,466	44,210,205	0.58	1
1p36 microdeletion syndrome	1	10,001	5,408,761	5.40	1
1q21.1 recurrent microdeletion (susceptibility locus for neurodevelopmental disorders)	1	146,512,930	147,737,500	1.22	3
1q21.1 recurrent microduplication (possible susceptibility locus for neurodevelopmental disorders)	1	146,512,930	147,737,500	1.22	3
1q21.1 susceptibility locus for Thrombocytopenia-Absent Radius (TAR) syndrome	1	145,401,253	145,928,123	0.53	3
22q11 deletion syndrome (Velocardiofacial / DiGeorge syndrome)	22	18,546,349	22,336,469	3.79	1
22q11 duplication syndrome	22	18,546,349	22,336,469	3.79	3
22q11.2 distal deletion syndrome	22	22,115,848	23,696,229	1.58	
22q13 deletion syndrome (Phelan-Mcdermid syndrome)	22	51,045,516	51,187,844	0.14	1
2p15-16.1 microdeletion syndrome	2	57,741,796	61,738,334	4.00	
2q33.1 deletion syndrome	2	196,925,089	205,206,940	8.28	1
2q37 monosomy	2	239,954,693	243,102,476	3.15	1
3q29 microdeletion	3	195,672,229	197,497,869	1.83	

Syndrome	Chromosome	Start	End	Interval (Mb)	Grade
syndrome					
3q29 microduplication syndrome	3	195,672,229	197,497,869	1.83	
7q11.23 duplication syndrome	7	72,332,743	74,616,901	2.28	
8p23.1 deletion syndrome	8	8,119,295	11,765,719	3.65	
9q subtelomeric deletion syndrome	9	140,403,363	141,153,431	0.75	1
Adult-onset autosomal dominant leukodystrophy (ADLD)	5	126,063,045	126,204,952	0.14	
Angelman syndrome (Type 1)	15	22,876,632	28,557,186	5.68	1
Angelman syndrome (Type 2)	15	23,758,390	28,557,186	4.80	1
ATR-16 syndrome	16	60,001	834,372	0.77	1
AZFa	Y	14,352,761	15,154,862	0.80	
AZFb	Y	20,118,045	26,065,197	5.95	
AZFb+AZFc	Y	19,964,826	27,793,830	7.83	
AZFc	Y	24,977,425	28,033,929	3.06	
Cat-Eye Syndrome (Type I)	22	1	16,971,860	16.97	
Charcot-Marie-Tooth syndrome type 1A (CMT1A)	17	13,968,607	15,434,038	1.47	1
Cri du Chat Syndrome (5p deletion)	5	10,001	11,723,854	11.71	1
Early-onset Alzheimer disease with cerebral amyloid angiopathy	21	27,037,956	27,548,479	0.51	
Familial Adenomatous Polyposis	5	112,101,596	112,221,377	0.12	
Hereditary Liability to Pressure Palsies (HNPP)	17	13,968,607	15,434,038	1.47	1
Leri-Weill dyschondrostosis (LWD) - SHOX deletion	X	751,878	867,875	0.12	
Leri-Weill dyschondrostosis	X	460,558	753,877	0.29	

Syndrome	Chromosome	Start	End	Interval (Mb)	Grade
(LWD) - SHOX deletion					
Miller-Dieker syndrome (MDS)	17	1	2,545,429	2.55	1
NF1-microdeletion syndrome	17	29,162,822	30,218,667	1.06	1
Pelizaeus-Merzbacher disease	X	102,642,051	103,131,767	0.49	
Potocki-Lupski syndrome (17p11.2 duplication syndrome)	17	16,706,021	20,482,061	3.78	
Potocki-Shaffer syndrome	11	43,985,277	46,064,560	2.08	1
Prader-Willi syndrome (Type 1)	15	22,876,632	28,557,186	5.68	1
Prader-Willi Syndrome (Type 2)	15	23,758,390	28,557,186	4.80	1
RCAD (renal cysts and diabetes)	17	34,907,366	36,076,803	1.17	
Rubinstein-Taybi Syndrome	16	3,781,464	3,861,246	0.08	1
Smith-Magenis Syndrome	17	16,706,021	20,482,061	3.78	1
Sotos syndrome	5	175,130,402	177,456,545	2.33	1
Split hand/foot malformation 1 (SHFM1)	7	95,533,860	96,779,486	1.25	
Steroid sulphatase deficiency (STS)	X	6,441,957	8,167,697	1.73	
WAGR 11p13 deletion syndrome	11	31,803,509	32,510,988	0.71	
Williams-Beuren Syndrome (WBS)	7	72,332,743	74,616,901	2.28	1
Wolf-Hirschhorn Syndrome	4	10,001	2,073,670	2.06	1
Xq28 (MECP2) duplication	X	152,749,900	153,390,999	0.64	

Grade 1 conditions often have one or more of the following characteristics; pathogenic anomaly; strong agreement amongst geneticists; highly penetrant; may still have variable phenotype but some common features; all cases in the literature have a clinical phenotype; no cases of healthy individuals with the anomaly; not reported on DVG databases or found in healthy population; functional data confirming single gene or multi-gene dosage effect; confirmed or strong candidate genes; clinical management implications defined; known cancer risk with implication for

surveillance; multiple sources of information (OMIM, GeneReviews, Orphanet, Unique, Wikipedia); and/or available for diagnostic use (reproductive counseling).

Grade 2 conditions often have one or more of the following characteristics; likely pathogenic anomaly; highly penetrant; variable phenotype with no consistent features other than DD; small number of cases/ reports in the literature; all reported cases have a clinical phenotype; no functional data or confirmed pathogenic genes; multiple sources of information (OMIM, Genereviews, Orphanet, Unique, Wikipedia); and/or may be used for diagnostic purposes and reproductive counseling.

Grade 3 conditions often have one or more of the following characteristics; susceptibility locus; healthy individuals or unaffected parents of a proband described; present in control populations; non penetrant; phenotype mild and not specific; features less consistent; no functional data or confirmed pathogenic genes; more limited sources of data; possibility of second diagnosis remains a possibility for cases deviating from the majority or if novel clinical finding present; and/or caution when using for diagnostic purposes and guarded advice for reproductive counseling.

Preeclampsia

In some embodiments, the presence or absence of preeclampsia is determined by using a method or apparatus described herein. Preeclampsia is a condition in which hypertension arises in pregnancy (i.e. pregnancy-induced hypertension) and is associated with significant amounts of protein in the urine. In some cases, preeclampsia also is associated with elevated levels of extracellular nucleic acid and/or alterations in methylation patterns. For example, a positive correlation between extracellular fetal-derived hypermethylated RASSF1A levels and the severity of pre-eclampsia has been observed. In certain examples, increased DNA methylation is observed for the H19 gene in preeclamptic placentas compared to normal controls.

Preeclampsia is one of the leading causes of maternal and fetal/neonatal mortality and morbidity worldwide. Circulating cell-free nucleic acids in plasma and serum are novel biomarkers with promising clinical applications in different medical fields, including prenatal diagnosis. Quantitative changes of cell-free fetal (cff)DNA in maternal plasma as an indicator for impending preeclampsia have been reported in different studies, for example, using real-time quantitative PCR for the male-specific SRY or DYS 14 loci. In cases of early onset preeclampsia, elevated levels may be seen in

the first trimester. The increased levels of cffDNA before the onset of symptoms may be due to hypoxia/reoxygenation within the intervillous space leading to tissue oxidative stress and increased placental apoptosis and necrosis. In addition to the evidence for increased shedding of cffDNA into the maternal circulation, there is also evidence for reduced renal clearance of cffDNA in preeclampsia. As the amount of fetal DNA is currently determined by quantifying Y-chromosome specific sequences, alternative approaches such as measurement of total cell-free DNA or the use of gender-independent fetal epigenetic markers, such as DNA methylation, offer an alternative. Cell-free RNA of placental origin is another alternative biomarker that may be used for screening and diagnosing preeclampsia in clinical practice. Fetal RNA is associated with subcellular placental particles that protect it from degradation. Fetal RNA levels sometimes are ten-fold higher in pregnant females with preeclampsia compared to controls, and therefore is an alternative biomarker that may be used for screening and diagnosing preeclampsia in clinical practice.

Pathogens

In some embodiments, the presence or absence of a pathogenic condition is determined by a method or apparatus described herein. A pathogenic condition can be caused by infection of a host by a pathogen including, but not limited to, a bacterium, virus or fungus. Since pathogens typically possess nucleic acid (e.g., genomic DNA, genomic RNA, mRNA) that can be distinguishable from host nucleic acid, methods and apparatus provided herein can be used to determine the presence or absence of a pathogen. Often, pathogens possess nucleic acid with characteristics unique to a particular pathogen such as, for example, epigenetic state and/or one or more sequence variations, duplications and/or deletions. Thus, methods provided herein may be used to identify a particular pathogen or pathogen variant (e.g. strain).

Cancers

In some embodiments, the presence or absence of a cell proliferation disorder (e.g., a cancer) is determined by using a method or apparatus described herein. For example, levels of cell-free nucleic acid in serum can be elevated in patients with various types of cancer compared with healthy patients. Patients with metastatic diseases, for example, can sometimes have serum DNA levels approximately twice as high as non-metastatic patients. Patients with metastatic diseases may also be identified by cancer-specific markers and/or certain single nucleotide polymorphisms or short tandem repeats, for example. Non-limiting examples of cancer types that may be

positively correlated with elevated levels of circulating DNA include breast cancer, colorectal cancer, gastrointestinal cancer, hepatocellular cancer, lung cancer, melanoma, non-Hodgkin lymphoma, leukemia, multiple myeloma, bladder cancer, hepatoma, cervical cancer, esophageal cancer, pancreatic cancer, and prostate cancer. Various cancers can possess, and can sometimes release into the bloodstream, nucleic acids with characteristics that are distinguishable from nucleic acids from non-cancerous healthy cells, such as, for example, epigenetic state and/or sequence variations, duplications and/or deletions. Such characteristics can, for example, be specific to a particular type of cancer. Thus, it is further contemplated that a method provided herein can be used to identify a particular type of cancer.

Examples

The examples set forth below illustrate certain embodiments and do not limit the technology.

Example 1: General methods for detecting conditions associated with genetic variations.

The methods and underlying theory described herein can be utilized to detect various conditions associated with genetic variation and determine the presence or absence of a genetic variation. Non-limiting examples of genetic variations that can be detected with the methods described herein include, segmental chromosomal aberrations (e.g., deletions, duplications), aneuploidy, gender, sample identification, disease conditions associated with genetic variation, the like or combinations of the foregoing.

Bin Filtering

The information content of a genomic region in a target chromosome can be visualized by plotting the result of the average separation between euploid and trisomy counts normalized by combined uncertainties, as a function of chromosome position. Increased uncertainty (see FIG. 1) or reduced gap between triploids and euploids (e.g. triploid pregnancies and euploid pregnancies)(see FIG. 2) both result in decreased Z-values for affected cases, sometimes reducing the predictive power of Z-scores.

FIG. 3 graphically illustrates a p-value profile, based on t-distribution, plotted as a function of chromosome position along chromosome 21. Analysis of the data presented in FIG. 3 identifies 36

uninformative chromosome 21 bins, each about 50 kilo-base pairs (kbp) in length. The uninformative region is located in the p-arm, close to centromere (21p11.2-21p11.1). Removing all 36 bins from the calculation of Z-scores, as schematically outlined in FIG. 4, sometimes can significantly increase the Z-values for all trisomy cases, while introducing only random variations into euploid Z-values.

The improvement in predictive power afforded by removal of the 36 uninformative bins can be explained by examining the count profile for chromosome 21 (see FIG. 5). In FIG. 5, two arbitrarily chosen samples demonstrate the general tendency of count versus (vs) bin profiles to follow substantially similar trends, apart from short-range noise. The profiles shown in FIG. 5 are substantially parallel. The highlighted region of the profile plot presented in FIG. 5 (e.g., the region in the ellipse), while still exhibiting parallelism, also exhibit large fluctuations relative to the rest of chromosome. Removal of the fluctuating bins (e.g., the 36 uninformative bins) can improve precision and consistency of Z statistics, in some embodiments.

Bin Normalization

Filtering out uninformative bins, as described in Example 1, sometimes does not provide the desired improvement to the predictive power of Z-values. When chromosome 18 data is filtered to remove uninformative bins, as described in Example 1, the z-values did not substantially improve (see FIG. 6). As seen with the chromosome 21 count profiles presented in Example 1, the chromosome 18 count profiles also are substantially parallel, disregarding short range noise. However, two chromosome 18 samples used to evaluate binwise count uncertainties (see the bottom of FIG. 6) significantly deviate from the general parallelism of count profiles. The dips in the middle of the two traces, highlighted by the ellipse, represent large deletions. Other samples examined during the course of the experiment did not exhibit this deletion. The deletion coincides with the location of a dip in p-value profiles for chromosome 18, illustrated in by the ellipse shown in FIG. 7. That is, the dip observed in the p-value profiles for chromosome 18 are explained by the presence of the deletion in the chromosome 18 samples, which cause an increase in the variance of counts in the affected region. The variance in counts is not random, but represents a rare event (e.g., the deletion of a segment of chromosome 18), which, if included with other, random fluctuations from other samples, decreases the predictive power bin filtering procedure.

Two questions arise from this example; (1) how are p-value signals determined to be meaningful and/or useful, and (2) can the p-value approach described herein be generalized for use with any bin data (e.g., from within any chromosome, not only bins from within chromosomes 13, 18 or 21). A generalized procedure could be used to remove variability in the total counts for the entire genome, which can often be used as the normalization factor when evaluating Z-scores. The data presented in FIG. 8 can be used to investigate the answers to the questions above by reconstructing the general contour of the data by assigning the median reference count to each bin, and normalizing each bin count in the test sample with respect to the assigned median reference count.

The medians are extracted from a set of known euploid references. Prior to computing the reference median counts, uninformative bins throughout the genome are filtered out. The remaining bin counts are normalized with respect to the total residual number of counts. The test sample is also normalized with respect to the sum of counts observed for bins that are not filtered out. The resulting test profile often centers around a value of 1, except in areas of maternal deletions or duplication, and areas in which the fetus is triploid (see FIG. 9). The bin-wise normalized profile illustrated in FIG. 10 confirms the validity of the normalization procedure, and clearly reveals the heterozygous maternal deletion (e.g., central dip in the gray segment of the profile tracing) in chromosome 18 and the elevated chromosomal representation of chromosome 18 of the tested sample (see the gray area of profile tracing in FIG. 10). As can be seen from FIG. 10, the median value for the gray segment of the tracing centers around about 1.1, where the median value for the black segment of the tracing centers around 1.0.

Peak Elevation

FIG. 11 graphically illustrates the results of analyzing multiple samples using bin-wise normalization, from a patient with a discernible feature or trait (e.g., maternal duplication, maternal deletion, the like or combinations thereof). The identities of the samples often can be determined by comparing their respective normalized count profiles. In the example illustrated in FIG. 11, the location of the dip in the normalized profile and its elevation, as well as its rarity, indicate that both samples originate from the same patient. Forensic panel data often can be used to substantiate these findings.

FIG. 12 and 13 graphically illustrate the results of the use of normalized bin profiles for identifying patient identity, or sample identity. The samples analyzed in FIG. 12 and 13 carry wide maternal aberrations in chromosomes 4 and 22, which are absent in the other samples in the profile tracings, confirming the shared origin of the top and bottom traces. Results such as this can lead to the determination that a particular sample belongs to a specific patient, and also can be used to determine if a particular sample has already been analyzed.

Bin-wise normalization facilitates the detection of aberrations, however, comparison of peaks from different samples often is further facilitated by analyzing quantitative measures of peak elevations and locations (e.g., peak edges). The most prominent descriptor of a peak often is its elevation, followed by the locations of its edges. Features from different count profiles often can be compared using the following non-limiting analysis.

(a) Determine the confidence in a features detected peaks in a single test sample. If the feature is distinguishable from background noise or processing artifacts, the feature can be further analyzed against the general population.
(b) Determine the prevalence of the detected feature in the general population. If the feature is rare, it can be used as a marker for rare aberrations. Features that are found frequently in the general population are less useful for analysis. Ethnic origins can play a role in determining the relevance of a detected features peak elevation. Thus, some features provide useful information for samples from certain ethnic origins.
(c) Derive the confidence in the comparison between features observed in different samples.

Illustrated in FIG. 14 are the normalized bin counts in chromosome 5, from a euploid subject. The average elevation generally is the reference baseline from which the elevations of aberrations are measured, in some embodiments. Small and/or narrow deviations are less reliable predictors than wide, pronounced aberrations. Thus, the background noise or variance from low fetal contribution and/or processing artifacts is an important consideration when aberrations are not large or do not have a significant peak elevation above the background. An example of this is presented in FIG. 15, where a peak that would be significant in the upper trace, can be masked in the background noise observed in the bottom profile trace. The confidence in the peak elevation (see FIG. 16) can be determined by the average deviation from the reference (shown as the delta symbol), relative to the width of the euploid distribution (e.g., combined with the variance (shown as the sigma symbol)

in the average deviation). The error in the average stretch elevation can be derived from the known formula for the error of the mean. If a stretch longer than one bin is treated as a random (non-contiguous) sample of all bins within a chromosome, the error in the average elevation decreases with the square root of the number of bins within the aberration. This reasoning neglects the correlation between neighboring bins, an assumption confirmed by the correlation function shown in FIG. 17 (e.g., the equation for G(n)). Non-normalized profiles sometimes exhibit strong medium-range correlations (e.g., the wavelike variation of the baseline), however, the normalized profiles smooth out the correlation, leaving only random noise. The close match between the standard error of the mean, the correction for autocorrelation, and the actual sample estimates of the standard deviation of the mean elevation in chromosome 5 (see FIG. 18) confirms the validity of the assumed lack of correlation. Z-scores (see FIG. 19) and p-values calculated from Z-scores associated with deviations from the expected elevation of 1 (see FIG. 20) can then be evaluated in light of the estimate for uncertainty in the average elevation. The p-values are based on a t-distribution whose order is determined by the number of bins in a peak. Depending on the desired level of confidence, a cutoff can suppress noise and allow unequivocal detection of the actual signal.

$$Z = \frac{\Delta_1 - \Delta_2}{\sqrt{\sigma_1^2\left(\frac{1}{N_1}+\frac{1}{n_1}\right)+\sigma_2^2\left(\frac{1}{N_2}+\frac{1}{n_2}\right)}} \quad (1)$$

Equation 1 can be used to directly compare peak elevation from two different samples, where N and n refer to the numbers of bins in the entire chromosome and within the aberration, respectively. The order of the t-test that will yield a p-value measuring the similarity between two samples is determined by the number of bins in the shorter of the two deviant stretches.

Peak Edge

In addition to comparing average elevations of aberrations in a sample, the beginning and end of the compared stretches also can provide useful information for statistical analysis. The upper limit of resolution for comparisons of peak edges often is determined by the bin size (e.g., 50 kbps in the examples described herein). FIG. 21 illustrates 3 possible peak edge scenarios; (a) a peak from one sample can be completely contained within the matching peak from another sample, (b) the edges from one sample can partially overlap the edges of another sample, or (c) the leading edge from one sample can just marginally touch or overlap the trailing edge of another sample. FIG. 22 illustrates and example of the scenario described in (c) (e.g., see the middle, light gray

trace, where the trailing edge of the middle trace marginally touches the leading edge of the upper trace).

The lateral tolerance associated with an edge often can be used to distinguish random variations from true, aberration edges. The position and the width of an edge can be quantified by numerically evaluating the first derivative of the aberrant count profile, as shown in FIG. 23. If the aberration is represented as a composite of two Heaviside functions, its derivative will be the sum of two Dirac's delta functions. The starting edge corresponds to an upward absorption-shaped peak, while the ending edge is a downward, 180 degree-shifted absorption peak. If the aberration is narrow, the two spikes are close to one another, forming a dispersion-like contour. The locations of the edges can be approximated by the extrema of the first derivative spikes, while the edge tolerance is determined by their widths.

Comparison between different samples often can be reduced to determining the difference between two matching edge locations, divided by the combined edge uncertainties. However, the derivatives sometimes are lost in background noise, as illustrated in FIG. 24. While the aberration itself benefits from the collective information contributed from all its bins, the first derivative only can afford information from the few points at the edge of the aberration, which can be insufficient to overcome the noise. Sliding window averaging, used to create FIG. 24, is of limited value in this situation. Noise can be suppressed by combining the first derivative (e.g., akin to a point estimate) with the peak elevation (e.g., comparable to an integral estimate). In some embodiments the first derivative and the peak elevation can be combined by multiplying them together, which is equivalent to taking the first derivative of a power of the peak elevation, as shown in FIG. 25. The results presented in FIG. 25 successfully suppress noise outside of the aberration, however, noise within the aberration is enhanced by the manipulation. The first derivative peaks are still clearly discernible, allowing them to be used to extract edge locations and lateral tolerances, thereby allowing the aberration to be clearly identified in the lower profile tracing.

Median chromosomal elevation

The median normalized elevation within the target chromosome in a euploid patient is expected to remain close to 1 regardless of the fetal fraction. However, as shown in FIG. 9 and 10, median elevations in trisomy patients increase with the fetal fraction. The increase generally is substantially linear with a slope of 0.5. Experimental measurements confirm these expectations. FIG. 26 illustrates a histogram of median elevations for 86 euploid samples (shown in black in FIG.

26). The median values are tightly clustered around 1 (median = 1.0000, median absolute deviation (MAD) = 0.0042, mean = 0.9996, standard deviation (SD) = 0.0046). None of the euploid median elevations exceeds 1.012, as shown in the histogram presented in FIG. 26. In contrast, out of 35 trisomy samples shown (the gray samples) in Fig 26, all but one have median elevations exceeding 1.02, significantly above the euploid range. The gap between the two groups of patients in this example is large enough to allow classification as euploid or aneuploid.

Fetal fraction as the limiting factor in classification accuracy

The ratio between the fetal fraction and the width of the distribution of median normalized counts in euploids (e.g. euploid pregnancies) can be used to determine the reliability of classification using median normalized elevations, in some embodiments. Since median normalized counts, as well as other descriptors such as Z-values, linearly increase with the fetal fraction with the proportionality constant of 0.5, the fetal fraction must exceed four standard deviations of the distribution of median normalized counts to achieve 95% confidence in classification, or six standard deviations to achieve 99% confidence in classification. Increasing the number of aligned sequences tags can serve to decrease the error in measured profiles and sharpen the distribution of median normalized elevations, in certain embodiments. Thus, the effect of increasingly precise measurements is to improve the ratio between fetal fraction and the width of the distribution of euploid median normalized elevations.

Area Ratio

The median of the distribution of normalized counts generally is a point estimate and, as such, often is a less reliable estimate than integral estimates, such as areas under the distribution (e.g., area under the curve. Samples containing high fetal level fractions are not as affected by using a point estimate, however at low fetal fraction values, it becomes difficult to distinguish a truly elevated normalized profile from a euploid sample that has a slightly increased median count due to random errors. A histogram illustrating the median distribution of normalized counts from a trisomy case with a relatively low fetal fraction (e.g., F = about 7%; F(7%)) is shown in FIG. 27. The median of the distribution is 1.021, not far from 1 + F/2 = 1.035. However, the width of the distribution (MAD = 0.054, SD = 0.082) far exceeds the deviation of the median from the euploid value of 1, precluding any claims that the sample is abnormal. Visual inspection of the distribution suggests an alternative analysis: although the shift of the peak to the right is relatively small, it

significantly perturbs the balance between the areas to the left (dark gray) and to the right (light gray) from the euploid expectation of 1. Thus the ratio between the two areas, being an integral estimate, can be advantageous in cases where classification is difficult due to low fetal fraction values. Calculation of the integral estimate for the light gray and dark gray areas under the curve is explained in more detail below.

If a Gaussian distribution of normalized counts is assumed, then

$$P(q) = \frac{1}{\sigma\sqrt{2\pi}} exp\left[-{}^{(q-q_0)}/_{(2\sigma^2)}\right] \quad (2).$$

In euploid cases, the expectation for the normalized counts is 1. For trisomy patients, the expectation is

$$q_0 = 1 + {}^{F}/_{2} \quad (3).$$

Since the reference point for calculating the area ratio is 1, the argument to the exponential function is z^2, where

$$z = -{}^{F}/_{(2\sigma\sqrt{2})} \quad (4).$$

The area to the left of the reference point is

$$B = \int_{-\infty}^{1} P(q)dq = \frac{1}{2}[1 + erf(z)] \quad (5).$$

The error function erf(z) can be evaluated using its Taylor expansion:

$$erf(z) = \frac{2}{\sqrt{\pi}} \sum_{n=0}^{\infty} \frac{(-1)^n z^{2n+1}}{n!\,(2n+1)} \quad (6).$$

The area to the right from the reference point is 1 – B. The ratio between two areas is therefore

$$R = \frac{1-B}{B} = \frac{1-erf(z)}{1+erf(z)} = \frac{1-erf\left[-{}^{F}/_{(2\sigma\sqrt{2})}\right]}{1+erf\left[-{}^{F}/_{(2\sigma\sqrt{2})}\right]} \quad (7).$$

Error propagation from measured fetal fractions into area ratios R can be estimated by simply replacing F in equation 7 with F – ΔF and F + ΔF. FIG. 28 shows the frequencies of euploid and

trisomy area ratios in a set of 480 samples. The overlap between two groups involves trisomy samples with low fetal fractions.

Combined classification criteria

FIG. 29 illustrates the interrelation and interdependence of median elevations and area ratios, both of which described substantially similar phenomena. Similar relationships connect median elevations and area ratios with other classification criteria, such as Z-scores, fitted fetal fractions, various sums of squared residuals, and Bayesian p-values (see FIG. 30). Individual classification criteria can suffer from ambiguity stemming from partial overlap between euploid and trisomy distributions in gap regions, however, a combination of multiple criteria can reduce or eliminate any ambiguities. Spreading the signal along multiple dimensions can have the same effect as measuring NMR frequencies of different nuclei, in some embodiments, resolving overlapping peaks into well-defined, readily identifiable entities. Since no attempt is made to quantitatively predict any theoretical parameter using mutually correlated descriptors, the cross-correlations observed between different classification criteria do not interfere. Defining a region in multidimensional space that is exclusively populated by euploids, allows classification of any sample that is located outside of the limiting surface of that region. Thus the classification scheme is reduced to a consensus vote for euploidy.

In some embodiments utilizing a combined classification criteria approach, classification criteria described herein can be combined with additional classification criteria known in the art. Certain embodiments can use a subset of the classification criteria listed here. Certain embodiments can mathematically combine (e.g., add, subtract, divide, multiply, and the like) one or more classification criteria among themselves and/or with fetal fraction to derive new classification criteria. Some embodiments can apply principal components analysis to reduce the dimensionality of the multidimensional classification space. Some embodiments can use one or more classification criteria to define the gap between affected and unaffected patients and to classify new data sets. Any combination of classification criteria can be used to define the gap between affected and unaffected patients and to classify new data sets. Non-limiting examples of classification criteria that can be used in combination with other classification criteria to define the gap between affected and unaffected patients and to classify new data sets include: linear discriminant analysis, quadratic discriminant analysis, flexible discriminant analysis, mixture

discriminant analysis, k Nearest Neighbors, classification tree, bagging, boosting, neural networks, support vector machines, and/or random forest.

Example 2: Methods for detection of genetic variations associated with fetal aneuploidy using measured fetal fractions and bin-weighted sums of squared residuals

Z-value statistics and other statistical analysis of sequence read data frequently are suitable for determining or providing an outcome determinative of the presence or absence of a genetic variation with respect to fetal aneuploidy, however, in some instances it can be useful to include additional analysis based on fetal fraction contribution and ploidy assumptions. When including fetal fraction contribution in a classification scheme, a reference median count profile from a set of known euploids (e.g. euploid pregnancies) generally is utilized for comparison. A reference median count profile can be generated by dividing the entire genome into N bins, where N is the number of bins. Each bin i is assigned two numbers: (i) a reference count F_i and (ii) the uncertainty (e.g., standard deviation or σ) for the bin reference counts.

The following relationship can be utilized to incorporate fetal fraction, maternal ploidy, and median reference counts into a classification scheme for determining the presence or absence of a genetic variation with respect to fetal aneuploidy,

$$y_i = (1 - F)M_i f_i + FXf_i \qquad (8)$$

where Y_i represents the measured counts for a bin in the test sample corresponding to the bin in the median count profile, F represents the fetal fraction, X represents the fetal ploidy, and M_i represents maternal ploidy assigned to each bin. Possible values used for X in equation (8) are: 1 if the fetus is euploid; 3/2, if the fetus is triploid; and, 5/4, if there are twin fetuses and one is affected and one is not. 5/4 is used in the case of twins where one fetus is affected and the other not, because the term F in equation (8) represents total fetal DNA, therefore all fetal DNA must be taken into account. In some embodiments, large deletions and/or duplications in the maternal genome can be accounted for by assigning maternal ploidy, M_i, to each bin or genomic section. Maternal ploidy often is assigned as a multiple of 1/2, and can be estimated using bin-wise normalization, in some embodiments. Because maternal ploidy often is a multiple of 1/2, maternal ploidy can be readily accounted for, and therefore will not be included in further equations to simplify derivations.

Fetal ploidy can be assessed using any suitable approach. In some embodiments, fetal ploidy can be assessed using equation (8), or derivations thereof. In certain embodiments, fetal ploidy can be classified using one of the following, equation (8) based, non-limiting approaches:

1) Measure fetal fraction F and use the value to form two sums of squared residuals. To calculate the sum of squared residuals, subtract the right hand side (RHS) of equation (8) from its left hand side (LHS), square the difference, and sum over selected genomic bins, or in those embodiments using all bins, sum over all bins. This process is performed to calculate each of the two sums of squared residuals. One sum of square residuals is evaluated with fetal ploidy set to 1 (e.g., $X = 1$) and the other sum of squared residuals is evaluated with fetal ploidy set to 3/2 (e.g., $X = 3/2$). If the fetal test subject is euploid, the difference between the two sums of squared residuals is negative, otherwise the difference is positive.

2) Fix fetal fraction at its measured value and optimize ploidy value. Fetal ploidy generally can take on only 1 of two discrete values, 1 or 3/2, however, the ploidy sometimes can be treated as a continuous function. Linear regression can be used to generate an estimate for ploidy. If the estimate resulting from linear regression analysis is close to 1, the fetal test sample can be classified as euploid. If the estimate is close to 3/2, the fetus can be classified as triploid.

3) Fix fetal ploidy and optimize fetal fraction using linear regression analysis. The fetal fraction can be measured and a restraint term can be included to keep the fitted fetal fraction close to the measured fetal fraction value, with a weighting function that is reciprocally proportional to the estimated error in the measure fetal fraction. Equation (8) is solved twice, once with ploidy set at 3/2, and once for fetal ploidy set to 1. When solving equation (8) with ploidy set to 1, the fetal fraction need not be fitted. A sum of square residuals is formed for each result and the sum of squared residuals subtracted. If the difference is negative, the fetal test subject is euploid. If the difference is positive, the fetal test subject is triploid.

The generalized approaches described in 1), 2) and 3) are described in further detail herein.

Fixed ploidy, fixed fetal fraction: sums of squared residuals

In some embodiments, fetal aneuploidy can be determined using a model which analyzes two variables, fetal ploidy (e.g., *X*) and fetal nucleic acid fraction (e.g., fetal fraction; *F*). In certain embodiments, fetal ploidy can take on discrete values, and in some embodiments, fetal fraction can be a continuum of values. Fetal fraction can be measured, and the measured valued used to generate a result for equation (8), for each possible value for fetal ploidy. Fetal ploidy values that can be used to generate a result for equation (8) include 1and 3/2 for a single fetus pregnancy, and in the case of a twin fetus pregnancy where one fetus is affected and the other fetus unaffected, 5/4 can be used. The sum of squared residuals obtained for each fetal ploidy value measures the success with which the method reproduces the measurements, in some embodiments. When evaluating equation (8) at $X = 1$, (e.g., euploid assumption), the fetal fraction is canceled out and the following equation results for the sum of squared residuals:

$$\varphi_E = \sum_{i=1}^{N} \frac{1}{\sigma_i^2}(y_i - f_i)^2 = \sum_{i=1}^{N} \frac{y_i^2}{\sigma_i^2} - 2\sum_{i=1}^{N} \frac{y_i f_i}{\sigma_i^2} + \sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} = \Xi_{yy} - 2\Xi_{fy} + \Xi_{ff} \quad (9)$$

To simplify equation (9) and subsequent calculations, the following notion is utilized:

$$\Xi_{yy} = \sum_{i=1}^{N} \frac{y_i^2}{\sigma_i^2} \quad (10)$$

$$\Xi_{ff} = \sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} \quad (11)$$

$$\Xi_{fy} = \sum_{i=1}^{N} \frac{y_i f_i}{\sigma_i^2} \quad (12)$$

When evaluating equation (8) at $X = 3/2$ (e.g., triploid assumption), the following equation results for the sum of the squared residuals:

$$\varphi_T = \sum_{i=1}^{N} \frac{1}{\sigma_i^2}\left(y_i - f_i - \frac{1}{2}F f_i\right)^2 = \Xi_{yy} - 2\Xi_{fy} + \Xi_{ff} + F\left(\Xi_{ff} - \Xi_{fy}\right) + \frac{1}{4}F^2\Xi_{ff} \quad (13)$$

The difference between equations (9) and (13) forms the functional result (e.g., phi) that can be used to test the null hypothesis (e.g., euploid, $X = 1$) against the alternative hypothesis (e.g., trisomy singleton, $X = 3/2$):

$$\varphi = \varphi_E - \varphi_T = F\left(\Xi_{fy} - \Xi_{ff}\right) - \frac{1}{4}F^2\,\Xi_{ff} \quad (14)$$

The profile of phi with respect to F is a parabola defined to the right of the ordinate (since F is greater than or equal to 0). Phi converges to the origin as F approaches zero, regardless of experimental errors and uncertainties in the model parameters.

In some embodiments, the functional Phi is dependent on the measured fetal fraction F with a negative second-order quadratic coefficient (see equation (14)). Phi's dependence on the measured fetal fraction would seem to imply a convex shape for both euploid and triploid cases. If this analysis were correct, trisomy cases would reverse the sign at high F values, however equation (12) depends on F. Combining equations (8) and (14), disregarding maternal ploidy, setting X = 3/2 and neglecting experimental errors, the equation for trisomy cases becomes:

$$\Xi_{fy} = \sum_{i=1}^{N} \frac{y_i f_i}{\sigma_i^2} = \sum_{i=1}^{N} \frac{f_i}{\sigma_i^2}\left[(1-F)f_i + FXf_i\right] = \left(1 + \frac{1}{2}F\right)\sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} = \left(1 + \frac{1}{2}F\right)\Xi_{ff} \quad (15)$$

The relationship between equations (11) and (12) for triploids holds under ideal circumstances, in the absence of any measurement errors. Combining equations (14) and (15) results in the following expression, which often yields a concave parabola in triploid cases:

$$\varphi = F\left(\Xi_{fy} - \Xi_{ff}\right) - \frac{1}{4}F^2\,\Xi_{ff} = F\left[\left(1 + \frac{1}{2}F\right)\Xi_{ff} - \Xi_{ff}\right] - \frac{1}{4}F^2\,\Xi_{ff} = \frac{1}{4}F^2\,\Xi_{ff} \quad \text{(Trisomy)} \quad (16)$$

For euploids, equations (11) and (12) should have the same value, with the exception of measurement errors, which sometimes yields a convex parabola:

$$\varphi = F\left(\Xi_{fy} - \Xi_{ff}\right) - \frac{1}{4}F^2\,\Xi_{ff} = -\frac{1}{4}F^2\,\Xi_{ff} \quad \text{(Euploids)} \quad (17)$$

Simulated functional phi profiles for typical model parameter values are shown in FIG. 31, for trisomy (gray) and euploid (blue) cases. FIG. 32 shows an example using actual data. In FIG. 31 and 32, data points below the abscissa generally represent cases classified as euploids. Data points above the abscissa generally represent cases classified as trisomy 21 (T21) cases. In FIG. 32, the solitary data point in the fourth quadrant (e.g., middle lower quadrant) is a twin pregnancy with one affected fetus. The data set utilized to generate FIG. 32 includes other affected twin samples as well, explaining the spread of T21 data points toward the abscissa.

Equations (9) and (10) often can be interpreted as follows: For triploids, the euploid model sometimes generates larger errors, implying that phi_E (see equation (9)) is greater than phi_T (see equation (13)). As a result, functional phi (see equation (7)) occupies the first quadrant (e.g., upper left quadrant). For euploids, the trisomy model sometimes generates larger errors, the rank of equations (2) and (6) reverses and functional phi (equation (7)) occupies in the fourth quadrant. Thus, in principle, classification of a sample as euploid or triploid sometimes reduces to evaluating the sign of phi.

In some embodiments, the curvature of the data points shown in FIG. 31 and 32 can be reduced or eliminated by replacing functional phi (equation (7)) with the square root of functional phi's absolute value, multiplied by its sign. The linear relationship generated with respect to *F* sometimes can improve separation between triploids and euploids at low fetal fraction values, as shown in FIG. 33. Linearizing the relationship with respect to *F* sometimes results in increase uncertainty intervals at low fetal fraction (e.g., *F*) values, therefore, the gains realized from this process are related to making visual inspection of the differences substantially easier; the gray area remains unchanged. Extension of the process to analysis of twin pregnancies is relatively straightforward. The reason used to generate equation (9) implies that in a twin pregnancy with one affected and one normal fetus, functional phi should reduce to zero, plus or minus experimental error, regardless of *F*. Twin pregnancies generally produce more fetal DNA than single pregnancies.

Optimized ploidy, fixed fetal fraction: linear regression

In certain embodiments, fetal aneuploidy can be determined using a model in which the fetal fraction is fixed at its measured value and ploidy is varied to optimize the sum of squared residuals. In some embodiments, the resulting fitted fetal fraction value can be used to classify a case as trisomy or euploid, depending on whether the value is close to 1, 3/2, or 5/4 in the case of twins.

Starting from equation (8), the sum of squared residuals can be formed as follows:

$$\varphi = \sum_{i=1}^{N} \frac{1}{\sigma_i^2} [y_i - (1-F)M_i f_i - FXf_i]^2$$

$$= \sum_{i=1}^{N} \frac{1}{\sigma_i^2} [y_i^2 - 2(1-F)M_i f_i y_i - 2FXf_i y_i + (1-F)^2 M_i^2 f_i^2 + 2F(1-F)XM_i f_i^2 + F^2 X^2 f_i^2]$$

(18)

To minimize phi as a function of X, the first derivative of phi with respect to X is generated, set equal to zero, and the resulting equation solved for X. The resulting expression is presented in equation (19).

$$\frac{1}{2}\left(\frac{d\varphi}{dX}\right) = 0 = XF^2 \sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} - F \sum_{i=1}^{N} \frac{f_i y_i}{\sigma_i^2} + F(1-F) \sum_{i=1}^{N} \frac{M_i f_i^2}{\sigma_i^2} \quad (19)$$

The optimal ploidy value sometimes is given by the following expression:

$$X = \frac{\sum_{i=1}^{N} \frac{f_i y_i}{\sigma_i^2} - (1-F) \sum_{i=1}^{N} \frac{M_i f_i^2}{\sigma_i^2}}{F \sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2}} \quad (20)$$

As noted previously, the term for maternal ploidy, M_i, can be omitted from further mathematical derivations. The resulting expression for X corresponds to the relatively simple, and often most frequently occurring, special case of when the mother has no deletions or duplications in the chromosome or chromosomes being evaluated. The resulting expression is presented in FIG. 21.

$$X = \frac{\Xi_{fy} - (1-F)\Xi_{ff}}{F\Xi_{ff}} = \frac{\Xi_{fy}}{F\Xi_{ff}} - \frac{1-F}{F} = 1 + \frac{1}{F}\left(\frac{\Xi_{fy}}{\Xi_{ff}} - 1\right) \quad (21)$$

Xi_{ff} and Xi_{fy} are given by equations (11) and (12), respectively. In embodiments where all experimental errors are negligible, solving equation (21) results in a value of 1 for euploids where $Xi_{ff} = Xi_{fy}$. In certain embodiments where all experimental errors are negligible, solving equation

(21) results in a value of 3/2 for triploids (see equation (15) for triploid relationship between Xi_{ff} and Xi_{fy}.

Optimized ploidy, fixed fetal fraction: error propagation

Optimized ploidy often is inexact due to various sources of error. Three, non-limiting examples of error sources include: reference bin counts f_i, measured bin counts y_i, and fetal fraction F. The contribution of the non-limiting examples of error will be examined separately.

Errors in measured fetal fractions: quality of fitted fetal fraction

Fetal fraction estimates based on the number of sequence tags mapped to the Y chromosome (e.g., Y-counts) sometimes show relatively large deviations with respect to FQA fetal fraction values (see FIG. 34). Z-values for triploid often also exhibit a relatively wide spread around the diagonal shown in FIG. 35. The diagonal line in FIG. 35 represents a theoretically expected increase of the chromosomal representation for chromosome 21 with increasing fetal fraction in trisomy 21 cases. Fetal fraction can be estimated using a suitable method. A non-limiting example of a method that can be utilized to estimate fetal fraction is the fetal quantifier assay (e.g., FQA). Other methods for estimating fetal fraction are known in the art. Various methods utilized to estimate fetal fraction sometimes also show a substantially similar spread around the central diagonal, as shown in FIG. 36-39. In FIG. 36, the deviations are substantially similar (e.g., negative at high F_0) to those observed in fitted fetal fraction (see equation (33)). In some embodiments, the slope of the linear approximation to the average chromosome Y (e.g., chromosome Y) fetal fraction (see the dark gray line in FIG. 36) in the range between 0% and 20% is about 3/4. In certain embodiments, the linear approximation for standard deviation (see FIG. 36, light gray line) is about $2/3 + F_0/6$. In some embodiments, fetal fraction estimates based on chromosome 21 (e.g., chromosome 21) are substantially similar to those obtained by fitting fetal fractions (see FIG. 37). Another qualitatively similar set of gender-based fetal fraction estimates is shown in FIG. 38. FIG. 39 illustrates the medians of normalized bin counts for T21 cases, which are expected to have a slope whose linear approximation is substantially similar to $1 + F_0/2$ (see gray line from origin to the midpoint of the top of the graph in FIG. 39).

FIG. 36-39 share the following common features:

a) slope not equal to 1(either greater or less than 1, depending on the method, with the exception of Z-values),
b) large spread fetal fraction estimation, and
c) the extent of spread increases with fetal fraction.

To account for these observations, errors in measured fetal fraction will be modeled using the formula $\Delta F = 2/3 + F_0/6$, in some embodiments.

Errors in measured fetal fractions: error propagation from measured fetal fractions to fitted ploidy

If the assumption is made that f_i and y_i are errorless, to simplify analysis, the measured fetal fraction F is composed of F_v (e.g., the true fetal fraction) and ΔF (e.g., the error in measured fetal fraction):

$$F = F_V + \Delta F \quad (22).$$

In some instances, uncertainties in fitted X values originate from errors in measured fetal fraction, F. Optimized values for X are given by equation (21), however the true ploidy value is given by X_V, where X_V =1 or 3/2. X_V varies discretely, whereas X varies continuously and only accumulates around X_V under favorable conditions (e.g., relatively low error).

Assuming again that f_i and y_i are errorless, equation (8) becomes:

$$y_i = (1 - F_V)M_i f_i + F_V X f_i \quad (23).$$

Combining equations (21) to (23) generates the following relationship between true ploidy X_V and the ploidy estimate X that includes the error ΔF. The relationship also includes the assumption that maternal ploidy equals 1 (e.g., euploid), and the term for maternal ploidy, M_i, is replaced by 1.

$$X = 1 + \frac{1}{F_V + \Delta F}\left\{\frac{\sum_{i=1}^{N}\frac{f_i}{\sigma_i^2}[(1-F_V)f_i + F_V X_V f_i]}{\sum_{i=1}^{N}\frac{f_i^2}{\sigma_i^2}} - 1\right\} = 1 + \frac{F_V(X_V - 1)}{F_V + \Delta F} \quad (24)$$

In some instances, the term $X_V - 1$ is substantially identical to zero in euploids, and ΔF does not contribute to errors in X. In triploid cases, the error term does not reduce to zero (e.g., is not substantially identical to zero). Thus, in some embodiments, ploidy estimates can be viewed as a function of the error ΔF:

$$X = g(\Delta F) \quad (25)$$

Simulated profiles of fitted triploid X as a function of F_0 with fixed errors ΔF = plus or minus 0.2% are shown in Fig 40. Results obtained using actual data are shown in FIG. 41. The data points generally conform to the asymmetric trumpet-shaped contour predicted by equation (24). Smaller fetal fractions often are qualitatively associated with larger ploidy errors. Underestimated fetal fraction sometimes is compensated by ploidy overestimates; overestimated fetal fraction often is linked to underestimates in ploidy. The effect frequently is stronger when fetal fraction is underestimated. This is consistent with the asymmetry seen in the graphs presented in FIG. 40 and 41, (e.g., as F decreases, the growth of the upper branch is substantially faster than the decay of the lower branch). Simulations with different levels of error in F follow the same pattern, with the extent of the deviations from X_V increasing with ΔF.

A probability distribution for X can be used to quantify these observations. In some embodiments, the distribution of ΔF can be used to derive the density function for X using the following expression:

$$f_Y(y) = \left| \frac{1}{g'(g^{-1}(y))} \right| f_X(g^{-1}(y)) \quad (26)$$

where,

$f_Y(y)$ is the unknown density function for $y = g(x)$

$f_X(x)$ is the given density function for x

$g'(x)$ is the first derivative of the given function $y = g(x)$

$g^{-1}(y)$ is the inverse of the given function $g : x = g^{-1}(y)$

$g'(g^{-1}(y))$ is the value of the derivative at the point $g^{-1}(y)$

In equation 26 x is ΔF, y is X (e.g., ploidy estimate), and $g(x)$ is given by equation (24). The derivative is evaluated according to the following expression:

$$\frac{dg}{d\Delta F} = -\frac{F_V(X_V-1)}{(F_V+\Delta F)^2} \quad (27)$$

The inverse $g^{-1}(y)$ can be obtained from equation (24), in some embodiments:

$$\Delta F = \frac{F_V(X_V-X)}{X-1} \quad (28)$$

If the error in F conforms to a Gaussian distribution, $f_x(x)$ in equation (26) can be replaced with the following expression:

$$P(\Delta F) = \frac{exp[-(\Delta F)^2/(2\sigma^2)]}{\sigma\sqrt{2\pi}} \quad (29)$$

In certain embodiments, combining equations (26) to (29) results in a probability distribution for X at different levels of ΔF, as shown in FIG. 42.

In some instances, a bias towards higher ploidy values, which sometimes are prominent at high levels of errors in F, often is reflected in the asymmetric shape of the density function: a relatively long, slowly decaying tail to the right of the light gray line, vertically in line with X, along the X axis, as shown in FIG. 42, panels A-C. In some embodiments, for any value of ΔF, the area under the probability density function to the left of the light gray line (X_V = 3/2) equals the area to the right of the light gray line. That is, one half of all fitted ploidy values often are overestimates, while the other half of all fitted ploidy values sometimes are underestimates. In some instances, the bias generally only concerns the extent of errors in X, not the prevalence of one or the other direction. The median of the distribution remains equal to X_V, in some embodiments. FIG. 43 illustrates euploid and trisomy distributions obtained for actual data. Uncertainties in measured fetal fraction values sometimes explain part of the variance seen in the fitted ploidy values for triploids, however errors in estimated X values for euploids often require examining error propagation from bin counts.

Fixed ploidy, optimized fetal fraction: linear regression

A continuously varying fetal fraction often can be optimized while keeping ploidy fixed at one of its possible values (e.g., 1 for euploids, 3/2 for singleton triploids, 5/4 for twin triploids), as opposed to fitting ploidy that often can take on a limited number of known discrete values. In embodiments in which the measured fetal fraction (F_0) is known, optimization of the fetal fraction can be restrained such that the fitted F remains close to F_0, within experimental error (e.g., ΔF). In some instances, the observed (e.g., measured) fetal fraction F_0, sometimes differs from fetal fraction, F_V, described in equations (22) to (28). A robust error propagation analysis should be able to distinguish between F_0 and F_V. To simplify the following derivations, difference between the observed fetal fraction and the true fetal fraction will be ignored.

Equation (8) is presented below in a rearranged format that also omits the maternal ploidy term (e.g., M_i).

$$y_i = F(X-1)f_i + f_i \quad (30)$$

A functional term that needs to be minimized is defined as follows, in some embodiments:

$$\varphi(F) = \frac{(F-F_0)^2}{(\Delta F)^2} + \sum_{i=1}^{N} \frac{1}{\sigma_i^2}[y_i - F(X-1)f_i - f_i]^2$$

$$= \frac{(F-F_0)^2}{(\Delta F)^2} + \sum_{i=1}^{N} \frac{1}{\sigma_i^2}\left[y_i^2 + F^2(X-1)^2 f_i^2 + f_i^2 - 2F(X-1)f_i y_i - 2f_i y_i + 2F(X-1)f_i^2\right]$$

$$= \frac{(F-F_0)^2}{(\Delta F)^2} + F^2(X-1)^2 \sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} + 2F(X-1)\sum_{i=1}^{N} \frac{f_i^2 - f_i y_i}{\sigma_i^2} + \sum_{i=1}^{N} \frac{(y_i - f_i)^2}{\sigma_i^2} \quad (31)$$

When equation (31) is evaluated for euploids (e.g., $X = 1$), the term $\frac{(F-F_0)^2}{(\Delta F)^2}$ often depends on F, thus fitted F frequently equals F_0. In some instances, when equation (24) is evaluated for euploids, the equation sometimes reduces to $\sum_{i=1}^{N} \frac{(y_i - f_i)^2}{\sigma_i^2}$.

When equation (24) is evaluated for singleton trisomy cases (e.g., $X = 3/2$), the coefficients that multiply F contain both fetal fraction measurements and bin counts, therefore the optimized value for F often depends on both parameters. The first derivative of equation (24) with respect to F reduces to zero in some instances:

$$\frac{1}{2}\left(\frac{d\varphi}{dF}\right) = 0 = \frac{(F-F_0)}{(\Delta F)^2} + F(X-1)^2 \sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} + (X-1)\sum_{i=1}^{N} \frac{f_i^2 - f_i y_i}{\sigma_i^2} \quad (32)$$

In some embodiments, replacing $X = 3/2$ and solving equation (32) for F yields an optimized value for F:

$$F = \frac{F_0 + \frac{(\Delta F)^2}{2}\sum_{i=1}^{N} \frac{1}{\sigma_i^2}(f_i y_i - f_i^2)}{1 + \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} f_i^2/\sigma_i^2} \quad (33).$$

To simplify further calculations and/or derivations, the following auxiliary variables will be utilized:

$$S_0 = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} \frac{1}{\sigma_i^2} \quad (34)$$

$$S_f = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} \frac{f_i}{\sigma_i^2} \quad (35)$$

$$S_y = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} \frac{y_i}{\sigma_i^2} \quad (36)$$

$$S_{yy} = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} \frac{y_i^2}{\sigma_i^2} \quad (37)$$

$$S_{ff} = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} \frac{f_i^2}{\sigma_i^2} \quad (38)$$

$$S_{fy} = \frac{(\Delta F)^2}{4} \sum_{i=1}^{N} \frac{y_i f_i}{\sigma_i^2} \quad (39)$$

Utilizing the auxiliary variables, the optimized fetal fraction for $X = 3/2$ for equation (33) then reduces to:

$$F = \frac{F_0 + 2S_{fy} - 2S_{ff}}{1 + S_{ff}} \quad (40)$$

Fitted F often is linearly proportional to the measured value F_0, but sometimes is not necessarily equal to F_0. The ratio between errors in fetal fraction measurements and uncertainties in bin counts determines the relative weight given to the measured F_0 versus individual bins, in some embodiments. In some instances, the larger the error ΔF, the stronger the influence that bin counts will exert on the fitted F. Alternatively, small ΔF generally implies that the fitted value F will be dominated by F_0. In some embodiments, if a data set comes from a trisomy sample, and all errors are negligible, equation (40) reduces to identity between F and F_0. By way of mathematic proof, using fetal ploidy set to X = 3/2, and assuming that F_0 (observed) and F_V (true) have the same value, equation (30) becomes:

$$y_i = \frac{1}{2} F_0 f_i + f_i \quad (41)$$

The assumption that F_0 and F_V generally is an acceptable assumption for the sake of the qualitative analysis presented herein. Combing equations (39) and (41) yields

$$S_{fy} = \frac{(\Delta F)^2}{4} \sum_{i=1}^{N} \frac{y_i f_i}{\sigma_i^2} = \frac{(\Delta F)^2}{4} \sum_{i=1}^{N} \frac{\left(\frac{1}{2} F_0 f_i + f_i\right) f_i}{\sigma_i^2} = \left(\frac{1}{2} F_0 + 1\right) S_{ff} \quad (42)$$

Combining equations (40) and (42) results in identity between F_0 and F_V:

$$F = \frac{F_0 + 2S_{fy} - 2S_{ff}}{1 + S_{ff}} = \frac{F_0 + 2\left(\frac{1}{2} F_0 + 1\right) S_{ff} - 2S_{ff}}{1 + S_{ff}} = \frac{F_0 (1 + S_{ff})}{1 + S_{ff}} \equiv F_0 \quad \text{QED} \quad (43)$$

To further illustrate the theoretical model, if the true ploidy is 1 (e.g., euploid) but the ploidy value use in equation (40) is set to X = 3/2 (e.g., triploid singleton), the resulting fitted *F* does not equal F_0, nor does it reduce to zero, and the following expression generally is true:

$$y_i = f_i \Rightarrow S_{fy} = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N}\frac{y_i f_i}{\sigma_i^2} = \frac{(\Delta F)^2}{4}\sum_{i=1}^{N}\frac{f_i^2}{\sigma_i^2} = S_{ff} \Rightarrow F = \frac{F_0 + 2S_{fy} - 2S_{ff}}{1+S_{ff}} = \frac{F_0}{1+S_{ff}}$$

(44).

Thus, application of triploid equations when testing a euploid case generally results in a non-zero fitted *F* that is proportional to F_0 with a coefficient of proportionality between 0 and 1 (exclusive), depending on reference bin counts and associated uncertainties (cf. equation (38)), in certain embodiments. A similar analysis is shown in FIG. 44, using actual data from 86 know euploids as reference. The slope of the straight line from equation (44) is close to 20 degrees, as shown in FIG. 44.

The solitary data point between euploid and T21 cases (e.g., measured fetal fraction approximately 40%, fitted fraction approximately 20%) represents a T21 twin. When a constant ΔF is assumed the euploid branch of the graph shown in FIG. 44 generally is sloped, however when $\Delta F = 2/3 + F_0/6$ is used the euploid branch of the graph often becomes substantially horizontal, as described herein in the section entitled “Fixed ploidy, optimized fetal fraction, error propagation: fitted fetal fractions”.

Fixed ploidy, optimized fetal fraction: sums of squared residuals

In some instances for euploid cases, were fitted *F* for equation (32) equals F_0 and X = 1, the sum of square residuals for a euploid model follows from equation (31):

$$\varphi_E = \sum_{i=1}^{N}\frac{1}{\sigma_i^2}(y_i - f_i)^2 = \Xi_{yy} - 2\Xi_{fy} + \Xi_{ff} \quad (45)$$

which is substantially the same result as equation (9). In certain instances for euploid cases, equation (40) can be combined into equation (31). The resulting mathematical expression quadratically depends on F_0, in some embodiments. In certain embodiments, classification of a genetic variation is performed by subtracting the triploid sum of squared residuals from the euploid sum of squared residuals. The result of the classification obtained by subtracting the triploid sum of squared residuals from the euploid sum of squared residuals also frequently depends on F_0:

$$\varphi_E - \varphi_T$$

$$= \frac{-1}{(\Delta F)^2}\left[\left(\frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}} - F_0\right)^2 + \left(\frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}}\right)^2 \frac{(\Delta F)^2}{4}\sum_{i=1}^{N}\frac{f_i^2}{\sigma_i^2} + \left(\frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}}\right)(\Delta F)^2\sum_{i=1}^{N}\frac{f_i^2-f_i y_i}{\sigma_i^2}\right]$$

$$= \frac{-1}{(\Delta F)^2}\left[\left(\frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}} - F_0\right)^2 + \left(\frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}}\right)^2 S_{ff} + 4\left(\frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}}\right)(S_{ff}-S_{fy})\right]$$

$$= \frac{-\left[(2S_{fy}-2S_{ff}-F_0S_{ff})^2+(F_0+2S_{fy}-2S_{ff})^2S_{ff}+4(F_0+2S_{fy}-2S_{ff})(1+S_{ff})(S_{ff}-S_{fy})\right]}{(\Delta F)^2(1+S_{ff})^2}$$

$$= \frac{-1}{(\Delta F)^2(1+S_{ff})^2}\left[\left(4S_{fy}^2+4S_{ff}^2+F_0^2S_{ff}^2-8S_{fy}S_{ff}-4F_0S_{fy}S_{ff}+4F_0S_{ff}^2\right)\right.$$

$$+\left(F_0^2S_{ff}+4S_{fy}^2S_{ff}+4S_{ff}^3+4F_0S_{fy}S_{ff}-4F_0S_{ff}^2-8S_{fy}S_{ff}^2\right)$$

$$+\left(4F_0S_{ff}+8S_{fy}S_{ff}-8S_{ff}^2-4F_0S_{fy}-8F_0S_{fy}+8S_{fy}S_{ff}\right.$$

$$\left.\left.+4F_0S_{ff}^2+8S_{fy}S_{ff}^2-8S_{ff}^3-4F_0S_{fy}S_{ff}-8S_{fy}^2S_{ff}+8S_{fy}S_{ff}^2\right)\right]$$

$$= \frac{-1}{(\Delta F)^2(1+S_{ff})}\left[F_0^2S_{ff}+4F_0(S_{ff}-S_{fy})-4(S_{ff}-S_{fy})^2\right]$$

(46)

The term S_{fy} generally depends on fetal fraction, as also seen for equation (14). The dependence of $\varphi_E - \varphi_T$ on the measured fetal fraction can be analyzed by accounting for the fetal fraction, in some embodiments. The fetal fraction often can be accounted for by assuming that measured fetal fraction F_0 equals true fetal fraction F_V. In some embodiments, if the sample's karyotype is euploid, S_{fy} and S_{ff} have the same values (e.g., with the exception of experimental errors). As a result, the difference between the two sums of squared residuals often reduces to:

$$\varphi_E - \varphi_T = \frac{-F_0^2S_{ff}}{(\Delta F)^2(1+S_{ff})} \qquad \text{(Euploids)} \tag{47}$$

In certain embodiments, if the sample's karyotype is triploid, equations (41) and (42) can be combined with equation (46), yielding:

$$\varphi_E - \varphi_T = \frac{F_0^2S_{ff}}{(\Delta F)^2} \qquad \text{(Triploids)} \tag{48}$$

Thus, if the difference of $\Phi_S - \Phi_T$ is positive, the fetus is triploid, in some embodiments, and in certain embodiments, if the difference is negative, the fetus is unaffected. The graphical representation for the positive or negative result frequently is a parabola; concave for triploids and convex for euploids. Both branches tend towards zero as F_0 decreases, with experimental error having little effect on the shape of the graph. Neither branch has a substantially linear or free term, but the second order coefficients differ in size in addition to having different signs, in many instances. With ΔF approximately 2%, the value of the term S_{ff} is close to 3.7, using the reference counts and uncertainties extracted from the 86 euploid set (see FIG. 45).

In the example shown in FIG. 45, the two branches often are asymmetric due to the different coefficients multiplying the square of the measured fetal fraction in equations (47) and (48). The triploid (e.g., positive) branch increases relatively quickly, becoming distinguishable from zero substantially earlier than the euploid branch. FIG. 46, obtained using a real data set, confirms the qualitative results shown in FIG. 45. In FIG. 46 the solitary dark gray point in the fourth quadrant (e.g., lower middle quadrant) is an affected twin. In the data set used to generate FIG. 46, both the euploid and T21 branches of the graph show curvature because both show quadratic dependence on F_0 from the trisomy version of equation (31)

In some embodiments, both branches of the graph can be linearized to facilitate visual inspection. The value of the linearization often is conditioned on the error propagation analysis. The results presented in FIG. 45 and 46 were based on the assumption that the error in measured fetal fractions is uniform the entire range of fetal fractions. However, the assumption is not always the case. In some instances, the more realistic assumption, based on a linear relationship between error ΔF and measured fetal fraction $F_0(\Delta F = 2/3 + F_0/6)$, produces the results presented in FIG. 47. In FIG. 47, the euploid branch is substantially flat, almost constant (e.g., the parabolic character is substantially lost), however, the trisomy branch remains parabolic. The three light gray points interspersed in the dark gray points of the trisomy branch represent data from twins. Twin data sometimes are elevated relative to the fixed error model.

Classification of whether or not a sample is affected by a genetic variation often is carried out using one of three processes: (1) classification based on parabolic differences of summed squares of residuals, (see FIG. 45 and 46), (2) classification based on linear differences of summed squares of residuals, (see FIG. 47 and 48), and (3) classification based on fitted fetal fraction (see equation (33)). In some embodiments, the chosen approach takes error propagation into account.

Fixed ploidy, optimized fetal fraction: systematic error – reference offset

Ideally, reference and measured bin counts should contain zero systematic error (e.g., offset), however, in practice, reference and measured bin counts sometimes are shifted with respect to one another. In some instances, the effect of the shift with respect to one another can be analyzed using equation (33), assuming the shift Δ is constant across the chromosome of interest. For euploid cases, if random errors are neglected, the following relationships hold, in some embodiments:

$$f_i = f_i^0 + \Delta \quad (49)$$

$$y_i = f_i^0 = f_i - \Delta \quad (50)$$

f_i^0 represents the true reference bin count i, and f_i represents the reference bin counts used, including any systematic error Δ. In certain embodiments, replacing equations (49) and (50) into equation (33) generates the following expression for the euploid branch of the fitted fetal fraction graph:

$$F_E = \frac{F_0 + \frac{(\Delta F)^2}{2}\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left(f_i y_i - f_i^2\right)}{1 + \frac{(\Delta F)^2}{4}\sum_{i=1}^{N} f_i^2/\sigma_i^2} = \frac{F_0 + \frac{(\Delta F)^2}{2}\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left[\left(f_i^0+\Delta\right)f_i^0 - \left(f_i^0+\Delta\right)^2\right]}{1 + \frac{(\Delta F)^2}{4}\sum_{i=1}^{N}\left(f_i^0+\Delta\right)^2/\sigma_i^2}$$

$$= \frac{F_0 - \frac{(\Delta F)^2}{2}\left(\Delta\sum_{i=1}^{N}\frac{f_i^0}{\sigma_i^2} + \Delta^2\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\right)}{1 + \frac{(\Delta F)^2}{4}\left(\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left(f_i^0\right)^2 + 2\Delta\sum_{i=1}^{N}\frac{f_i^0}{\sigma_i^2} + \Delta^2\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\right)} = \frac{F_0 - 2S_f^0\Delta - 2S_0^0\Delta^2}{1 + S_{ff}^0 + 2S_f^0\Delta + S_0^0\Delta^2} \quad (51)$$

The coefficients S_0^0, S_f^0 and S_{ff}^0, are generated from equations (33) to (39) by replacing f_i with f_i^0, in some embodiments. In certain embodiments, the reciprocal slope of the linear functional relationship between fitted euploid value F_E and measured F_0 equals $1 + S_{ff}^0 + 2\,S_f^0\,\Delta + S_0^0\,\Delta^2$, which often allows estimation of the systematic error Δ by solving a relatively simple quadratic equation. For triploids, assuming F_0 equals F_V, measured bin counts sometimes become:

$$y_i = f_i^0 + \frac{1}{2}F_0 f_i^0 \qquad (52)$$

Combining equations (52), (49) and (33) generates the following expression for the triploid branch of the fitted fetal fraction graph:

$$F_T = \frac{F_0 + \frac{(\Delta F)^2}{2}\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left(f_i N_i - f_i^2\right)}{1+\frac{(\Delta F)^2}{4}\sum_{i=1}^{N} f_i^2/\sigma_i^2} = \frac{F_0 + \frac{(\Delta F)^2}{2}\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left[\left(f_i^0+\Delta\right)\left(f_i^0+\frac{1}{2}F_0 f_i^0\right)-\left(f_i^0+\Delta\right)^2\right]}{1+\frac{(\Delta F)^2}{4}\sum_{i=1}^{N}\left(f_i^0+\Delta\right)^2/\sigma_i^2}$$

$$= \frac{F_0 + \frac{(\Delta F)^2}{2}\left(\frac{1}{2}F_0\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left(f_i^0\right)^2+\frac{1}{2}F_0\Delta\sum_{i=1}^{N}\frac{f_i^0}{\sigma_i^2}-\Delta\sum_{i=1}^{N}\frac{f_i^0}{\sigma_i^2}-\Delta^2\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\right)}{1+\frac{(\Delta F)^2}{4}\left(\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left(f_i^0\right)^2+2\Delta\sum_{i=1}^{N}\frac{f_i^0}{\sigma_i^2}+\Delta^2\sum_{i=1}^{N}\frac{1}{\sigma_i^2}\right)} = \frac{F_0\left(1+S_{ff}^0+S_f^0\Delta\right)-S_f^0\Delta-S_0^0\Delta^2}{1+S_{ff}^0+2S_f^0\Delta+S_0^0\Delta^2} \qquad (53)$$

In some embodiments, equations (51) and (53) predict that fitted triploid and euploid fetal fractions will behave as shown in FIG. 48. In FIG. 48 black lines (e.g., upper lines in each set of 3 lines) correspond to negative offset Δ, dark gray lines (e.g., bottom lines in each set of 3 lines) correspond to positive offset Δ, and light gray lines (e.g., middle lines in each set of 3 lines), correspond to the absence of offset. FIG. 49 illustrates the effects of simulated systematic errors Δ artificially imposed on actual data.

FIG. 50 illustrates the dependence of fitted fetal fraction on systematic error offset for euploid and triploid data sets. For both euploid and triploid cases, the theoretical expressions of equations (51) and (53) often capture the qualitative dependence of fitted fetal fraction on measured fetal fraction and on systematic error offset. Coefficients used for the graphs in FIG. 49 and 50 were obtained from raw reference bin counts, without removing any potential systematic bias.

Fixed ploidy, optimized fetal fraction, error propagation: fitted fetal fraction

Contributions to errors in fitted fetal fractions often fall into one of two types of errors: 1) from measured fetal fractions, and 2) from measured and reference bin counts. The two types of errors will be analyzed separately, using different approaches, and later combined to generate final error ranges. Errors propagated from measure fetal fractions can be evaluated by replacing F_0 in equation (40) first with $F_0 - 2\Delta F$ (e.g., for the lower error boundary) and then with $F_0 + 2\Delta F$ (e.g., for

the upper error boundary). This relatively simple approach produces correct qualitative behavior at 95% confidence intervals, in certain embodiments. For a different desired level of confidence, a more general pair of bounds, $F_0 - n\Delta F$ and $F_0 + n\Delta F$, can be utilized. The terms used to generate upper and lower error boundaries sometimes underestimates the total error because the contributions from errors in measure and reference bin counts often are neglected.

To better assess the contribution from measured and reference bin counts on error in fitted fetal fraction, equations (38) to (40) can be utilized, in some embodiments. In certain embodiments, equation (33) can be expanded for fitted fetal fraction into a Taylor series with respect to f_i and y_i, truncated to the first order, square and average. In some instances, it can be assumed that uncertainties in y_i often are the same as uncertainties in f_i. To simply analysis, cross-terms and higher-order terms are assumed to reduce to zero upon averaging. Taylor expansion coefficients often are obtained utilizing the chain rule. The mean squared variation in the fitted fetal fraction is then given by equation (54) shown below. The model represented by equation ignores contributions from estimates for ΔF, in some embodiments. Partial derivatives can be evaluated using the expressions presented below equation (54).

$$(\delta F)^2 = \sum_{i=1}^{N} \left(\frac{\partial F}{\partial f_i}\right)^2 \sigma_i^2 + \sum_{i=1}^{N} \left(\frac{\partial F}{\partial y_i}\right)^2 \sigma_i^2$$

$$= \sum_{i=1}^{N} \left[\left(\frac{\partial F}{\partial S_{ff}}\right)\left(\frac{\partial S_{ff}}{\partial f_i}\right) + \left(\frac{\partial F}{\partial S_{fy}}\right)\left(\frac{\partial S_{fy}}{\partial f_i}\right)\right]^2 \sigma_i^2 + \sum_{i=1}^{N} \left[\left(\frac{\partial F}{\partial S_{fy}}\right)\left(\frac{\partial S_{fy}}{\partial y_i}\right)\right]^2 \sigma_i^2 \quad (54)$$

$$\left(\frac{\partial F}{\partial S_{ff}}\right) = -\frac{F_0 + 2S_{fy} + 2}{(1+S_{ff})^2} \quad (55)$$

$$\left(\frac{\partial F}{\partial S_{fy}}\right) = \frac{2}{1+S_{ff}} \quad (56)$$

$$\left(\frac{\partial S_{ff}}{\partial f_i}\right) = \frac{(\Delta F)^2}{2}\left(\frac{f_i}{\sigma_i^2}\right) \quad (57)$$

$$\left(\frac{\partial S_{fy}}{\partial f_i}\right) = \frac{(\Delta F)^2}{4}\left(\frac{y_i}{\sigma_i^2}\right) \quad (58)$$

$$\left(\frac{\partial S_{fy}}{\partial y_i}\right) = \frac{(\Delta F)^2}{4}\left(\frac{f_i}{\sigma_i^2}\right) \quad (59)$$

Combining equations (54) to (59) generates the following expression:

$$(\delta F)^2 = \left[\frac{(\Delta F)^2}{4}\right]^2 \left\{\sum_{i=1}^{N} \frac{1}{\sigma_i^2}\left[\frac{2y_i}{1+S_{ff}} - 2f_i\frac{F_0+2S_{fy}+2}{(1+S_{ff})^2}\right]^2 + \sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left(\frac{2f_i}{1+S_{ff}}\right)^2\right\}$$

$$= \left[\frac{(\Delta F)^2}{4}\right]^2 \sum_{i=1}^{N}\frac{1}{\sigma_i^2}\left[\left(\frac{2y_i}{1+S_{ff}}\right)^2 - 8f_iy_i\frac{F_0+2S_{fy}+2}{(1+S_{ff})^3} + 4f_i^2\frac{(F_0+2S_{fy}+2)^2}{(1+S_{ff})^4} + \left(\frac{2f_i}{1+S_{ff}}\right)^2\right]$$

$$= \left[\frac{(\Delta F)^2}{4}\right]^2 \left\{\frac{4}{(1+S_{ff})^2}\sum_{i=1}^{N}\frac{y_i^2}{\sigma_i^2} - 8\frac{F_0+2S_{fy}+2}{(1+S_{ff})^3}\sum_{i=1}^{N}\frac{f_iy_i}{\sigma_i^2} + 4\left[\frac{(F_0+2S_{fy}+2)^2}{(1+S_{ff})^4} + \frac{1}{(1+S_{ff})^2}\right]\sum_{i=1}^{N}\frac{f_i^2}{\sigma_i^2}\right\}$$

$$= (\Delta F)^2\left\{\frac{S_{yy}}{(1+S_{ff})^2} - 2S_{fy}\frac{F_0+2S_{fy}+2}{(1+S_{ff})^3} + S_{ff}\left[\frac{(F_0+2S_{fy}+2)^2}{(1+S_{ff})^4} + \frac{1}{(1+S_{ff})^2}\right]\right\}$$

(60)

To evaluate equation (60) at a 95% confidence interval, the following upper and lower bounds can be used, in some embodiments:

$$\begin{bmatrix}F_{Lower}\\F_{Upper}\end{bmatrix} = \frac{F_0+2S_{fy}-2S_{ff}}{1+S_{ff}} + \begin{bmatrix}-2\\2\end{bmatrix}\Delta F\left\{\frac{1}{1+S_{ff}} + \sqrt{\frac{S_{yy}}{(1+S_{ff})^2} - 2S_{fy}\frac{F_0+2S_{fy}+2}{(1+S_{ff})^3} + S_{ff}\left[\frac{(F_0+2S_{fy}+2)^2}{(1+S_{ff})^4} + \frac{1}{(1+S_{ff})^2}\right]}\right\}$$

(61)

In embodiments in which substantially all possible sources of error (e.g., F_0, f_i, y_i) are included in the Taylor expansion series, the same equation often is obtained. In some instances, dependence of F on F_o, can be accounted for through S_{fy}. In some embodiments, power series terms corresponding to F_0 often take the form;

$\left[\left(\frac{\partial F}{\partial F_0}\right)+\left(\frac{\partial F}{\partial S_{fy}}\right)\left(\frac{\partial S_{fy}}{\partial F_0}\right)\right]^2 (\Delta F)^2$, but $\left[\left(\frac{\partial F}{\partial F_0}\right)+\left(\frac{\partial F}{\partial S_{fy}}\right)\left(\frac{\partial S_{fy}}{\partial F_0}\right)\right]^2$ equals 1 for triploids. Thus, relatively simple subtraction and addition of ΔF to F_0 often is justified, even though ΔF often increases with F_0 and becomes large at high F_0. The outcome is due to both F and S_{fy} depending linearly on F_0, in some embodiments. Simulations based on equation (61) are shown in FIG. 51, along with fitted fetal fractions obtained from test subject derived data. In the simulations presented in Fig, 51, $\Delta F = 2/3 + F_0/6$, as described herein.

Example 3: Sliding window analysis and cumulative sums as a function of genomic position

Identification of recognizable features (e.g., regions of genetic variation, regions of copy number variation) in a normalized count profile sometimes is a relatively time consuming and/or relatively expensive process. The process of identifying recognizable features often is complicated by data sets containing noisy data and/or low fetal nucleic acid contribution. Identification of recognizable features that represent true genetic variations or copy number variations can help avoid searching large, featureless regions of a genome. Identification of recognizable features can be achieved by removing highly variable genomic sections from a data set being searched and obtaining, from the remaining genomic sections, data points that deviate from the mean profile elevation by a predetermined multiple of the profile variance.

In some embodiments, obtaining data points that deviate from the mean profile elevation by a predetermined multiple of the profile variance can be used to reduce the number of candidate genomic sections from greater than 50,000 or 100, 000 genomic sections to in the range of about 100 to about 1000 candidate genomic sections that represent true signals or solitary noise spikes (e.g., about 100 genomic sections, about 200 genomic sections, about 300 genomic sections, about 400 genomic sections, about 500 genomic sections, about 600 genomic sections, about 700 genomic sections, about 800 genomic sections, about 900 genomic sections, or about 1000 genomic sections). The reduction in the number of candidate genomic sections can be achieved relatively quickly and easily and often speeds up the search for and/or identification of genetic aberrations by two or more orders of magnitude. Reduction in the number of genomic sections searched for the presence or absence of candidate regions of genomic variation often reduces the complexity and/or dimensionality of a data set.

After a reduced data set containing data points that deviate from the mean profile elevation by a predetermined multiple of the profile variance is generated, the reduced data set is filtered to eliminate solitary noise spikes, in some embodiments. Filtering a reduced data set to remove solitary noise spikes often generates a filtered, reduced data set. In some embodiments, a filtered, reduced data set retains contiguous clusters of data points, and in certain embodiments, a filtered, reduced data set retains clusters of data points that are largely contiguous with allowance for a predetermined number and/or size of gaps. Data points from the filtered, reduced data set that deviate from the average profile elevation in substantially the same direction are grouped together, in some embodiments.

Due to the background noise often present in nucleic acid samples (e.g., ratio of regions of interest compared to the total nucleic acid in a sample), distinguishing regions of genetic variation or genetic aberration from background noise often is challenging. Methods that improve the signal-to-noise ratio often are useful for facilitating the identification of candidate regions representative of regions of true genetic variation and/or genetic aberration. Any method that improves the signal-to-noise ratio of regions of true genetic variation with respect to the genomic background noise can be used. A non-limiting example of a method suitable for use in improving the signal-to-noise ratio of regions of true genetic variation with respect to the genomic background noise is the use of integrals over the suspected aberration and its immediate surroundings. In some embodiments, the use of integrals over the suspected aberration and its immediate surroundings is beneficial, because summation cancel out random noise. After noise has been reduced or eliminated, even relatively minor signals can become readily detectable using a cumulative sum of the candidate peak and its surroundings, in some embodiments. A cumulative sum sometimes is defined with respect to an arbitrarily chosen origin outside (e.g., on one side or the other) of the peak. A cumulative sum often is a numerical estimate of the integral of the normalized count profile over the selected genetic section or sections.

In the absence of aberrations, the cumulative sum as a function of the genomic position often behaves as a straight line with unit slope (e.g., slope equal to 1). If deletions or duplications are present, the cumulative sum profile often consists of two or more line segments. In some embodiments, areas outside of aberrations map to line segments with unit slopes. For areas within aberrations, the line segments are connected by other line segments whose slopes equal the count profile elevation or depression within the aberration, in certain embodiments.

In those samples having maternal aberrations, the slopes (e.g., equivalent to the count profile elevation) are relatively easily determined: 0 for homozygous maternal deletions, 0.5 for heterozygous maternal deletions, 1.5 for heterozygous duplications, 2.0 for homozygous duplications. In those samples having fetal aberrations, the actual slopes depend both on the type of the aberration (e.g., homozygous deletion, heterozygous deletion, homozygous duplication or heterozygous duplication) and on the fetal fraction. In some embodiments, inheritance of a maternal aberration by the fetus also is taken into account when evaluating fetal samples for genetic variations.

In some embodiments, line segments with unit slopes, corresponding to normal genomic areas to the left and to the right of an aberration, are vertically shifted with respect to one another. The difference (e.g., subtractive result) between their intercepts equals the product between the width of the aberration (number of affected genomic sections) and the aberration level (e.g., -1 for homozygous maternal deletion, -0.5 for heterozygous maternal deletion, +0.5 for heterozygous duplication, +1 for homozygous duplication, and the like). Refer to FIGS. 52-61F for examples of data sets processed using cumulative sums as a function of genomic position (e.g., sliding window analysis).

Example 4: Parameterized Error Removal and Unbiased Normalization (PERUN)

Variability of Measured Counts

Ideally, the measured chromosomal elevation is a straight horizontal line with the elevation of 1 for euploids, as in FIG. 62. For trisomy pregnancies, the desired behavior of the measured chromosomal elevation is a step-function, with the deviation from 1 proportional to the fetal fraction, as simulated in FIG. 63 for fetal fraction equal to 15%. Exceptions arise out of maternal deletions/duplications, which are readily recognized and distinguished from fetal abnormalities based on their magnitudes, which are multiples of one-half.

What was actually measured was not ideal. FIG. 64 shows overlaid raw counts for chromosomes 20, 21, and 22 collected from 1093 euploid pregnancies and FIG. 65 shows overlaid raw counts for chromosomes 20, 21, and 22 collected from 134 trisomy 21 pregnancies. Visual inspection of the two sets of profiles failed to confirm that chromosome 21 traces in trisomy cases were elevated. Stochastic noise and systematic bias both made the elevation of chromosome 21 difficult to

visualize. Furthermore, the far right segment of chromosome 21 incorrectly suggested that euploid chromosome 21 traces were elevated, rather than the trisomy profiles. A large part of the systematic bias originated from the GC content associated with a particular genomic region.

Attempts to remove the systematic bias due to GC content included multiplicative LOESS GC smoothing, Repeat Masking (RM), combination of LOESS and RM (GCRM), and others, such as cQN. FIG. 66 shows the results of a GCRM procedure as applied to 1093 euploid traces and FIG. 67 shows the GCRM profiles for 134 trisomy cases. GCRM successfully flattened the elevated, GC-rich, rightmost segment of chromosome 21 in euploids. However, the procedure evidently increased the overall stochastic noise. Moreover, it created a new systematic bias, absent from the raw measurements (leftmost region of chromosome 20 (Chr20)). The improvements that were due to GCRM were offset by increased noise and bias, rendering the usefulness of the procedure questionable. The tiny elevation from chromosome 21 as observed in FIG. 63 was lost in the high noise as shown in FIG. 66 and FIG. 67.

PERUN (Parameterized Error Removal and Unbiased Normalization) was developed as a viable alternative to previously described GC normalization methods. FIG. 68 and FIG. 69 contrast the PERUN method results against those presented in FIG. 64 through 67. PERUN results were obtained on the same two subpopulations of data that was analyzed in FIG. 64 through 67. Most of the systematic bias was absent from PERUN traces, only leaving stochastic noise and biological variation, such as the prominent deletion in chromosome 20 of one of the euploid samples (FIG. 68). The chromosome 20 deletion was also observable in raw count profiles (FIG. 64), but completely masked in the GCRM traces. The inability of GCRM to reveal this huge deviation clearly disqualifies it for the purposes of measuring the miniscule fetal T21 elevations. PERUN traces contain fewer bins than raw or GCRM profiles. As shown in FIG. 62-63, the PERUN results look at least as good as the measurement errors permit.

Normalization with Respect to Reference Median Count Profile

Conventional GC normalization procedures can perform suboptimally. A part of the reason has been that GC bias is not the only source of variation. A stack plot of many individual raw count profiles revealed parallelism between different samples. While some genomic regions were consistently over-represented, others were consistently under-represented, as illustrated by the traces from a 480v2 study (FIG. 6). While GC bias varied from one sample to another, the systematic, bin-specific bias observed in these profiles followed the same pattern for all samples.

All the profiles in FIG. 6 zigzagged in a coordinated fashion. The only exceptions were the middle portions of the bottom two samples, which turned out to originate from maternal deletions. To correct for this bin-specific bias, a median reference profile was used. The median reference profile was constructed from a set of known euploids (e.g. euploid pregnancies) or from all the samples in a flow cell. The procedure generated the reference profile by evaluating median counts per bin for a set of reference samples. The MAD associated with a bin measured the reliability of a bin. Highly variable bins and bins that consistently have vanishing representations were removed from further analysis (FIG. 4). The measured counts in a test data set were then normalized with respect to the median reference profile, as illustrated in FIG. 8. The highly variable bins are removed from the normalized profile, leaving a trace that is approximately 1 in the diploid sections, 1.5 in the regions of heterozygous duplication, 0.5 in the areas of heterozygous deletion, and so on (FIG. 9). The resulting normalized profiles reasonably reduced the variability, enabling detection of maternal deletions and duplications and tracing of sample identities (FIG. 12, 22, 13, 11). Normalization based on median count profile can clarify outcomes, but GC bias still has a negative effect on such methods. PERUN methods described here can be used to address GC bias and provide outcomes with higher sensitivity and specificity.

Detrimental Effects of Multiplicative LOESS Correction

FIG. 11. illustrated why binwise counts fluctuate more after application of GC-LOESS or GCRM (FIG. 66-67) than before (FIG. 64-65). LOESS GC correction removed the trend from the raw counts (FIG. 70, upper panel) by dividing the raw counts with the regression line (straight line, FIG. 70, upper panel). The point defined by the median counts and the median genome GC content was kept immobile. On average, counts below the median count were divided by small numbers, while counts exceeding the median count were divided by large numbers. In either case, on average, counts were scaled up or down to match 1 (FIG. 70, lower panel). The scaling of small counts, in addition to inflating the counts, also inflated their variability. The end result (FIG. 70, lower panel) to the left from the median GC genome content displayed a larger spread than the corresponding raw counts (FIG. 70, upper panel), forming the typical triangular shape (FIG. 70, lower panel, triangle). To detrend the counts, GC LOESS/GCRM sacrificed precision as such corrective processes generally are multiplicative and not additive. Normalization provided by PERUN generally is additive in nature and enhances precision over multiplicative techniques.

Inadequacy of a Genome-Wide Pivot for GC-Bias Scaling

An alternative approach applied the LOESS correction separately to individual chromosomes instead of subjecting the entire genome to a collective GC-Bias scaling. The scaling of individual chromosomes was impractical for purposes of classifying samples as euploid or trisomy because it canceled out the signal from over-represented chromosomes. However, the conclusions from this study were eventually useful as catalyzers for developing the PERUN algorithm. FIG. 71 illustrates the fact that LOESS curves obtained for the same chromosome from multiple samples share a common intersection (pivot).

FIG. 72 demonstrated that tilting chromosome-specific LOESS curves around the pivot by an angle proportional to the GC bias coefficients measured in those samples caused all the curves to coalesce. The tilting of the chromosome-specific LOESS curves by the sample-specific GC bias coefficients significantly reduced the spread of the family of LOESS curves obtained for multiple samples, as shown in FIG. 73 (black v-shaped line (before tilting) and grey bottom line (after tilting)). The point where the black and grey curves touch coincided with the pivot. In addition, it became evident that the location on the GC content axis of the chromosome-specific pivot coincided with the median GC content of the given chromosome (FIG. 74, left vertical grey line: median, right vertical bold line: mean). Similar results were obtained for all chromosomes, as shown in FIG. 75A through FIG. 75F (left vertical grey line: median, right vertical bold line: mean). All autosomes and chromosome X were ordered according to their median GC content.

The genome-wide GC LOESS scaling pivoted the transformation on the median GC content of the entire genome, as shown in FIG. 76. That pivot was acceptable for chromosomes that have median GC content similar to the GC content of the entire genome, but became suboptimal for chromosomes with extreme GC contents, such as chromosomes 19, 20, 17, and 16 (extremely high GC content). The pivoting of those chromosomes centered on the median GC content of the entire genome maintained the spread observed within the left box in FIG. 76, missing the low-variability region enclosed by the right box in FIG. 76 (the chromosome-specific pivot).

Pivoting on the chromosome-specific median GC content, however, significantly reduced the variability (FIG. 75). The following observations were made:

1) GC correction should be done on small genomic sections or segments, rather than on the entire genome, to reduce the variability. The smaller the section or segment, the more focused GC correction becomes, minimizing the residual error.

2) In this particular instance, those small genomic sections or segments are identical to chromosomes. In principle, the concept is more general: the sections or segments could be any genomic regions, including 50 kbp bins.

3) The GC bias within individual genomic regions can be rectified using the sample-specific, genome-wide GC coefficient evaluated for the entire genome. This concept is important: while some descriptors of the genomic sections (such as the location of the pivot point, GC content distribution, median GC content, shape of the LOESS curve, and so on) are specific to each section and independent of the sample, the GC coefficient value used to rectify the bias is the same for all the sections and different for each sample.

These general conclusions guided the development of PERUN, as will become apparent from the detailed description of its processes.

Separability of Sources of Systematic Bias

Careful inspection of a multitude of raw count profiles measured using different library preparation chemistries, clustering environments, sequencing technologies, and sample cohorts consistently confirmed the existence of at least two independent sources of systematic variability:

1) sample-specific bias based on GC-content, affecting all bins within a given sample in the same manner, varying from sample to sample, and

2) bin-specific attenuation pattern common to all samples.

The two sources of variability are intermingled in the data. Thorough removal of both required their deconvolution. The deficiencies of the error-removal procedures predating PERUN stem from the fact that they only correct for one of the two sources of systematic bias, while neglecting the other.

For example, the GCRM (or GC LOESS) method treated identically all the bins with GC content values falling within a narrow GC content range. The bins belonging to that subset may be characterized by a wide range of different *intrinsic* elevations, as reflected by the reference median count profile. However, GCRM was blind to their inherent properties other than their GC content. GCRM therefore maintains (or even enlarges) the spread already present in the bin subset. On the other hand, the binwise reference median count disregarded the modulation of the bin-specific attenuation pattern by the GC bias, maintaining the spread caused by the varying GC content.

The sequential application of the methods dealing with the opposite extremes of the error spectrum unsuccessfully attempts to resolve the two biases globally (genome-wide), ignoring the need to dissociate the two biases on the bin elevation. Without being limited by theory, PERUN apparently owes its success to the fact that it separates the two sources of bias locally, on the bin elevation.

Removal of Uninformative Bins

Multiple attempts to remove uninformative bins have indicated that bin selection has the potential to improve classification. The first such approach evaluated the mean chromosome 21, chromosome 18, and chromosome 13 counts per bin for all 480v2 trisomy cases and compared it with the mean counts per bin for all 480v2 euploids. The gap between affected and unaffected cases was scaled with the combined binwise uncertainty derived from bin counts measured in both groups. The resulting t-statistic was used to evaluate binwise p-value profile, shown in FIG. 77. In the case of chromosome 21, the procedure identified 36 uninformative bins (center panel, labeled with ellipse on FIG. 77). Elimination of those bins from calculation of Z scores noticeably increased the Z-values for affected cases, while randomly perturbing the unaffected Z-scores (FIG. 78), thereby increasing the gap between euploids and trisomy 21 cases.

In chromosome 18, the procedure only improved Z scores for two affected cases (FIG. 79).

A post-hoc analysis showed that the improvement of the Z-scores in those two samples resulted from removal of the large maternal deletion in chromosome 18 (FIG. 11) and that the two samples actually come from the same patient. These improvements were sample-specific, with no generalizing power. In chromosome 13, the procedure did not lead to any improvements of Z-scores.

An alternative bin filtering scheme removes bins with extremely low or extremely high GC content. This approach yielded mixed results, with noticeably reduced variance in chromosomes 9, 15, 16, 19, and 22 (depending on the cutoffs), but adverse effects on chromosomes 13 and 18.

Yet another simple bin selection scheme eliminates bins with consistently low counts. The procedure corrected two LDTv2CE chromosome 18 false negatives (FIG. 80) and two chromosome 21 false negatives (FIG. 81). It also corrected at least three chromosome 18 false positives, but created at least one new chromosome 18 false positive (FIG. 80):

In conclusion, the different criteria used to filter out uninformative bins made it clear that data processing will benefit from bin selection based on how much useful information the bins contribute to the classification.

Separation of GC Bias from Systematic Binwise Bias

To resolve and eliminate the different systematic biases found in the measured counts, the data processing workflow needed to optimally combine the partial procedures described from the previous section entitled "Normalization with Respect to Reference Median Count Profile" to the section entitled "Removal of Uninformative Bias". The first step is to order different samples according to their GC bias coefficient values and then stack their plots of counts-vs.-GC content. The result is a three-dimensional surface that twists like a propeller, schematically shown on FIG. 82.

Thus arranged, the measurements suggest that a set of sample-specific GC bias coefficient can be applied to rectify errors within an individual genomic section or segment. In FIG. 82, the sections or segments are defined by their GC content. An alternative partition of the genome gives contiguous, non-overlapping bins. The successive starting locations of the bins uniformly cover the genome. For one such 50 kbp long bin, FIG. 83 explores the behavior of the count values measured within that bin for a set of samples. The counts are plotted against the GC bias coefficients observed in those samples. The counts within the bin evidently increase linearly with the sample-specific GC bias. The same pattern in observed in an overwhelming majority of bins. The observations can be modeled using the simple linear relationship:

$$M = LI + GS \qquad \text{(A)}$$

The various terms in Eq. A have the following meanings:

- ***M***: measured counts, representing the primary information polluted by unwanted variation.
- ***L***: chromosomal elevation – this is the desired output from the data processing procedure. *L* indicates fetal and/or maternal aberrations from euploidy. This is the quantity that is masked both by stochastic errors and by the systematic biases. The chromosomal elevation *L* is both sample specific and bin-specific.
- ***G***: GC bias coefficient measured using linear model, LOESS, or any equivalent approach. *G* represents secondary information, extracted from M and from a set of bin-specific GC

content values, usually derived from the reference genome (but may be derived from actually observed GC contents as well). *G* is sample specific and does not vary along the genomic position. It encapsulates a portion of the unwanted variation.

- ***I***: Intercept of the linear model (green line in FIG. 83). This model parameter is fixed for a given experimental setup, independent on the sample, and bin-specific.

- ***S***: Slope of the linear model (green line in FIG. 83). This model parameter is fixed for a given experimental setup, independent on the sample, and bin specific.

The quantities *M* and *G* are measured. Initially, the bin-specific values *I* and *S* are unknown. To evaluate unknown *I* and *S*, we must assume that $L = 1$ for all bins in euploid samples. The assumption is not always true, but one can reasonably expect that any samples with deletions/duplications will be overwhelmed by samples with normal chromosomal elevations. A linear model applied to the euploid samples extracts the *I* and *S* parameter values specific for the selected bin (assuming $L = 1$). The same procedure is applied to all the bins in the human genome, yielding a set of intercepts *I* and slopes *S* for every genomic location. Cross-validation randomly selects a work set containing 90% of all LDTv2CE euploids and uses that subset to train the model. The random selection is repeated 100 times, yielding a set of 100 slopes and 100 intercepts for every bin. The previous section entitled “Cross-Validation of PERUN Parameters” describes the cross-validation procedure in more detail.

FIG. 84-85 show 100 intercept values and 100 slope values, respectively, evaluated for bin # 2404 in chromosome 2. The two distributions correspond to 100 different 90% subsets of 1093 LDTv2CE euploids shown in FIG. 83. Both distributions are relatively narrow and irregularly shaped. Their spreads are similar to the errors in the coefficient as reported by the linear model. As a rule, the slope is less reliable than the intercept because fewer samples populate the extreme sections of the GC-bias range.

Interpretation of PERUN Parameters I and S

The meaning of the intercept *I* is illustrated by FIG. 86. The graph correlates the estimated bin intercepts with the data extracted from a set of technical replicates, obtained when one LDTv2CE flow cell was subjected to three separate sequencing runs. The y-axis contains median values of binwise counts from those three measurements. These median values are related conceptually to the median reference profile, previously used to normalize profiles as described in the section

entitled “Normalization with Respect to Reference Median Count Profile”. The binwise intercepts are plotted along the x-axis. The striking correlation between the two quantities reveals the true meaning of the intercepts as the expected counts per bin in the absence of GC bias. The problem with the median reference count profile is that it fails to account for the GC bias (see section entitled “Normalization with Respect to Reference Median Count Profile”). In PERUN, without being limited by theory, the task of an intercept *I* is to deal with the bin-specific attenuation, while the GC bias is relegated to the other model parameter, the slope *S*.

FIG. 86 excludes chromosome Y from the correlation because the set of technical replicates does not reflect the general population of male pregnancies.

The distribution of the slope *S* (FIG. 87) illustrates the meaning of that model parameter.

The marked semblance between the distribution from FIG. 87 and the distribution of the genome-wide GC content (FIG. 88) indicates that the slope *S* approximates the GC content of a bin, shifted by the median GC content of the containing chromosome. The thin vertical line in FIG. 88 marks the median GC content of the entire genome.

FIG. 89 reaffirms the close relationship between the slope *S* and the GC content per bin. While slightly bent, the observed trend is extremely tight and consistent, with only a handful of notable outlier bins.

Extraction of Chromosomal Elevation from Measured Counts

Assuming that the model parameter values *I* and *S* are available for every bin, measurements *M* collected on a new test sample are used to evaluate the chromosomal elevation according to the following expression:

$$L = (M - GS)/I \qquad \text{(B)}$$

As in Eq. A, the GC bias coefficient *G* is evaluated as the slope of the regression between the binwise measured raw counts *M* and the GC content of the reference genome. The chromosomal elevation *L* is then used for further analyses (Z-values, maternal deletions/duplications, fetal microdeletions/ microduplications, fetal gender, sex aneuploidies, and so on). The procedure

encapsulated by Eq. B is named Parameterized Error Removal and Unbiased Normalization (PERUN).

Cross-Validation of PERUN Parameters

As inferred in the section entitled "Separation of GC Bias from Systematic Binwise Bias", the evaluation of *I* and *S* randomly selects 10% of known euploids (a set of 1093 LDTv2 in FIG. 83) and sets them aside for cross-validation. Linear model applied to the remaining 90% of euploids extracts the *I* and *S* parameter values specific for the selected bin (assuming $L = 1$). Cross validation then uses the *I* and *S* estimates for a given bin to reproduce measured *M* values from measured *G* values both in the work set and in the remaining 10% euploids (again assuming $L = 1$). The random selection of the cross-validation subset is repeated many times (100 times in FIG. 83, although 10 repetitions would suffice). 100 diagonal straight lines in FIG. 83 represent the linear models for 100 different 90% work subset selections. The same procedure is applied to all the bins in the human genome, yielding a set of intercepts *I* and slopes *S* for every genomic location.

To quantify the success of the model and avoid biasing the results, we use the *R*-factor, defined as follows:

$$R = \frac{\sum_{i=1}^{N} |M_i - P_i|}{\sum_{i=1}^{N} |M_i|} \qquad \text{(C)}$$

The numerator in Eq. B sums up the absolute deviations of the predicted count values (*P*, Eq. B) from the actual measurements (*M*). The numerator simply sums up the measurements. The *R* factor may be interpreted as the residual error in the model, or the unexplained variation. The *R* factor is directly borrowed from the crystallographic model refinement practice, which is vulnerable to bias. In crystallography, the bias is detected and measured by the R-factor evaluated within the cross-validation subset of observables. The same concepts are applied in the context of genome-wide count bias removal.

FIG. 90 shows the *R*-factors evaluated for the cross-validation subset (y-axis) plotted against *R*-factors evaluated for the work (training) set for bin # 2404 from chromosome 2. There are 100 data points since the random selection of the cross-validation subset was repeated 100 times.

Typical linear relationship is observed, with the increasing R_{cv} values (measuring bias) accompanying the decreasing R_{work}.

FIG. 90 may be interpreted in terms of the percentage error (or relative error) of the model for this particular bin. R_{cv} always exceeds R_{work}, usually by ~1%. Here, both R_{cv} and R_{work} remain below 6%, meaning that one can expect ~6% error in the predicted *M* values using the measured GC bias coefficient *G* and the model parameters *I* and *S* from the procedure described above.

Cross-Validation Error Values

FIG. 90-91 show cross-validation errors for bins chr2_2404 and chr2_2345, respectively. For those and many other bins, the errors never exceed 6%. Some bins, such as chr1_31 (FIG. 92) have cross-validation errors approaching 8%. Still others (FIG. 93-95) have much larger cross-validation errors, at times exceeding 100% (40% for chr1_10 in FIG. 93, 350% for chr1_9 in FIG. 94, and 800% for chr1_8 in FIG. 95).

FIG. 96 shows the distribution of max(R_{cv}, R_{work}) for all bins. Only a handful of bins have errors below 5%. Most bins have errors below 7% (48956 autosomes out of 61927 total including X and Y). A few bins have errors between 7% and 10%. The tail consists of bins with errors exceeding 10%.

FIG. 97 correlates the cross-validation errors with the relative errors per bin estimated from the set of technical replicates. Data points in the blue region correspond to cross-validation errors between 7% and 10%. Data points in the red region denote bins with cross-validation error exceeding 10%. Data points in the grey region (error < 7%) represent the bulk of bins.

In FIG. 91-95, the number in parentheses following the bin name above the top right inset indicates the ratio between the intercept found for that particular bin and the genome-wise median count per bin. The cross-validation errors evidently increase with the decreasing value of that ratio. For example, the bin chr1_8 never gets more than 3 counts and its relative error approaches 800%. The smaller the expected number of counts for a given bin, the less reliable that bin becomes.

Bin Selection Based on Cross-Validation

Based on the observations described in the previous section entitled "Removal of Uninformative Bins" (FIG. 78 and FIG. 80-81), cross-validation errors were used as a criterion for bin filtering. The selection procedure throws away all bins with cross-validation errors exceeding 7%. The filtering also eliminates all bins that consistently contain zero counts. The remaining subset contains 48956 autosomal bins. Those are the bins used to evaluate chromosomal representations and to classify samples as affected or euploid. The cutoff of 7% is justified by the fact that the gap separating euploid Z-scores from trisomy Z-scores plateaus at the 7% cross-validation error (FIG. 98).

FIG. 99A (all bins) and 99B (cross-validated bins) demonstrate that the bin selection described above mostly removes bins with low mappability.

As expected, most removed bins have intercepts far smaller than the genome-wide median bin count. Not surprisingly, the bin selection largely overlaps with the selection described in the previous section entitled "Removal of Uninformative Bins" (FIG. 25 and 27-28).

Errors in Model Parameters

FIG. 100-101 show the 95% confidence intervals (curved lines) of the fitted linear model (thin straight line) for two bins (chr18_6 and chr18_8). The thick grey straight lines are obtained by replacing the S parameter with the difference between the GC contents of these two bins and the median GC content of chromosome 18. The error range is evaluated based on errors in the model parameters I and S for those two bins, as reported by the linear model. In addition, larger GC bias coefficients also contain larger errors. The large uncertainty corresponding to extremely large GC bias coefficients suggests that the range of applicability of the unmodified PERUN is limited to modest GC bias coefficients. Beyond that range, additional measures need to be taken to remove the residual GC bias. Fortunately, only very few samples are affected (roughly 10% of the LDTv2CE population).

FIG. 102-104 show the errors in the model parameters I and S and the correlation between the error in S and the value of the intercept.

Secondary Normalization

High values of GC bias coefficients exceed the linear range assumed by the PERUN model and are remedied by an additional LOESS GC normalization step after PERUN normalization. The multiplicative nature of the LOESS procedure does not significantly inflate the variability since the normalized counts are already very close to 1. Alternatively, LOESS can be replaced with an additive procedure that subtracts residuals. The optional secondary normalization often is utilized only required for a minority of samples (roughly 10%).

Hole Padding (Padding)

FIG. 68-69 confirm the presence of a large number of maternal deletions and duplications that have the potential to create false positives or false negatives, depending on their sizes and locations. An optional procedure called hole-padding has been devised to eliminate the interferences from these maternal aberrations. The procedure simply pads the normalized profile to remain close to 1 when it deviates above 1.3 or below 0.7. In LDTv2CE, hole padding (i.e., padding) did not significantly affect the classification. However, FIG. 105 shows a WI profile that contains a large deletion in chromosome 4. Hole padding converts that profile from chromosome 13 false positive to chromosome 13 true negative.

Results

This section discusses PERUN results for trisomy 13, trisomy 18 and trisomy 21 (T13, T18 and T21, respectively), gender determination, and sex aneuploidy.

Reduced Variability

FIG. 106 compares the distribution of standard deviations of the binwise count profiles before and after PERUN normalization. The resulting distributions of chromosome representations for euploids and trisomy cases are shown in FIG. 107.

Improved T13, T18, and T21 Classification

FIG. 108-111 compare LDTv2CE PERUN classification results with those obtained using GCRM counts. In addition to removing two chromosome 18 false positives, two chromosome 18 false negatives, and two chromosome 21 false negatives, PERUN almost doubles the gap between the

euploids and the affected cases, in spite of the fact that the higher plexing elevation decreased the number of counts per sample (ELAND data). Similar results are obtained when PERUN parameters trained on LDTv2CE Eland data are applied to WI measurements. Bowtie alignments require a different set of parameters and additional bin filtering, accounting for low mappability in some bins, but its results approach those seen with ELAND alignments.

Example 5: Additional Description of PERUN

Examples of parameterized Error Removal and Unbiased Normalization (PERUN) methods are described in Example 4, and an additional description of such methods is provided in this Example 5.

Massive parallel sequencing of cell-free circulating DNA (e.g. from maternal plasma) can, under ideal conditions, quantify chromosomal elevations by counting sequenced reads if unambiguously aligned to a reference human genome. Such methods that incorporate massive amounts of replicate data can, in some cases, show statistically significant deviations between the measured and expected chromosomal elevations that can imply aneuploidy [Chiu et al., Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci U S A*. 2008;105:20458–20463; Fan et al., Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci U S A*. 2008;105:16266–16271; Ehrich et al., Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting, *American Journal of Obstetrics and Gynecology - AMER J OBSTET GYNECOL*, vol. 204, no. 3, pp. 205.e1-205.e11, 2011 DOI: 10.1016/j.ajog.2010.12.060]. Ideally, the distribution of aligned reads should cover euploid sections of the genome at a constant level (FIG. 62 and FIG. 63). In practice, uniformity can be difficult to attain because multiplexed Next Generation Sequencing (NGS) measurements typically yield low coverage (about 0.1) with sparsely scattered read start positions. In some embodiments, this problem is partially overcome by partitioning the genome into non-overlapping sections (bins) of equal lengths and assigning to each bin the number of the reads that align within it. In some embodiments, residual unevenness stemming from GC bias [Dohm JC, Lottaz C, Borodina T, Himmelbauer H. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. Nucleic Acids Res. 2008 Sep;36(16):e105. Epub 2008 Jul 26.] is largely suppressed using multiplicative detrending with respect to the binwise GC content (Fan HC, Quake SR (2010) Sensitivity of Noninvasive Prenatal Detection of Fetal Aneuploidy from Maternal Plasma Using

Shotgun Sequencing Is Limited Only by Counting Statistics. PLoS ONE 5(5): e10439. doi:10.1371/journal.pone.0010439). In some embodiments, the resulting flattening of the count profile allows for successful classification of fetal trisomies in a clinical setting using quadruplex barcoding [Palomaki et al., DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med.,* 2011 Nov;13(11):913-20.].

The transition from a quadruplex (i.e. 4 simultaneous sample reads) to higher sample plexing levels (e.g., dodecaplex (i.e. 12 simultaneous sample reads)) pushes the limits of NGS-based detection of genetic variations (e.g. aneuploidy, trisomy, and the like) in a test subject (e.g. a pregnant female), reducing both the number of reads per sample and the gap separating genetic variations (e.g. euploid from trisomy samples). The downsampling driven by increased multiplexing can impose new, more stringent requirements on data processing algorithms (FIG. 64, FIG. 65 and Example 4). In some embodiments, GC detrending, even when coupled with repeat masking, requires some improvement (FIG. 66, FIG. 67 and Example 4). In some embodiments, to maintain the sensitivity achieved with quadruplex barcoding (e.g., quadruplex indexing), methods and algorithms are presented that are capable of extracting a minute signal of interest from an overwhelming background noise as illustrated and described below and in FIG. 7, FIG. 8 and Example 4. In some embodiments, a novel method termed “PERUN” (Parameterized Error Removal and Unbiased Normalization) is described.

Conventional GC detrending can be multiplicative in nature (FIG. 17 and Example 4) and may not address additional sources of systematic bias, illustrated in FIG. 6. In some cases, a reference median count profile constructed from a set of known euploid samples can eliminate additional bias and lead to qualitative improvements. In some cases, a reference median count profile constructed from a set of known euploid samples can inherit a mixture of residual GC biases from the reference samples. In some embodiments, a normalization removes one or more orthogonal types of bias by separating them from one another at the bin elevation, rather than tackling them in bulk. In some embodiments GC bias is removed and binwise separation of the GC bias from the position-dependent attenuation is achieved (FIG. 68. FIG. 69 and Example 4). In some embodiments, substantially increased gaps between euploid and trisomy Z-scores are obtained relative to both quadruplex and dodecaplex GCRM results. In some embodiments, maternal and fetal microdeletions and duplications are detected. In some embodiments fetal fractions are accurately measured. In some embodiments gender is determined reliably. In some embodiments sex aneuploidy (e.g. fetal sex aneuploidy) is identified.

PERUN Method and Definitions

In some embodiments the entire reference genome is partitioned into an ordered set *B* of *J* bins:

$$B = \{b_j | j = 1, \ldots, J\} \quad \text{(D)}$$

Bin lengths can be constrained to accommodate genomic stretches of relatively uniform GC content. In some embodiments adjacent bins can overlap. In some embodiments adjacent bins do not overlap. In some embodiments the bin edges can be equidistant or can vary to offset systematic biases, such as nucleotide composition or signal attenuation. In some embodiments a bin comprises genomic positions within a single chromosome. Each bin b_j is characterized by the GC content g_j^0 of the corresponding portion of the reference genome. In some embodiments, the entire genome is assigned a reference GC content profile:

$$g^0 = \begin{bmatrix} g_1^0 & g_2^0 & \cdots & g_J^0 \end{bmatrix} \quad \text{(E)}$$

The same g^0 profile can apply to all samples aligned to the chosen reference genome.

A proper or trivial subset of bins b,

$$b \subseteq B \quad \text{(F)}$$

can be selected to satisfy certain criteria, such as to exclude bins with $g_j^0 = 0$, bins with extreme g_j^0 values, bins characterized by low complexity or low mappability (Derrien T, Estelle´ J, Marco Sola S, Knowles DG, Raineri E, et al. (2012) Fast Computation and Applications of Genome Mappability. PLoS ONE 7(1): e30377, doi:10.1371/journal.pone.0030377), highly variable or otherwise uninformative bins, regions with consistently attenuated signal, observed maternal aberrations, or entire chromosomes (X, Y, triploid chromosomes, and/or chromosomes with extreme GC content). The symbol $\|b\|$ denotes the size of b.

All sequenced reads from sample *i* unambiguously aligned within a bin b_j form a set a_{ij} whose cardinality M_{ij} represents raw measured counts assigned to that bin. In some embodiments, the vector of measured bin counts for sample *i* constitutes the raw count profile for that sample. In some embodiments this is the primary observation for the purposes of PERUN:

$$M_i = [M_{i1} \quad M_{i2} \quad \cdots \quad M_{iJ}] \tag{G}$$

To enable comparisons among different samples, the scaling constant N_i is evaluated as the sum of raw bin counts over a subset of the bins:

$$N_i = \sum_{b \subseteq B} M_{ij} \tag{H}$$

In some embodiments b in Eq. H is restricted to autosomal bins. In some embodiments b in Eq. H is not restricted to autosomal bins. Division of M_i by the total counts N_i yields the scaled raw bin counts m_{ij}:

$$m_i = [m_{i1} \quad m_{i2} \quad \cdots \quad m_{iJ}] = M_i / N_i \tag{I}$$

The nucleotide composition of the set a_{ij} is described by the bin's observed GC content g_{ij}. The sample-specific observed GC content profile g_i gathers individual bin-specific GC contents into a vector:

$$g_i = [g_{i1} \quad g_{i2} \quad \cdots \quad g_{iJ}] \tag{J}$$

In some embodiments, $g_i \neq g^0$ and $g_{i_1} \neq g_{i_2 \neq i_1}$. The symbol *g* denotes the GC content profile regardless of its origin, i.e. whether it is derived from the reference genome or from the sample-specific read alignments. In some embodiments model equations use g. In some embodiments, actual implementations can substitute g with either g^0 or g_i.

For a single sample *i*, a linear relationship between m_i and g is assumed, with G_i and r_i denoting the sample-specific slope of the regression line and the array of residuals, respectively:

$$m_i = G_i g + r_i \qquad \text{(K)}$$

The regression can extend over the entire set B (Eq. D) or its proper subset b (Eq. F). The observed slope G_i is also referred to as the scaled GC bias coefficient. G_i expresses the bulk of the vulnerability of the sample *i* to the systematic GC bias. In some embodiments, to minimize the number of model parameters, higher-order terms, linked with curvature of the relationship $m_i(g)$ and encapsulated in the residuals r_i are not explicitly addressed. In some embodiments, since sample-specific total counts N_i confound the interactions among observables recorded on different samples, the unscaled equivalent of G_i, relating M_i to g, is less useful and will not be considered.

The vector of true chromosomal elevations l_{ij} corresponding to bins $b_j \in b$ in sample *i* form the sample-specific chromosomal elevation profile:

$$l_i = [l_{i1} \quad l_{i2} \quad \cdots \quad l_{iJ}] \qquad \text{(L)}$$

In some embodiments, the goal is to derive estimates for l_i from m_i by removing systematic biases present in m_i.

The values l_{ij} are bin-specific and also sample-specific. They comprise both maternal and fetal contributions, proportional to their respective ploidies P_{ij}^M and P_{ij}^F. The bin-specific and sample-specific ploidy P_{ij} can be defined as an integral multiple of one-half, with the values of 1, 1/2, 0, 3/2, and 2 representing euploidy, heterozygous deletion, homozygous deletion, heterozygous duplication, and homozygous duplication, respectively. In some instances, trisomy of a given chromosome implies ploidy values of 3/2 along the entire chromosome or its substantial portion.

When both the mother and the fetus are diploid ($P_{ij}^{M} = P_{ij}^{F} = 1$), l_{ij} equals some arbitrarily chosen euploid elevation E. In some embodiments, a convenient choice sets E to $1/\|b\|$, thus ensuring that the profile l_i is normalized. In the absence of bin selection, $\|b\| = \|B\| = J \Rightarrow E = 1/J$. In some embodiments, E can be set to 1 for visualization. In some embodiments, the following relationship is satisfied:

$$l_{ij} = E\left[(1-f_i)P_{ij}^{M} + f_i P_{ij}^{F}\right] \quad \text{(M)}$$

The symbol f_i stands for the fraction of the fetal DNA present in the cell-free circulating DNA from maternal plasma in sample i. Any deviations from euploidy, either fetal ($P_{ij}^{F} \neq 1$) or maternal ($P_{ij}^{M} \neq 1$), cause differences between l_{ij} and E that can be exploited to estimate f_i and detect microdeletions/microduplications or trisomy.

To achieve the goal of extracting l_i from m_i, a linear relationship is postulated between the bin-specific scaled raw counts m_{ij} measured on a given sample and the sample-specific scaled GC bias coefficients:

$$m_i = l_i I + G_i S \quad \text{(N)}$$

The diagonal matrix I and the vector S gather bin-specific intercepts and slopes of the set of linear equations summarized by Eq. N:

$$I = \begin{bmatrix} I_1 & 0 & \cdots & 0 \\ 0 & I_2 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & I_J \end{bmatrix} \quad \text{(O)}$$

$$S = \begin{bmatrix} S_1 & S_2 & \cdots & S_J \end{bmatrix} \quad \text{(P)}$$

Both I and S are sample-independent. The intercepts I_j can be viewed as expected euploid values for scaled row counts in the absence of GC bias (i.e. when $G_i = 0$). Their actual values

reflect the convention adopted for E (*vide supra*). The intercepts S_j are non-linearly related to the differences $g_j^0 - \langle g_k^0 \rangle$, where $\langle g_k^0 \rangle$ represents the median GC content of the chromosome containing the bin j.

Once the values for the parameters I and S are known, the true chromosomal elevation profile l_i is estimated from the scaled raw count profile m_i and the scaled GC bias coefficient G_i by rearranging Eq. N:

$$l_i = (m_i - G_i S) I^{-1} \qquad \text{(Q)}$$

The diagonal character of the intercept matrix I provides for the matrix inversion in Eq. Q.

Parameter estimation

Model parameters I and S are evaluated from a set of N scaled raw count profiles collected on samples karyotyped as euploid pregnancies. N is of the order of 10^3. Scaled GC bias coefficients G_i are determined for each sample $(i = 1,..., N)$. All samples are segregated into a small number of classes according to the sizes and signs of their G_i values. The stratification balances the opposing needs to include sufficiently large numbers of representatives and a sufficiently small range of G_i values within each shell. The compromise of four strata accommodates negative, near-zero, moderately positive, and extreme positive GC biases, with the near-zero shell being most densely populated. A fraction of samples (typically 10%) from each stratum can be randomly selected and set aside for cross-validation. The remaining samples make up the work set, used to train the model. Both the training and the subsequent cross-validation assume that all samples are free of maternal and fetal deletions or duplications along the entire genome:

$$P_{ij}^M = P_{ij}^F = 1, \forall i = 1,\ldots N, \forall j = 1,\ldots, J \qquad \text{(R)}$$

The large number of samples compensates for the occasional maternal deviations from the assumption R. For each bin j, l_{ij} is set to E, allowing evaluation of the intercept I_j and the slope

S_j as the coefficients of the linear regression applied to the training set according to Eq. N. The uncertainty estimates for I_j and S_j are recorded as well.

The random partitioning into the working and the cross-validation subsets is repeated multiple times (e.g. 10^2), yielding distributions of values for the I_j and S_j parameters. In some embodiments the random partitioning is repeated between about 10 and about 10^5 times. In some embodiments the random partitioning is repeated about 10, about 10^2, about 10^3, about 10^4 or about 10^5 times.

Cross-validation

Once derived from the work set, the model parameters I_j and S_j are employed to back-calculate scaled raw counts from the scaled GC bias coefficients using Eq. N and assumption R. The symbol p_{ij} denotes the predicted scaled raw counts for the bin b_j in the sample i. The indices W and CV in further text designate the work and the cross-validation subsets, respectively. The back-calculation is applied to all samples, both from W and CV. R-factors, borrowed from the crystallographic structure refinement practice (Brünger, Free R value: a novel statistical quantity for assessing the accuracy of crystal structures, *Nature* 355, 472 - 475 (30 January 1992); doi:10.1038/355472a0), are separately defined for the two subsets of samples:

$$R_j^W = \frac{\sum_{i \in W} \left| m_{ij} - p_{ij} \right|}{\sum_{i \in W} \left| m_{ij} \right|} \qquad \text{(S)}$$

$$R_j^{CV} = \frac{\sum_{i \in CV} \left| m_{ij} - p_{ij} \right|}{\sum_{i \in CV} \left| m_{ij} \right|} \qquad \text{(T)}$$

Both R-factors are bin-specific. As in crystallography, R-factors 16-17 can be interpreted as residual relative errors in the model. Having been excluded from the parameter estimation, the cross-validation R-factor R_j^{CV} provides a true measure of the error for the given W/CV division, while the difference between R_j^{CV} and R_j^W reflects the model bias for the bin j. A separate pair of

R-values is evaluated for each bin and for each random partitioning of the set of samples into W and CV. The maximum of all R_j^{CV} and R_j^W values obtained for the different random partitionings into W and CV is assigned to the bin j as its overall model error ε_j.

Bin selection

All the bins with zero GC content g_j^0 are eliminated from further consideration, as is the set $\{b_j : M_{ij} \equiv 0, \forall i = 1,\ldots,N\}$ of bins that consistently receive zero counts across a large number of samples. In addition, a maximum tolerable cross-validation error value ε can be imposed on all bins. In some embodiments the bins with model errors ε_j exceeding the upper limit ε are rejected. In some embodiments, filtering uses bin mappability scores $\mu_j \in [0,1]$ and imposes a minimum acceptable mappability μ, rejecting bins with $\mu_j < \mu$ (Derrien T, Estelle´ J, Marco Sola S, Knowles DG, Raineri E, et al. (2012) Fast Computation and Applications of Genome Mappability. PLoS ONE 7(1): e30377, doi:10.1371/journal.pone.0030377). For the purposes of determining fetal trisomy of chromosomes 21, 18, and 13, the sex chromosomes can be excluded as well. The subset β of bins that survive all the phases of the bin selection can undergo further computations. In some embodiments, the same subset β is used for all samples.

Normalization and standardization

In some embodiments, for a given sample i, the chromosomal elevations l_{ij} corresponding to the bin selection β are estimated according to Eq. Q. In some embodiments, a secondary normalization is applied to remove any curvature from the l_{ij} -vs.-GC content correlation. In some embodiments l_{ij} is already nearly unbiased, the secondary detrending is robust and is immune to error boosting. In some embodiments, standard textbook procedures suffice.

In some embodiments, the results of the normalization are summed up within each chromosome:

$$L_{in} = \sum_{b_j \in \beta \cap Chr_n} l_{ij} \ , \ n = 1,\ldots,22 \qquad \text{(U)}$$

The total autosomal material in sample *i* can be evaluated as the sum of all individual L_{in} terms:

$$L_i = \sum_{n=1}^{22} L_{in} \quad \text{(V)}$$

The chromosomal representation of each chromosome of interest can be obtained by dividing L_{in} with L_i:

$$\chi_{in} = L_{in} / L_i \quad \text{(W)}$$

The variability σ_n of the representation of the chromosome n can be estimated as an uncensored MAD of χ_{in} values across a selection of samples spanning multiple flow cells. In some embodiments, the expectation $\langle \chi_n \rangle$ is evaluated as the median of χ_{in} values corresponding to a selection of samples from the same flow cell as the tested sample. Both sample selections can exclude high positive controls, low positive controls, high negative controls, blanks, samples that fail QC criteria, and samples with SD(l_i) exceeding a predefined cutoff (typically 0.10). Together, the values σ_n and $\langle \chi_n \rangle$ can provide the context for standardization and comparison of chromosomal representations among different samples using Z-scores:

$$Z_{in} = (\chi_{in} - \langle \chi_n \rangle) / \sigma_n \quad \text{(X)}$$

In some embodiments, aberrations such as trisomies 13, 18, and 21 are indicated by Z-values exceeding a predefined value, dictated by the desired confidence level.

Example 6: Examples of formulas

Provided below are non-limiting examples of mathematical and/or statistical formulas that can be used in methods described herein.

$$Z = \frac{\Delta_1 - \Delta_2}{\sqrt{\sigma_1^2\left(\frac{1}{N_1}+\frac{1}{n_1}\right)+\sigma_2^2\left(\frac{1}{N_2}+\frac{1}{n_2}\right)}}$$

$$P(q) = \frac{1}{\sigma\sqrt{2\pi}} exp[-(q - q_0)/(2\sigma^2)]$$

$$q_0 = 1 + F/2$$

$$z = -F/(2\sigma\sqrt{2})$$

$$B = \int_{-\infty}^{1} P(q)dq = \frac{1}{2}[1 + erf(z)]$$

$$erf(z) = \frac{2}{\sqrt{\pi}} \sum_{n=0}^{\infty} \frac{(-1)^n z^{2n+1}}{n!(2n+1)}$$

$$R = \frac{1-B}{B} = \frac{1-erf(z)}{1+erf(z)} = \frac{1-erf[-F/(2\sigma\sqrt{2})]}{1+erf[-F/(2\sigma\sqrt{2})]}$$

Example 7: Identifying and Adjusting (Padding) Elevations

Maternal deletions and duplications, often represented as first elevations in a profile, can be removed from count profiles normalized with PERUN to reduce variability when detecting T21, T18, or T13. The removal of deletions and duplication from a profile can reduce the variability (e.g., biological variability) found in measured chromosomal representations that originates from maternal aberrations.

All bins that significantly deviate from the expected chromosomal elevation of 1 are first identified. In this example some isolated bins are removed from the selection. This is optional. In this example only large enough groups of contiguous outlier bins are kept. This is also optional. Depending on the elevation assigned to an outlier bin or a group of contiguous outlier bins, a correction factor is added to the measured elevation to adjust it closer to the expected elevation of 1. The PAV values used in this example are +1 (for homozygous maternal deletions), +0.5 (for heterozygous maternal deletions), -0.5 (for heterozygous duplications), -1 (for homozygous duplications), or more (for large spikes). Large spikes are often not identified as maternal deletions and duplications.

This padding procedure corrected the classification (e.g., the classification as an aneuploidy, e.g., a trisomy) for samples that contains large maternal aberrations. Padding converted the WI sample

from false positive T13 to true negative due to removal of a large maternal deletion in Chr4 (FIG. 112 - 115).

Past simulations with experimental data have shown that depending on the chromosome, fetal fraction, and the type of aberration (homozygous or heterozygous, duplication or deletion), maternal aberrations in 20-40 bins long may push the Z-value over the classification edge (e.g., threshold) and result in a false positive or a false negative. Padding (e.g., adjusting) can circumvent this risk.

This padding procedure can remove uninteresting maternal aberrations (a confounding factor), reduce euploid variability, create tighter sigma-values used to standardize Z-scores and therefore enlarge the gap between euploids and trisomy cases.

Example 8: Determining Fetal Fractions from Maternal and/or Fetal Copy Number Variations

A distinguishing feature of the method described herein is the use of maternal aberrations (e.g., maternal and/or fetal copy number variations) as a probe providing insight into the fetal fraction in the case of a pregnant female bearing a fetus (e.g., a euploid fetus). The detection and quantitation of maternal aberrations typically is aided by normalization of raw counts. In this example raw counts are normalized using PERUN. Alternatively, normalization with respect to a reference median count profile can be used in a similar manner and for the same purpose.

PERUN normalization of raw counts yields sample-specific binwise chromosomal levels l_{ij} (*i* counts samples, *j* counts bins). They comprise both maternal and fetal contributions, proportional to their respective ploidy P_{ij}^{M} and P_{ij}^{F}. The bin-specific and sample-specific ploidy P_{ij} is defined as an integral multiple of 1/2, with the values of 1, 1/2, 0, 3/2, and 2 representing euploidy, heterozygous deletion, homozygous deletion, heterozygous duplication, and homozygous duplication, respectively. In particular, trisomy of a given chromosome implies ploidy values of 3/2 along the entire chromosome or its substantial portion.

When both the mother and the fetus are diploid ($P_{ij}^{M} = P_{ij}^{F} = 1$), l_{ij} equals some arbitrarily chosen euploid level *E*. A convenient choice sets *E* to $1/\|b\|$, where *b* denotes a proper or trivial subset of the set of all bins (*B*). thus ensuring that the profile l_i is normalized. In the absence of bin

selection, $\|b\| = \|B\| = J \Rightarrow E = 1/J$. Alternatively and preferentially, *E* may be set to 1 for visualization. In general, the following relationship is satisfied:

$$l_{ij} = E\left[(1-f_i)P_{ij}^M + f_i P_{ij}^F\right] \quad \text{(Y)}$$

The symbol f_i stands for the fraction of the fetal DNA present in the cell-free circulating DNA from maternal plasma in sample *i*. Any deviations from euploidy, either fetal ($P_{ij}^F \neq 1$) or maternal ($P_{ij}^M \neq 1$), cause differences between l_{ij} and *E* that can be exploited to estimate f_i and detect microdeletions/microduplications or trisomy.

Four different types of maternal aberrations are considered separately. All four account for possible fetal genotypes, as the fetus may (or in homozygous cases must) inherit the maternal aberration. In addition, the fetus may inherit a matching aberration from the father as well. In general, fetal fraction can only be measured when $P_{ij}^M \neq P_{ij}^F$.

A) Homozygous maternal deletion ($P_{ij}^M = 0$). Two possible accompanying fetal ploidies include:
   a. $P_{ij}^F = 0$, in which case $l_{ij} = 0$ and the fetal fraction cannot be evaluated from the deletion.
   b. $P_{ij}^F = 1/2$, in which case $l_{ij} = f_i/2$ and the fetal fraction is evaluated as twice the average elevation within the deletion.

B) Heterozygous maternal deletion ($P_{ij}^M = 1/2$). Three possible accompanying fetal ploidies include:
   a. $P_{ij}^F = 0$, in which case $l_{ij} = (1-f_i)/2$ and the fetal fraction is evaluated as twice the difference between ½ and the average elevation within the deletion.
   b. $P_{ij}^F = 1/2$, in which case $l_{ij} = 1/2$ and the fetal fraction cannot be evaluated from the deletion.

c. $P_{ij}^{F}=1$, in which case $l_{ij}=(1+f_i)/2$ and the fetal fraction is evaluated as twice the difference between ½ and the average elevation within the deletion.

C) Heterozygous maternal duplication ($P_{ij}^{M}=3/2$). Three possible accompanying fetal ploidies include:

a. $P_{ij}^{F}=1$, in which case $l_{ij}=(3-f_i)/2$ and the fetal fraction is evaluated as twice the difference between 3/2 and the average elevation within the duplication.

b. $P_{ij}^{F}=3/2$, in which case $l_{ij}=3/2$ and the fetal fraction cannot be evaluated from the duplication.

c. $P_{ij}^{F}=2$, in which case $l_{ij}=(3+f_i)/2$ and the fetal fraction is evaluated as twice the difference between 3/2 and the average elevation within the duplication.

D) Homozygous maternal duplication ($P_{ij}^{M}=2$). Two possible accompanying fetal ploidies include:

a. $P_{ij}^{F}=2$, in which case $l_{ij}=2$ and the fetal fraction cannot be evaluated from the duplication.

b. $P_{ij}^{F}=3/2$, in which case $l_{ij}=2-f_i/2$ and the fetal fraction is evaluated as twice the difference between 2 and the average elevation within the duplication.

The following LDTv2CE samples (FIG. 116 – 131) illustrate the application of determining fetal fraction from maternal and/or fetal copy number variations. The patients were not selected randomly and any agreement with FQA fetal fraction values should not be construed as the measure of merit of either technique.

Example 9: Binary genome

To generate a binary genome, a human reference genome (hg19) was converted to a two nucleotide system by converting all guanine nucleotides to adenine (i.e., all purines were converted to adenine). Similarly, all cytosine nucleotides were converted to thymidine (i.e., all pyrimidines were converted to thymidine). The human reference genome was indexed according to Bowtie 2 version 2.0.0-beta3 default instructions (see World Wide Web URL bowtie-bio.sourceforge.net/bowtie2/manual.shtml). 36 bp sequence reads from 87 maternal plasma

samples from RDFC010224 were converted to a binary genome by using a custom script that converts all purines to adenines and all pyrimidines to thymidines. The sequence quality for each base was left unchanged. Of the 87 maternal plasma samples, there were 4 samples having a chromosome 13 fetal aneuploidy, 9 samples having a chromosome 18 fetal aneuploidy, and 15 samples having a chromosome 21 fetal aneuploidy. The sequence reads were aligned against the converted binary genome using Bowtie 2 along with the "--very-fast" alignment option.

Results obtained using the binary genome were compared to the original four nucleotide data and alignments. Comparative metrics included the following: GC bias, relative coverage, bin profile variance, total autosomal counts, and chromosome representation. GC bias, as described by a GC coefficient, is measured by the slope of a linear model between the percent GC in the discreet 50kb bin of the human reference genome and the observed number of alignments to that bin. Positive (or negative) slopes indicate a slight relationship with counts and GC content per 50kb bin. GC bias, as described by a GC curve, also is measured by the second order of the linear model to reflect the overall concavity or convexity of the relationship between GC content and counts per 50kb bin. Relative coverage is the relative error in PCR duplicate rates (where 2 or more reads begin in the exact same chromosome and position) from simulated expectation given total depth of coverage. Bin profile variance is the standard deviation of counts per bin after PERUN with secondary LOESS normalization. Total autosomal counts reflects the total alignable data, and chromosome representation is the ratio of reads mapped to a specific chromosome normalized by the total autosomal counts.

Figures 132 to 143 illustrate a similarity between the originally processed four nucleotide genome against the in silico converted two nucleotide (i.e., binary) genome. GC bias and total autosomal counts, for example, were highly concordant. Raw chromosome representation also were correlated. PERUN with secondary LOESS and padding attenuated underlying GC bias of the sequencing technology and were thus able to detect fetal aneuploidy events.

To evaluate the performance of fetal aneuploidy detection in the absence of bias (i.e. GC biases, sequencing errors, non-uniformity of coverage), next-generation sequence reads were simulated via WGSIM (World Wide Web URL github.com/lh3/wgsim) with complete uniformity of coverage and no mutations/sequencing errors (parameters: -d=0 and –e=0). After generating 14 million 36 base pair reads for 7 euploids and 2 aneuploids (chromosome 21 fetal aneuploidies) at 8% fetal fraction, the same analyses work flow described above were implemented after converting all

bases into a binary code. To generate an artificial chromosome 21 fetal aneuploidy, chromosome 21 reads were increased by doubling sampled reads proportional to the fetal fraction.

Figure 144 illustrates that in the absence of bias (GC bias, mutations, non-uniformity of coverage), raw (non-normalized) chromosome 21 representation as generated by simulation can detect aneuploidy without the need of further normalization. Nevertheless, if PERUN with secondary LOESS without padding was applied, chromosome 21 representation remained concordant (Figure 145).

The total number of unique sequences of a specific length was determined by the total number of unique nucleotides raised to a length of sequence (i.e., number of nucleotides). For example, a 4 nucleotide genome of length 3 has 4^3=64 unique sequences (Figure 146). Assuming the human reference genome is approximately 3.1 billion bases, every start position could have a unique sequence if the read length is 15.76 for a 4 nucleotide genome and 31.53 for a 2 nucleotide (i.e., binary) genome. Thus, for a two nucleotide system (i.e., binary genome) a 36 bp read length, for example, is sufficiently long enough to uniquely map anywhere in the genome.

Example 10: Unary genome

To generate a unary genome, a human reference genome (hg19) was converted to a unary nucleotide system (e.g., A, not A; T, not T; C, not C; or G, not G) using four separate conversion schemes. For the first scheme, also referred to as unary genome A (A, not A), all non-adenine nucleotides were converted to guanine. For the second scheme, also referred to as unary genome G (G, not G), all non-guanine nucleotides were converted to adenine. For the third scheme, also referred to as unary genome C (C, not C), all non-cytosine nucleotides were converted to thymidine. For the fourth scheme, also referred to as unary genome T (T, not T), all non-thymidine nucleotides were converted to cytosine. The human reference genome was indexed according to Bowtie 2 version 2.0.0-beta3 default instructions (see World Wide Web URL bowtie-bio.sourceforge.net/bowtie2/manual.shtml). 36 bp sequence reads from 87 maternal plasma samples from RDFC010224 were converted to a unary genome using the nucleotide conversion criteria as outline above. The data was processed as described in Example 9 for the binary genome. Results obtained using the unary genome were compared to the original four nucleotide data and alignments by comparative analyses identical to those described in Example 9 for the binary genome study.

Figures 147 to 154 illustrate the similarity of GC coefficient and total autosomal counts between the originally processed four nucleotide genome against the in silico converted unary genome. Raw chromosome 21 representations for each unary genome are presented in Figures 155 to 158. As shown in Figures 159 to 170, PERUN normalization reduced systematic biases and allowed for some distinction between euploids and fetal aneuploids. Figures 171 and 172 illustrate the similarity between the four base genome and the unary genome when comparing counts per bin per GC content. Figures 173 to 184 demonstrate that in the absence of bias, reads converted to any of the four unary genomes retained uniqueness such that fetal aneuploidy detection is feasible.

Example 11: Examples of embodiments

1. A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) obtaining partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) mapping the partial nucleotide sequence reads to reference genome sections,

(c) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(d) comparing the number of counts of the partial nucleotide sequence reads mapped in (c), or derivative thereof, to a reference, thereby making a comparison, and

(e) determining the presence or absence of a fetal aneuploidy based on the comparison.

1.1 A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) obtaining partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) mapping the partial nucleotide sequence reads to reference genome sections,

(c) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(d) comparing the number of counts of the partial nucleotide sequence reads mapped in (c), or derivative thereof, to a reference, or portion thereof, thereby making a comparison, and

(e) determining the presence or absence of a fetal aneuploidy based on the comparison.

2. A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, thereby making a comparison, and

(d) determining the presence or absence of a fetal aneuploidy based on the comparison.

2.1 A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, or portion thereof, thereby making a comparison, and

(d) determining the presence or absence of a fetal aneuploidy based on the comparison.

3. A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) obtaining a sample comprising circulating, cell-free nucleic acid from a pregnant female,

(b) isolating sample nucleic acid from the sample,

(c) obtaining partial nucleotide sequence reads from the sample nucleic acid, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(d) mapping the partial nucleotide sequence reads to reference genome sections,

(e) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(f) comparing the number of counts of the partial nucleotide sequence reads mapped in (e), or derivative thereof, to a reference, thereby making a comparison, and

(g) determining the presence or absence of a fetal aneuploidy based on the comparison.

3.1 A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) obtaining a sample comprising circulating, cell-free nucleic acid from a pregnant female,

(b) isolating sample nucleic acid from the sample,

(c) obtaining partial nucleotide sequence reads from the sample nucleic acid, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(d) mapping the partial nucleotide sequence reads to reference genome sections,

(e) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(f) comparing the number of counts of the partial nucleotide sequence reads mapped in (e), or derivative thereof, to a reference, or portion thereof, thereby making a comparison, and

(g) determining the presence or absence of a fetal aneuploidy based on the comparison.

4. A method for detecting the presence or absence of a genetic variation comprising:

(a) obtaining partial nucleotide sequence reads from a sample comprising nucleic acid from a subject, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) mapping the partial nucleotide sequence reads to reference genome sections,

(c) comparing the partial nucleotide sequence reads mapped in (b) to a reference, thereby making a comparison, and

(d) determining the presence or absence of a genetic variation based on the comparison.

4.1 A method for detecting the presence or absence of a genetic variation comprising:

(a) obtaining partial nucleotide sequence reads from a sample comprising nucleic acid from a subject, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) mapping the partial nucleotide sequence reads to reference genome sections,

(c) comparing the partial nucleotide sequence reads mapped in (b) to a reference, or portion thereof, thereby making a comparison, and

(d) determining the presence or absence of a genetic variation based on the comparison.

5. A method for detecting the presence or absence of a genetic variation comprising:

(a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising nucleic acid from a subject, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) comparing the partial nucleotide sequence reads mapped in (a) to a reference, thereby making a comparison, and

(c) determining the presence or absence of a genetic variation based on the comparison.

5.1 A method for detecting the presence or absence of a genetic variation comprising:

(a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising nucleic acid from a subject, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) comparing the partial nucleotide sequence reads mapped in (a) to a reference, or portion thereof, thereby making a comparison, and

(c) determining the presence or absence of a genetic variation based on the comparison.

6. A method for detecting the presence or absence of a genetic variation comprising:

(a) obtaining a sample comprising nucleic acid from a subject,

(b) isolating sample nucleic acid from the sample,

(c) obtaining partial nucleotide sequence reads from the sample nucleic acid, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(d) mapping the partial nucleotide sequence reads to reference genome sections,

(e) comparing the partial nucleotide sequence reads mapped in (d) to a reference, thereby making a comparison, and

(f) determining the presence or absence of a genetic variation based on the comparison.

6.1 A method for detecting the presence or absence of a genetic variation comprising:

(a) obtaining a sample comprising nucleic acid from a subject,

(b) isolating sample nucleic acid from the sample,

(c) obtaining partial nucleotide sequence reads from the sample nucleic acid, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(d) mapping the partial nucleotide sequence reads to reference genome sections,

(e) comparing the partial nucleotide sequence reads mapped in (d) to a reference, or portion thereof, thereby making a comparison, and

(f) determining the presence or absence of a genetic variation based on the comparison.

7. A method for detecting the presence or absence of a genetic variation, comprising:

(a) obtaining counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii),

(b) normalizing the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(c) detecting the presence or absence of a genetic variation based on the normalized counts.

7.01 The method of embodiment 7, wherein the genetic variation is a nucleic acid sequence variation.

7.02 The method of embodiment 7 or 7.01, wherein the genetic variation is a copy number variation.

7.03 The method of embodiment 7.02, wherein the test sample is from a pregnant female and the genetic variation is a fetal aneuploidy.

7.04 The method of any one of embodiments 7 to 7.03, comprising comparing the normalized counts to a reference, thereby making a comparison, wherein determining the presence or absence of the genetic variation in (c) is based on the normalized counts and the comparison.

7.05 The method of embodiment 7.04, wherein the reference is counts of sequence reads mapped to a reference chromosome or segment thereof.

7.06 The method of embodiment 7.05, wherein the reference chromosome is chromosome 1, chromosome 14, chromosome 19 or combination thereof.

7.07 The method of any one of embodiments 7.04 to 7.06, wherein the counts of the partial nucleotide sequence reads obtained in (a) comprise counts of partial nucleotide sequence reads mapped to a test chromosome or segment thereof.

7.08 The method of embodiment 7.07, wherein the test chromosome is chromosome 13, chromosome 18, chromosome 21 or combination thereof.

7.09 The method of any one of embodiment 7.04 to 7.08, wherein the counts are expressed as a ratio of counts for genomic sections in a test chromosome or segment thereof to counts for genomic sections in autosomes or segment thereof, thereby providing a count representation.

7.10 The method of any one of embodiments 7 to 7.09, wherein the normalizing in (b) comprises normalizing according to guanine and cytosine (GC) content of the genomic sections, and providing calculated genomic section levels.

7.11 The method of any one of embodiments 7 to 7.10, wherein the normalizing in (b) comprises:

(i) determining a guanine and cytosine (GC) bias for each of the genomic sections for multiple samples from a fitted relation for each sample between (1) the counts of the partial nucleotide sequence reads mapped to each of the genomic sections, and (2) GC content for each of the genomic sections; and

(ii) calculating a genomic section level for each of the genomic sections from a fitted relation between (1) the GC bias and (2) the counts of the partial nucleotide sequence reads mapped to each of the genomic sections, thereby providing calculated genomic section levels, whereby bias in the counts of the partial nucleotide sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels, and wherein the normalized counts in (b) comprise the calculated genomic section levels.

7.12 The method of embodiment 7.11, wherein the normalized counts in (b) are the calculated genomic section levels.

7.13 The method of any one of embodiments 7 to 7.12, wherein the normalized counts are adjusted for a first level for a first set of genomic sections which first level is significantly different than a second level for a second set of genomic sections, thereby providing adjusted normalized

counts, wherein determining the presence or absence of the genetic variation in (c) is based on the adjusted normalized counts.

7.14 The method of embodiment 7.13, comprising:

(i) identifying a first elevation of the normalized counts significantly different than a second elevation of the normalized counts in a normalized counts profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections;

(ii) determining an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the genome; and

(iii) adjusting the first elevation by a predetermined value, or adjusting the first elevation to the second elevation, when the first elevation is within one of the expected elevation ranges, thereby providing an adjustment of the first elevation.

7.15 The method of embodiment 7.14, wherein the segment of the genome comprises the first elevation or the second elevation, or the first elevation and the second elevation.

7.16 The method of any one of embodiments 7 to 7.15, wherein the normalizing in (b) comprises performing a local regression on the counts of the partial nucleotide sequence reads or the calculated genomic section levels, or the counts of the partial nucleotide sequence reads and the calculated genomic section levels.

7.17 The method of embodiment 7.16, wherein the local regression comprises a weighted least squares fit.

7.18 The method of embodiment 7.17, wherein the local regression comprises a LOESS regression.

7.19 The method of any one of embodiments 7 to 7.18, wherein the partial nucleotide sequence reads are unary partial reads, for which unary partial reads one nucleotide species is known at known positions and the other positions can be any one of three other nucleotide species.

7.20 The method of embodiment 7.19, wherein the partial nucleotide sequence reads are about 30 base pairs or more.

7.21 The method of any one of embodiments 7 to 7.18, wherein the partial nucleotide sequence reads are binary partial reads, for which binary partial reads a first nucleotide class consisting of two possible bases is known at known positions and a second nucleotide class consisting of two possible bases is known at known positions, wherein the bases of the first nucleotide class are different than the bases of the second nucleotide class.

7.22 The method of embodiment 7.21, wherein the partial nucleotide sequence reads are about 30 base pairs or more.

7.23 The method of any one of embodiments 7 to 7.18, wherein the partial nucleotide sequence reads are ternary partial reads, for which ternary partial reads a first nucleotide species is known at known positions, a second nucleotide species is known at other known positions and the other positions are any one of two nucleotide species other than the first nucleotide species and the second nucleotide species.

7.24 The method of embodiment 7.23, wherein the partial nucleotide sequence reads are about 20 base pairs or more.

7.25 The method of any one of embodiments 7 to 7.24, comprising determining partial nucleotide sequence reads of the nucleic acid from the test sample.

7.26 The method of embodiment 7.25, which partial nucleotide sequence reads are determined using a method comprising a massively parallel sequencing (MPS) process or a nanopore process, or a massively parallel sequencing (MPS) process and a nanopore process.

7.27 The method of embodiment 7.25 or 7.26, comprising mapping the partial nucleotide sequence reads to genomic sections of the reference genome.

7.28 The method of any one of embodiments 7 to 7.27, comprising isolating the nucleic acid from the test sample.

7.29 The method of any one of embodiments 7 to 7.28, comprising obtaining the test sample.

7.30 The method of embodiment 7.29, wherein the test sample is obtained from a pregnant female.

7.31 The method of any one of embodiments 7 to 7.30, wherein the test sample is blood plasma, blood serum or urine.

8. The method of any one of embodiments 4 to 7.31, wherein the genetic variation is a nucleic acid sequence variation.

8.1 The method of embodiment 8, wherein the nucleotide sequences of the partial nucleotide sequence reads are compared to a reference.

9. The method of embodiment 8.1, wherein a sequence match or mismatch is determined.

10. The method of any one of embodiments 4 to 6.1, wherein the genetic variation is a nucleic acid copy number variation.

11. The method of embodiment 10, wherein the method further comprises after the mapping of partial nucleotide sequence reads, counting the number of partial nucleotide sequence reads mapped to each reference genome section.

12. The method of embodiment 11, wherein the number of counts of the partial nucleotide sequence reads, or derivative thereof, are compared to a reference.

13. The method of any one of embodiments 4 to 12, wherein the subject is a fetus and the sample is from a pregnant female that bears a fetus.

14. The method of embodiment 13, wherein the sample comprises circulating, cell-free nucleic acid.

15. The method of embodiment 14, wherein the sample nucleic acid comprises maternal and fetal nucleic acid.

16. The method of any one of embodiments 1 to 15, wherein the sample is blood.

16.1 The method of any one of embodiments 1 to 15, wherein the sample is urine.

16.2 The method of any one of embodiments 1 to 15, wherein the sample is saliva.

16.3 The method of any one of embodiments 1 to 15, wherein the sample is a cervical swab.

17. The method of any one of embodiments 1 to 15, wherein the sample is serum.

18. The method of any one of embodiments 1 to 15, wherein the sample is plasma.

19. The method of any one of embodiments 1 to 18, wherein the partial nucleotide sequence reads comprise relative positional information for one or more nucleobase species.

20. The method of embodiment 19, wherein the partial nucleotide sequence reads contain relative positional information for adenine.

21. The method of embodiment 19 or 20, wherein the partial nucleotide sequence reads contain relative positional information for guanine.

22. The method of any one of embodiments 19 to 21, wherein the partial nucleotide sequence reads contain relative positional information for thymine.

23. The method of any one of embodiments 19 to 22, wherein the partial nucleotide sequence reads contain relative positional information for cytosine.

24. The method of any one of embodiments 19 to 23, wherein the partial nucleotide sequence reads contain relative positional information for methyl-cytosine.

25. The method of embodiment 19, wherein the partial nucleotide sequence reads contain relative positional information for two nucleobase species selected from the group consisting of adenine, guanine, thymine, cytosine or methyl-cytosine.

26. The method of embodiment 19, wherein the partial nucleotide sequence reads contain relative positional information for three nucleobase species selected from the group consisting of adenine, guanine, thymine, cytosine or methyl-cytosine.

27. The method of embodiment 19, wherein the one or more nucleobase species comprise one or more detectable labels.

28. The method of embodiment 19, wherein the partial nucleotide sequence reads contain relative positional information for sequences complementary to one or more hybridized probe species.

29. The method of embodiment 28, wherein the one or more hybridized probe species comprise one or more detectable labels.

30. The method of any one of embodiments 1 to 18, wherein the nucleobase class is purine.

31. The method of any one of embodiments 1 to 18, wherein the nucleobase class is pyrimidine.

32. The method of embodiment 30 or 31, wherein purines are distinguished from pyrimidines in the partial nucleotide sequence reads.

33. The method of any one of embodiments 1 to 18, wherein the sample nucleic acid comprises single stranded nucleic acid.

34. The method of any one of embodiments 1 to 18, wherein the sample nucleic acid comprises double stranded nucleic acid.

35. The method of embodiment 34, wherein the nucleobase class is a nucleobase pair species in a duplex nucleic acid.

36. The method of any one of embodiments 1 to 35, wherein the obtaining partial nucleotide sequence reads includes subjecting the sample nucleic acid to a sequencing process using a sequencing device.

37. The method of embodiment 36, wherein the partial nucleotide sequence reads are obtained by nanopore sequencing.

38. The method of embodiment 36, wherein the partial nucleotide sequence reads are obtained by reversible terminator-based sequencing.

39. The method of embodiment 36, wherein the partial nucleotide sequence reads are obtained by pyrosequencing.

40. The method of embodiment 36, wherein the partial nucleotide sequence reads are obtained by real time sequencing.

41. The method of embodiment 36, wherein the partial nucleotide sequence reads are obtained by oligonucleotide probe ligation sequencing.

41.1 The method of embodiment 36, wherein the partial nucleotide sequence reads are obtained by sequencing by hybridization.

42. The method of any one of embodiments 1 to 41, wherein the partial nucleotide sequence reads comprise a number of discrete position identities sufficient to map to a reference genome section.

43. The method of any one of embodiments 1 to 41, wherein the partial nucleotide sequence read is of sufficient length to map to a reference genome section.

44. The method of embodiment 43, wherein the partial nucleotide sequence read length is at least about 36 nucleobases.

44.1 The method of embodiment 43, wherein the partial nucleotide sequence read length is at least about 72 nucleobases.

45. The method of embodiment 43, wherein the partial nucleotide sequence read length is at least about 108 nucleobases.

46. The method of any one of embodiments 1 to 45, wherein the nucleobase gaps in each partial nucleotide sequence read independently are about 1 to about 100 sequential nucleobases.

47. The method of any one of embodiments 1 to 46, which comprises obtaining full nucleotide sequence reads, which nucleotide sequence reads do not contain nucleobase gaps between identified nucleobases or a nucleobase class comprising a subset of nucleobases present in the sample nucleic acid.

48. The method of any one of embodiments 1 to 3, wherein the fetal aneuploidy is trisomy 13.

49. The method of any one of embodiments 1 to 3, wherein the fetal aneuploidy is trisomy 18.

50. The method of any one of embodiments 1 to 3, wherein the fetal aneuploidy is trisomy 21.

51. The method of any one of embodiments 4 to 6.1, wherein the genetic variation is associated with a medical condition.

52. The method of embodiment 51, wherein the medical condition is cancer.

53. The method of embodiment 51, wherein the medical condition is an aneuploidy.

54. A computer program product, comprising a computer usable medium having a computer readable program code embodied therein, the computer readable program code comprising distinct software modules comprising a sequence receiving module, a logic processing module, and a data display organization module, the computer readable program code adapted to be executed to implement a method for identifying the presence or absence of a fetal aneuploidy, the method comprising:

(a) obtaining, by the sequence receiving module, partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii);

(b) receiving, by the logic processing module, the partial nucleotide sequence reads;

(c) mapping, by the logic processing module, the partial nucleotide sequence reads to reference genome sections;

(d) counting, by the logic processing module, the number of partial nucleotide sequence reads mapped to each reference genome section;

(e) comparing, by the logic processing module, the number of counts of the partial nucleotide sequence reads, or derivative thereof, to a reference, or portion thereof, thereby making a comparison;

(f) providing, by the logic processing module, an outcome determinative of the presence or absence of a fetal aneuploidy based on the comparison; and

(g) organizing, by the data display organization module in response to being determined by the logic processing module, a data display indicating the presence or absence of a fetal aneuploidy.

55. An apparatus, comprising memory in which a computer program product of embodiment 54 is stored.

56. The apparatus of embodiment 55, which comprises a processor that implements one or more functions of the computer program product specified in embodiment 54.

57. A system comprising a nucleic acid sequencing apparatus and a processing apparatus, wherein the sequencing apparatus obtains sequence reads from a sample, and the processing apparatus obtains the sequence reads from the sequencing apparatus and carries out a method comprising:

(a) mapping partial nucleotide sequence reads from the sequencing apparatus that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, or portion thereof, thereby making a comparison, and

(d) determining the presence or absence of a fetal aneuploidy based on the comparison.

58. A system comprising one or more processors and memory,
which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii); and

which instructions executable by the one or more processors are configured to:

(a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(b) detect the presence or absence of a genetic variation based on the normalized counts.

59. An apparatus comprising one or more processors and memory,
which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii); and

which instructions executable by the one or more processors are configured to:

(a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(b) detect the presence or absence of a genetic variation based on the normalized counts.

60. A computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to:

(a) access counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free

nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii),

(b) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(c) detect the presence or absence of a genetic variation based on the normalized counts.

* * *

The entirety of each patent, patent application, publication and document referenced herein hereby is incorporated by reference. Citation of the above patents, patent applications, publications and documents is not an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

Modifications may be made to the foregoing without departing from the basic aspects of the technology. Although the technology has been described in substantial detail with reference to one or more specific embodiments, those of ordinary skill in the art will recognize that changes may be made to the embodiments specifically disclosed in this application, yet these modifications and improvements are within the scope and spirit of the technology.

The technology illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising,” “consisting essentially of,” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and use of such terms and expressions do not exclude any equivalents of the features shown and described or portions thereof, and various modifications are possible within the scope of the technology claimed. The term “a” or “an” can refer to one of or a plurality of the elements it modifies (e.g., “a reagent” can mean one or more reagents) unless it is contextually clear either one of the elements or more than one of the elements is described. The term “about” as used herein refers to a value within 10% of the underlying parameter (i.e., plus or

minus 10%), and use of the term “about” at the beginning of a string of values modifies each of the values (i.e., “about 1, 2 and 3” refers to about 1, about 2 and about 3). For example, a weight of “about 100 grams” can include weights between 90 grams and 110 grams. Further, when a listing of values is described herein (e.g., about 50%, 60%, 70%, 80%, 85% or 86%) the listing includes all intermediate and fractional values thereof (e.g., 54%, 85.4%). Thus, it should be understood that although the present technology has been specifically disclosed by representative embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and such modifications and variations are considered within the scope of this technology.

Certain embodiments of the technology are set forth in the claim(s) that follow(s).

What is claimed is:

1. A method for detecting the presence or absence of a genetic variation, comprising:

(a) obtaining counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii),

(b) normalizing the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(c) detecting the presence or absence of a genetic variation based on the normalized counts.

2. The method of claim 1, wherein the genetic variation is a nucleic acid sequence variation.

3. The method of claim 1 or 2, wherein the genetic variation is a copy number variation.

4. The method of claim 3, wherein the test sample is from a pregnant female and the genetic variation is a fetal aneuploidy.

5. The method of any one of claims 1 to 4, comprising comparing the normalized counts to a reference, thereby making a comparison, wherein determining the presence or absence of the genetic variation in (c) is based on the normalized counts and the comparison.

6. The method of claim 5, wherein the reference is counts of sequence reads mapped to a reference chromosome or segment thereof.

7. The method of claim 6, wherein the reference chromosome is chromosome 1, chromosome 14, chromosome 19 or combination thereof.

8. The method of any one of claims 5 to 7, wherein the counts of the partial nucleotide sequence reads obtained in (a) comprise counts of partial nucleotide sequence reads mapped to a test chromosome or segment thereof.

9. The method of claim 8, wherein the test chromosome is chromosome 13, chromosome 18, chromosome 21 or combination thereof.

10. The method of any one of claim 5 to 9, wherein the counts are expressed as a ratio of counts for genomic sections in a test chromosome or segment thereof to counts for genomic sections in autosomes or segment thereof, thereby providing a count representation.

11. The method of any one of claims 1 to 10, wherein the normalizing in (b) comprises normalizing according to guanine and cytosine (GC) content of the genomic sections, and providing calculated genomic section levels.

12. The method of any one of claims 1 to 11, wherein the normalizing in (b) comprises:

(i) determining a guanine and cytosine (GC) bias for each of the genomic sections for multiple samples from a fitted relation for each sample between (1) the counts of the partial nucleotide sequence reads mapped to each of the genomic sections, and (2) GC content for each of the genomic sections; and

(ii) calculating a genomic section level for each of the genomic sections from a fitted relation between (1) the GC bias and (2) the counts of the partial nucleotide sequence reads mapped to each of the genomic sections, thereby providing calculated genomic section levels, whereby bias in the counts of the partial nucleotide sequence reads mapped to each of the portions of the reference genome is reduced in the calculated genomic section levels, and wherein the normalized counts in (b) comprise the calculated genomic section levels.

13. The method of claim 12, wherein the normalized counts in (b) are the calculated genomic section levels.

14. The method of any one of claims 1 to 13, wherein the normalized counts are adjusted for a first level for a first set of genomic sections which first level is significantly different than a second level for a second set of genomic sections, thereby providing adjusted normalized counts, wherein determining the presence or absence of the genetic variation in (c) is based on the adjusted normalized counts.

15. The method of claim 14, comprising:

(i) identifying a first elevation of the normalized counts significantly different than a second elevation of the normalized counts in a normalized counts profile, which first elevation is for a first set of genomic sections, and which second elevation is for a second set of genomic sections;

(ii) determining an expected elevation range for a homozygous and heterozygous copy number variation according to an uncertainty value for a segment of the genome; and

(iii) adjusting the first elevation by a predetermined value, or adjusting the first elevation to the second elevation, when the first elevation is within one of the expected elevation ranges, thereby providing an adjustment of the first elevation.

16. The method of claim 15, wherein the segment of the genome comprises the first elevation or the second elevation, or the first elevation and the second elevation.

17. The method of any one of claims 1 to 16, wherein the normalizing in (b) comprises performing a local regression on the counts of the partial nucleotide sequence reads or the calculated genomic section levels, or the counts of the partial nucleotide sequence reads and the calculated genomic section levels.

18. The method of claim 17, wherein the local regression comprises a weighted least squares fit.

19. The method of claim 18, wherein the local regression comprises a LOESS regression.

20. The method of any one of claims 1 to 19, wherein the partial nucleotide sequence reads are unary partial reads, for which unary partial reads one nucleotide species is known at known positions and the other positions can be any one of three other nucleotide species.

21. The method of claim 20, wherein the partial nucleotide sequence reads are about 30 base pairs or more.

22. The method of any one of claims 1 to 19, wherein the partial nucleotide sequence reads are binary partial reads, for which binary partial reads a first nucleotide class consisting of two possible bases is known at known positions and a second nucleotide class consisting of two possible bases is known at known positions, wherein the bases of the first nucleotide class are different than the bases of the second nucleotide class.

23. The method of claim 22, wherein the partial nucleotide sequence reads are about 30 base pairs or more.

24. The method of any one of claims 1 to 19, wherein the partial nucleotide sequence reads are ternary partial reads, for which ternary partial reads a first nucleotide species is known at known positions, a second nucleotide species is known at other known positions and the other positions are any one of two nucleotide species other than the first nucleotide species and the second nucleotide species.

25. The method of claim 24, wherein the partial nucleotide sequence reads are about 20 base pairs or more.

26. The method of any one of claims 1 to 25, comprising determining partial nucleotide sequence reads of the nucleic acid from the test sample.

27. The method of claim 26, which partial nucleotide sequence reads are determined using a method comprising a massively parallel sequencing (MPS) process or a nanopore process, or a massively parallel sequencing (MPS) process and a nanopore process.

28. The method of claim 26 or 27, comprising mapping the partial nucleotide sequence reads to genomic sections of the reference genome.

29. The method of any one of claims 1 to 28, comprising isolating the nucleic acid from the test sample.

30. The method of any one of claims 1 to 29, comprising obtaining the test sample.

31. The method of claim 30, wherein the test sample is obtained from a pregnant female.

32. The method of any one of claims 1 to 31, wherein the test sample is blood plasma, blood serum or urine.

33. A system comprising one or more processors and memory,

which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii); and

which instructions executable by the one or more processors are configured to:

(a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(b) detect the presence or absence of a genetic variation based on the normalized counts.

34. An apparatus comprising one or more processors and memory,

which memory comprises instructions executable by the one or more processors and which memory comprises counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are

reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii); and

which instructions executable by the one or more processors are configured to:

(a) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(b) detect the presence or absence of a genetic variation based on the normalized counts.

35. A computer program product tangibly embodied on a computer-readable medium, comprising instructions that when executed by one or more processors are configured to:

(a) access counts of partial nucleotide sequence reads mapped to genomic sections of a reference genome, which partial nucleotide sequence reads are reads of circulating cell-free nucleic acid from a test sample, wherein at least some of the partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

a combination of (i) and (ii),

(b) normalize the counts of the partial nucleotide sequence reads, thereby providing normalized counts, and

(c) detect the presence or absence of a genetic variation based on the normalized counts.

36. A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) obtaining partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) mapping the partial nucleotide sequence reads to reference genome sections,

(c) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(d) comparing the number of counts of the partial nucleotide sequence reads mapped in (c), or derivative thereof, to a reference, thereby making a comparison, and

(e) determining the presence or absence of a fetal aneuploidy based on the comparison.

37. A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, thereby making a comparison, and

(d) determining the presence or absence of a fetal aneuploidy based on the comparison.

38. A method for detecting the presence or absence of a fetal aneuploidy comprising:

(a) obtaining a sample comprising circulating, cell-free nucleic acid from a pregnant female,

(b) isolating sample nucleic acid from the sample,

(c) obtaining partial nucleotide sequence reads from the sample nucleic acid, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(d) mapping the partial nucleotide sequence reads to reference genome sections,

(e) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(f) comparing the number of counts of the partial nucleotide sequence reads mapped in (e), or derivative thereof, to a reference, thereby making a comparison, and

(g) determining the presence or absence of a fetal aneuploidy based on the comparison.

39. A method for detecting the presence or absence of a genetic variation comprising:

(a) obtaining partial nucleotide sequence reads from a sample comprising nucleic acid from a subject, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) mapping the partial nucleotide sequence reads to reference genome sections,

(c) comparing the partial nucleotide sequence reads mapped in (b) to a reference, thereby making a comparison, and

(d) determining the presence or absence of a genetic variation based on the comparison.

40. A method for detecting the presence or absence of a genetic variation comprising:

(a) mapping partial nucleotide sequence reads that have been obtained from a sample comprising nucleic acid from a subject, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(b) comparing the partial nucleotide sequence reads mapped in (a) to a reference, thereby making a comparison, and

(c) determining the presence or absence of a genetic variation based on the comparison.

41. A method for detecting the presence or absence of a genetic variation comprising:

(a) obtaining a sample comprising nucleic acid from a subject,

(b) isolating sample nucleic acid from the sample,

(c) obtaining partial nucleotide sequence reads from the sample nucleic acid, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii),

(d) mapping the partial nucleotide sequence reads to reference genome sections,

(e) comparing the partial nucleotide sequence reads mapped in (d) to a reference, thereby making a comparison, and

(f) determining the presence or absence of a genetic variation based on the comparison.

42. The method of any one of claims 39 to 41, wherein the genetic variation is a nucleic acid sequence variation.

43. The method of claim 42, wherein the nucleotide sequences of the partial nucleotide sequence reads are compared to a reference.

44. The method of claim 43, wherein a sequence match or mismatch is determined.

45. The method of any one of claims 39 to 41, wherein the genetic variation is a nucleic acid copy number variation.

46. The method of claim 45, wherein the method further comprises after the mapping of partial nucleotide sequence reads, counting the number of partial nucleotide sequence reads mapped to each reference genome section.

47. The method of claim 46, wherein the number of counts of the partial nucleotide sequence reads, or derivative thereof, are compared to a reference.

48. The method of any one of claims 39 to 47, wherein the subject is a fetus and the sample is from a pregnant female that bears a fetus.

49. The method of claim 48, wherein the sample comprises circulating, cell-free nucleic acid.

50. The method of claim 49, wherein the sample nucleic acid comprises maternal and fetal nucleic acid.

51. The method of any one of claims 1 to 50, wherein the sample is blood.

52. The method of any one of claims 1 to 50, wherein the sample is urine.

53. The method of any one of claims 1 to 50, wherein the sample is saliva.

54. The method of any one of claims 1 to 50, wherein the sample is a cervical swab.

55. The method of any one of claims 1 to 50, wherein the sample is serum.

56. The method of any one of claims 1 to 50, wherein the sample is plasma.

57. The method of any one of claims 1 to 56, wherein the partial nucleotide sequence reads comprise relative positional information for one or more nucleobase species.

58. The method of claim 57, wherein the partial nucleotide sequence reads contain relative positional information for adenine.

59. The method of claim 57 or 58, wherein the partial nucleotide sequence reads contain relative positional information for guanine.

60. The method of any one of claims 57 to 59, wherein the partial nucleotide sequence reads contain relative positional information for thymine.

61. The method of any one of claims 57 to 60, wherein the partial nucleotide sequence reads contain relative positional information for cytosine.

62. The method of any one of claims 57 to 61, wherein the partial nucleotide sequence reads contain relative positional information for methyl-cytosine.

63. The method of claim 57, wherein the partial nucleotide sequence reads contain relative positional information for two nucleobase species selected from the group consisting of adenine, guanine, thymine, cytosine or methyl-cytosine.

64. The method of claim 57, wherein the partial nucleotide sequence reads contain relative positional information for three nucleobase species selected from the group consisting of adenine, guanine, thymine, cytosine or methyl-cytosine.

65. The method of claim 57, wherein the one or more nucleobase species comprise one or more detectable labels.

66. The method of claim 57, wherein the partial nucleotide sequence reads contain relative positional information for sequences complementary to one or more hybridized probe species.

67. The method of claim 66, wherein the one or more hybridized probe species comprise one or more detectable labels.

68. The method of any one of claims 1 to 56, wherein the nucleobase class is purine.

69. The method of any one of claims 1 to 56, wherein the nucleobase class is pyrimidine.

70. The method of claim 68 or 69, wherein purines are distinguished from pyrimidines in the partial nucleotide sequence reads.

71. The method of any one of claims 1 to 56, wherein the sample nucleic acid comprises single stranded nucleic acid.

72. The method of any one of claims 1 to 56, wherein the sample nucleic acid comprises double stranded nucleic acid.

73. The method of claim 72, wherein the nucleobase class is a nucleobase pair species in a duplex nucleic acid.

74. The method of any one of claims 1 to 73, wherein obtaining partial nucleotide sequence reads includes subjecting the sample nucleic acid to a sequencing process using a sequencing device.

75. The method of claim 74, wherein the partial nucleotide sequence reads are obtained by nanopore sequencing.

76. The method of claim 74, wherein the partial nucleotide sequence reads are obtained by reversible terminator-based sequencing.

77. The method of claim 75, wherein the partial nucleotide sequence reads are obtained by pyrosequencing.

78. The method of claim 74, wherein the partial nucleotide sequence reads are obtained by real time sequencing.

79. The method of claim 74, wherein the partial nucleotide sequence reads are obtained by oligonucleotide probe ligation sequencing.

80. The method of claim 80, wherein the partial nucleotide sequence reads are obtained by sequencing by hybridization.

81. The method of any one of claims 1 to 80, wherein the partial nucleotide sequence reads comprise a number of discrete position identities sufficient to map to a reference genome section.

82. The method of any one of claims 1 to 80, wherein the partial nucleotide sequence read is of sufficient length to map to a reference genome section.

83. The method of claim 82, wherein the partial nucleotide sequence read length is at least about 36 nucleobases.

84. The method of claim 82, wherein the partial nucleotide sequence read length is at least about 72 nucleobases.

85. The method of claim 82, wherein the partial nucleotide sequence read length is at least about 108 nucleobases.

86. The method of any one of claims 1 to 85, wherein the nucleobase gaps in each partial nucleotide sequence read independently are about 1 to about 100 sequential nucleobases.

87. The method of any one of claims 1 to 86, which comprises obtaining full nucleotide sequence reads, which nucleotide sequence reads do not contain nucleobase gaps between identified nucleobases or a nucleobase class comprising a subset of nucleobases present in the sample nucleic acid.

88. The method of any one of claims 36 to 38, wherein the fetal aneuploidy is trisomy 13.

89. The method of any one of claims 36 to 38, wherein the fetal aneuploidy is trisomy 18.

90. The method of any one of claims 36 to 38, wherein the fetal aneuploidy is trisomy 21.

91. The method of any one of claims 39 to 41, wherein the genetic variation is associated with a medical condition.

92. The method of claim 91, wherein the medical condition is cancer.

93. The method of claim 91, wherein the medical condition is an aneuploidy.

94. A computer program product, comprising a computer usable medium having a computer readable program code embodied therein, the computer readable program code comprising distinct software modules comprising a sequence receiving module, a logic processing module, and a data display organization module, the computer readable program code adapted to be executed to implement a method for identifying the presence or absence of a fetal aneuploidy, the method comprising:

(a) obtaining, by the sequence receiving module, partial nucleotide sequence reads from a sample comprising circulating, cell-free nucleic acid from a pregnant female, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or combination of (i) and (ii);

(b) receiving, by the logic processing module, the partial nucleotide sequence reads;

(c) mapping, by the logic processing module, the partial nucleotide sequence reads to reference genome sections;

(d) counting, by the logic processing module, the number of partial nucleotide sequence reads mapped to each reference genome section;

(e) comparing, by the logic processing module, the number of counts of the partial nucleotide sequence reads, or derivative thereof, to a reference, or portion thereof, thereby making a comparison;

(f) providing, by the logic processing module, an outcome determinative of the presence or absence of a fetal aneuploidy based on the comparison; and

(g) organizing, by the data display organization module in response to being determined by the logic processing module, a data display indicating the presence or absence of a fetal aneuploidy.

95. An apparatus, comprising memory in which a computer program product of claim 94 is stored.

96. The apparatus of claim 95, which comprises a processor that implements one or more functions of the computer program product specified in claim 94.

97. A system comprising a nucleic acid sequencing apparatus and a processing apparatus, wherein the sequencing apparatus obtains sequence reads from a sample, and the processing apparatus obtains the sequence reads from the sequencing apparatus and carries out a method comprising:

(a) mapping partial nucleotide sequence reads from the sequencing apparatus that have been obtained from a sample comprising circulating, cell-free nucleic acid from a pregnant female, to reference genome sections, wherein at least some partial nucleotide sequence reads comprise:

i) multiple nucleobase gaps between identified nucleobases, or

ii) one or more nucleobase classes, wherein each nucleobase class comprises a subset of nucleobases present in the sample nucleic acid, or

combination of (i) and (ii),

(b) counting the number of partial nucleotide sequence reads mapped to each reference genome section,

(c) comparing the number of counts of the partial nucleotide sequence reads mapped in (b), or derivative thereof, to a reference, or portion thereof, thereby making a comparison, and

(d) determining the presence or absence of a fetal aneuploidy based on the comparison.

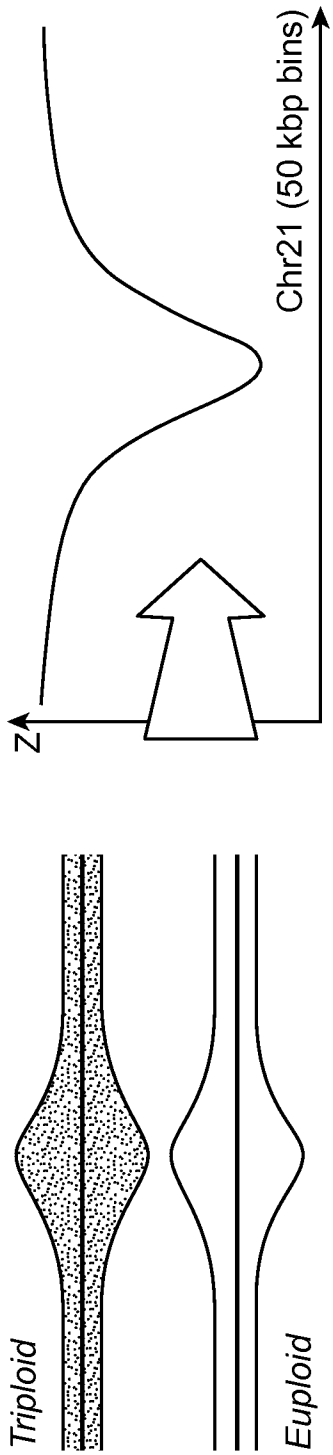


FIG. 1

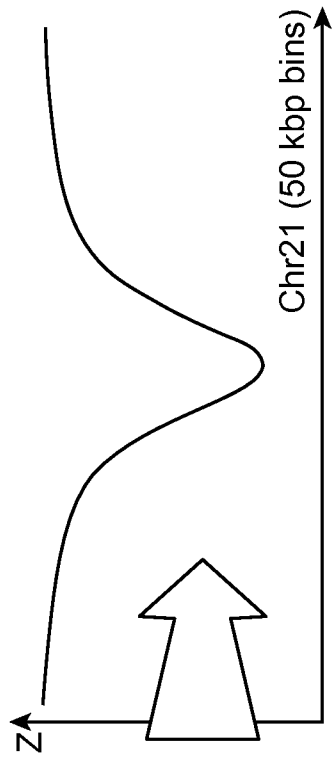
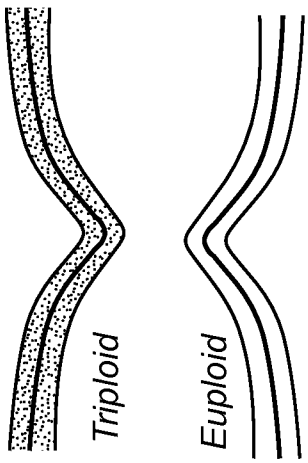


FIG. 2



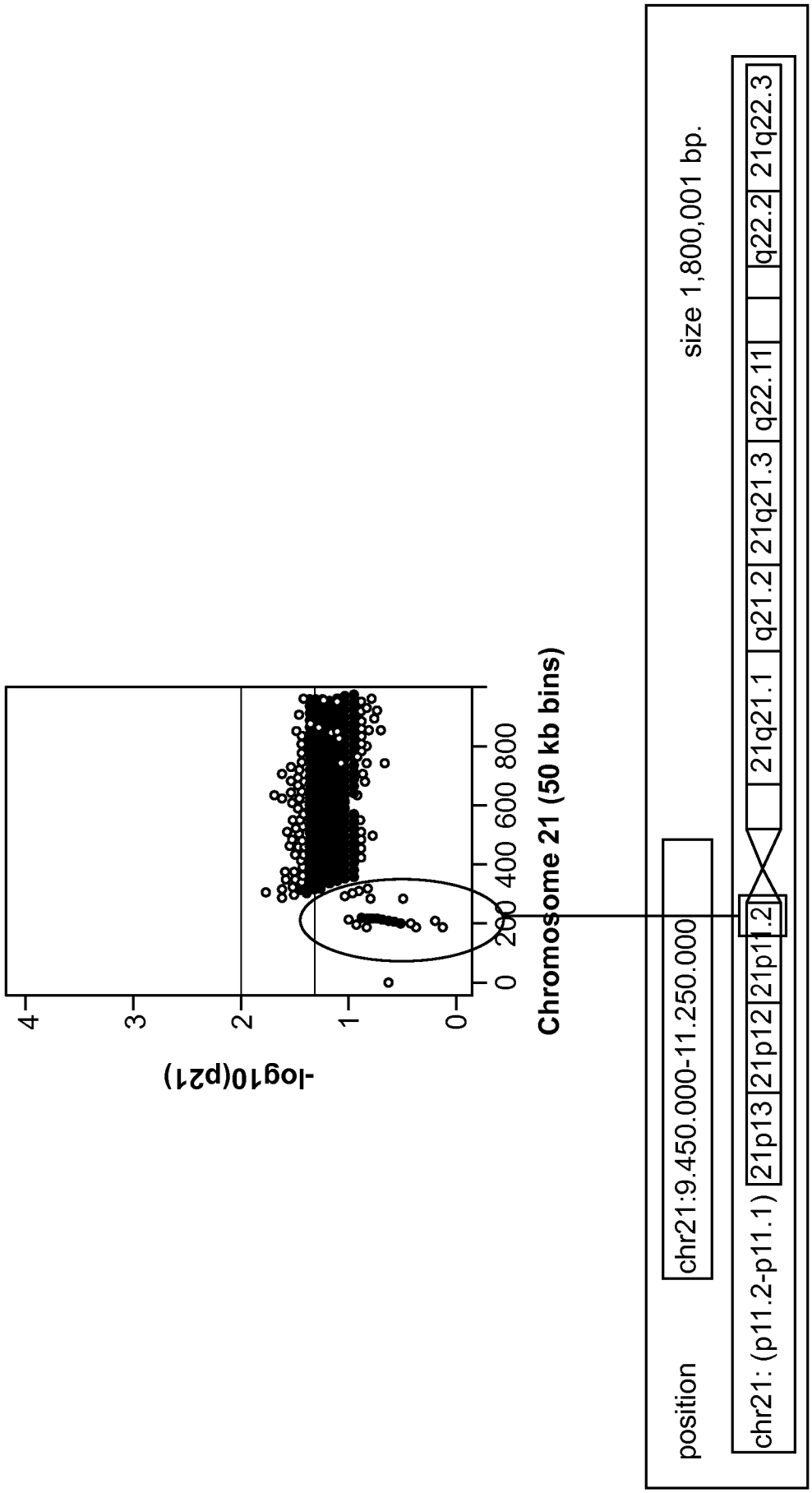

-log10(p21)
0
1
2
3
4
0
200
400
600
800
Chromosome 21 (50 kb bins)
position
chr21:9.450.000-11.250.000
size 1,800,001 bp.
chr21: (p11.2-p11.1)
21p13
21p12
21p11.2
21q21.1
q21.2
21q21.3
q22.11
q22.2
21q22.3

FIG. 3

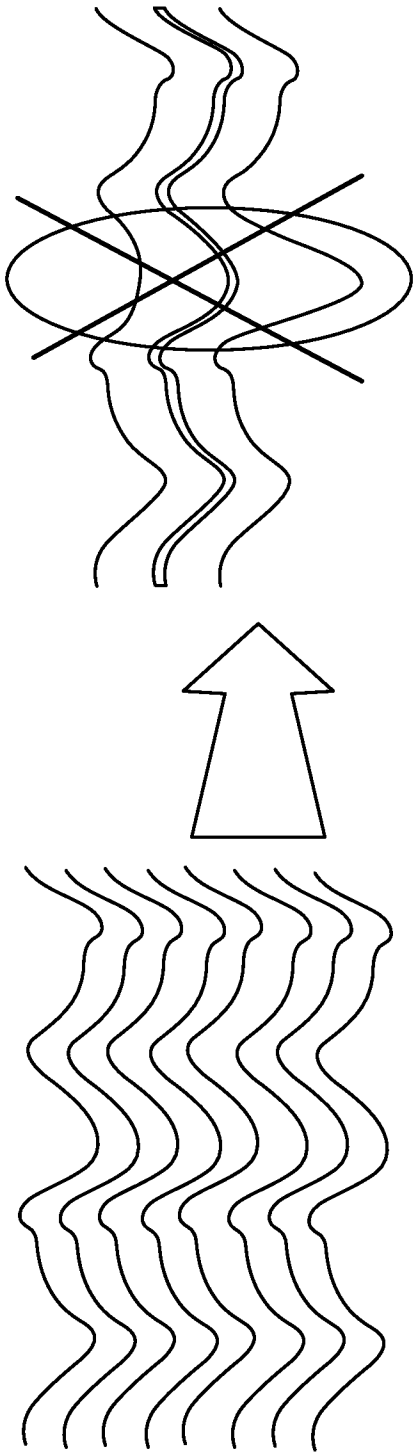


FIG. 4

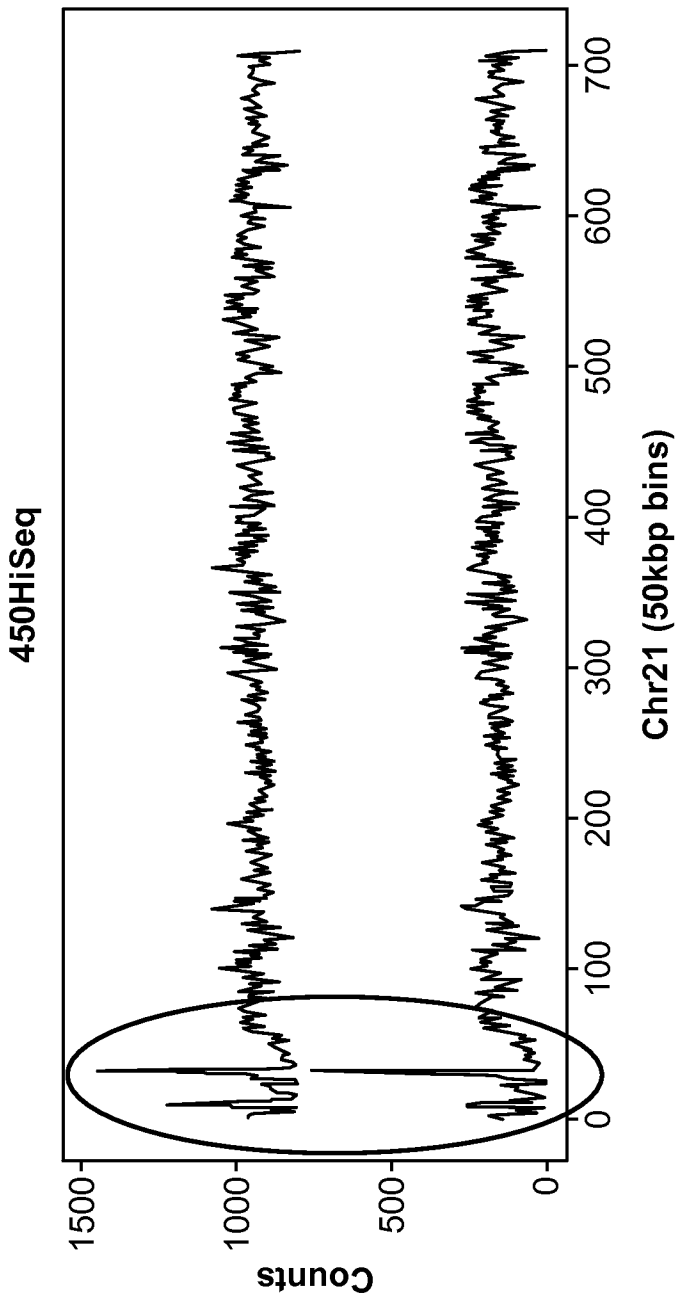

450HiSeq
Counts
1500
1000
500
0
0
100
200
300
400
500
600
700
Chr21 (50kbp bins)

FIG. 5

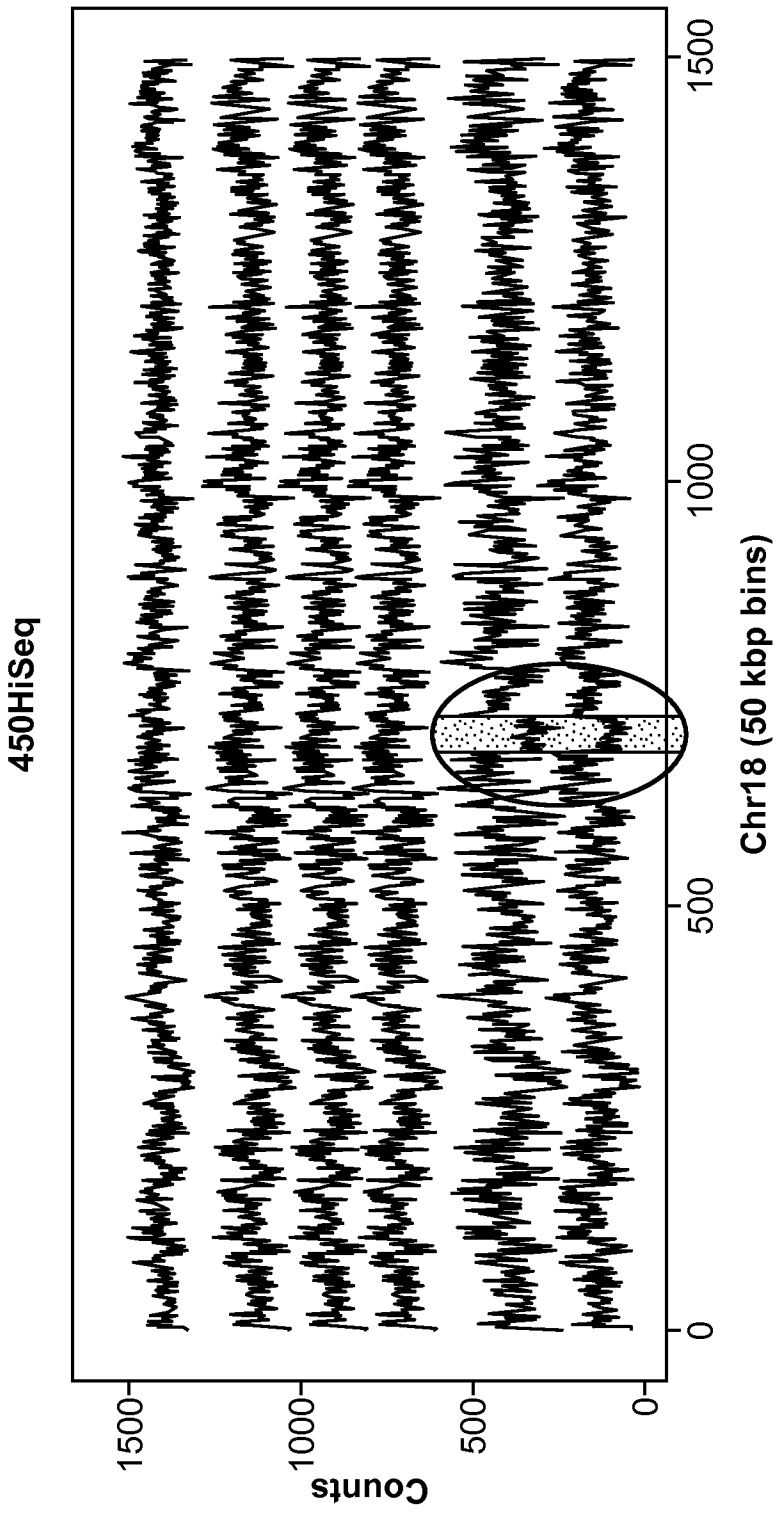

450HiSeq
Counts
1500
1000
500
0
0
500
1000
1500
Chr18 (50 kbp bins)

FIG. 6

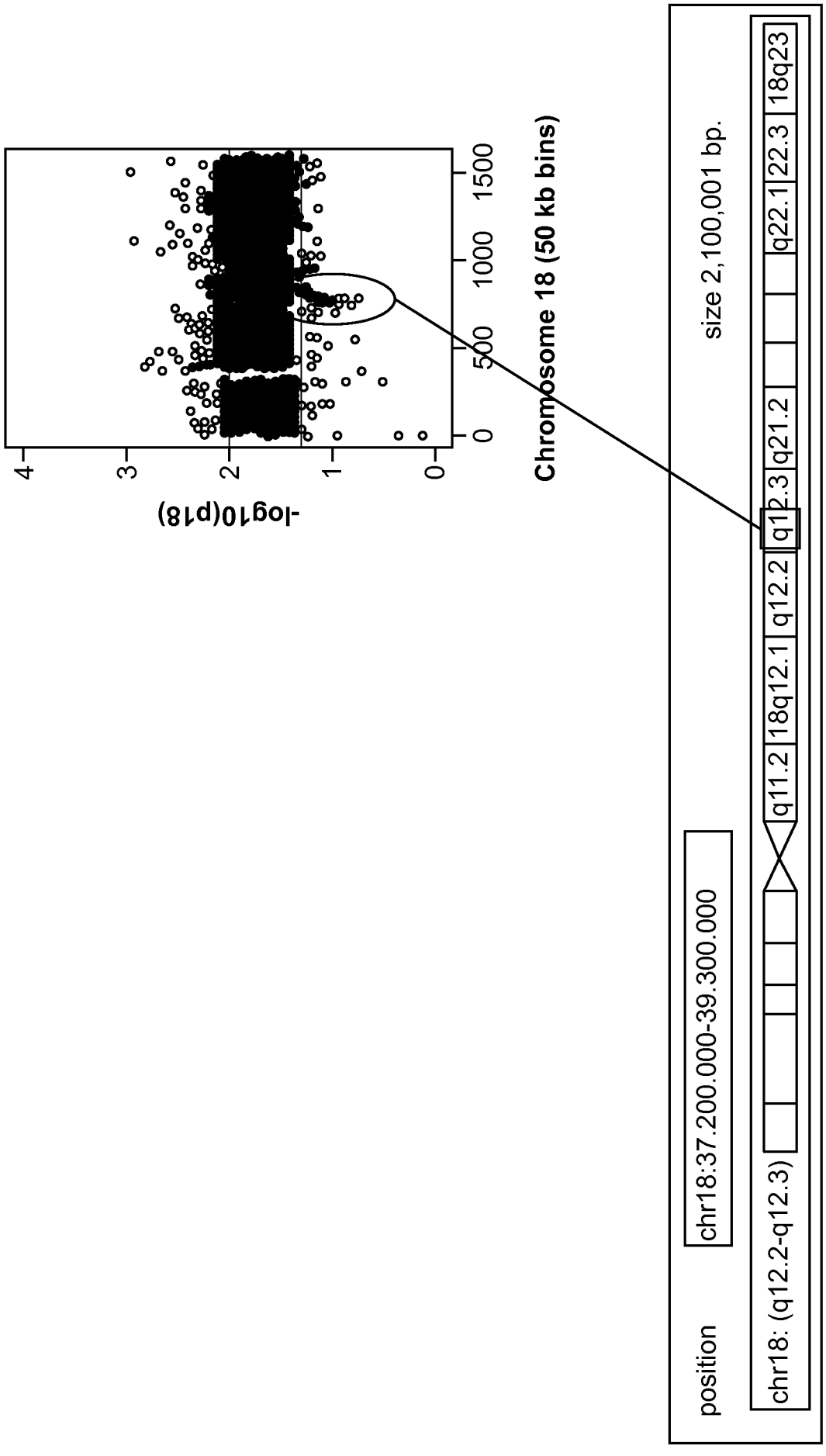

-log10(p18)
0
1
2
3
4
0
500
1000
1500
Chromosome 18 (50 kb bins)
size 2,100,001 bp.
position
chr18:37.200.000-39.300.000
chr18: (q12.2-q12.3)
q11.2
18q12.1
q12.2
q12.3
q21.2
q22.1
22.3
18q23

FIG. 7

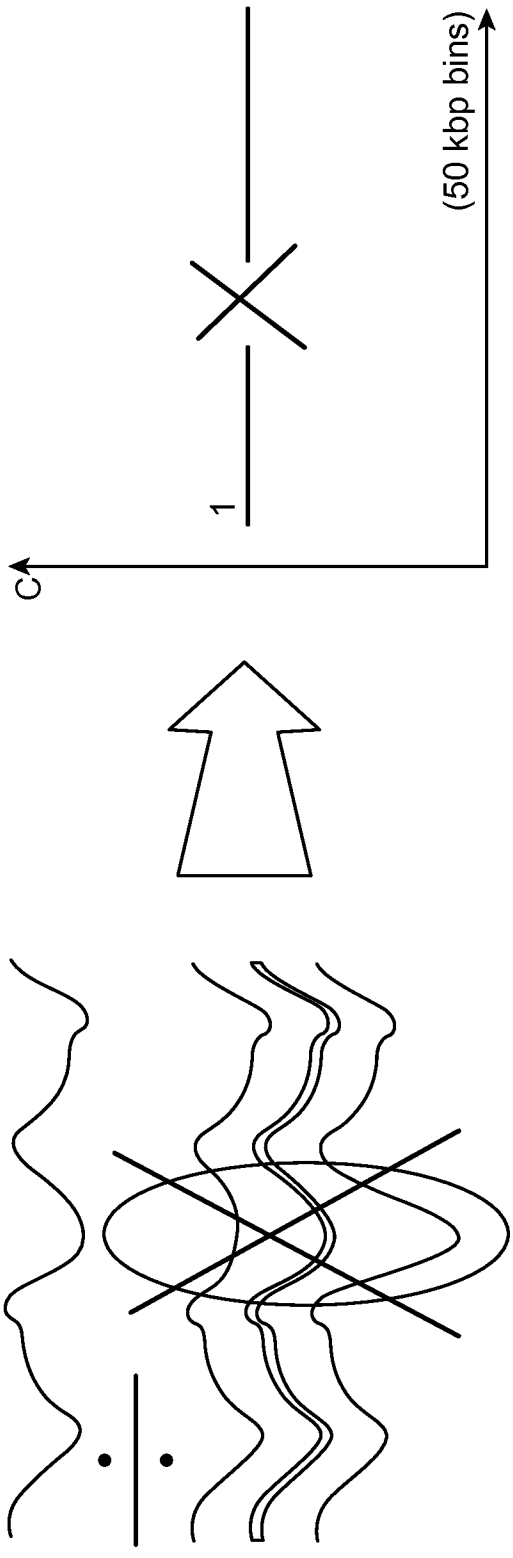


FIG. 8

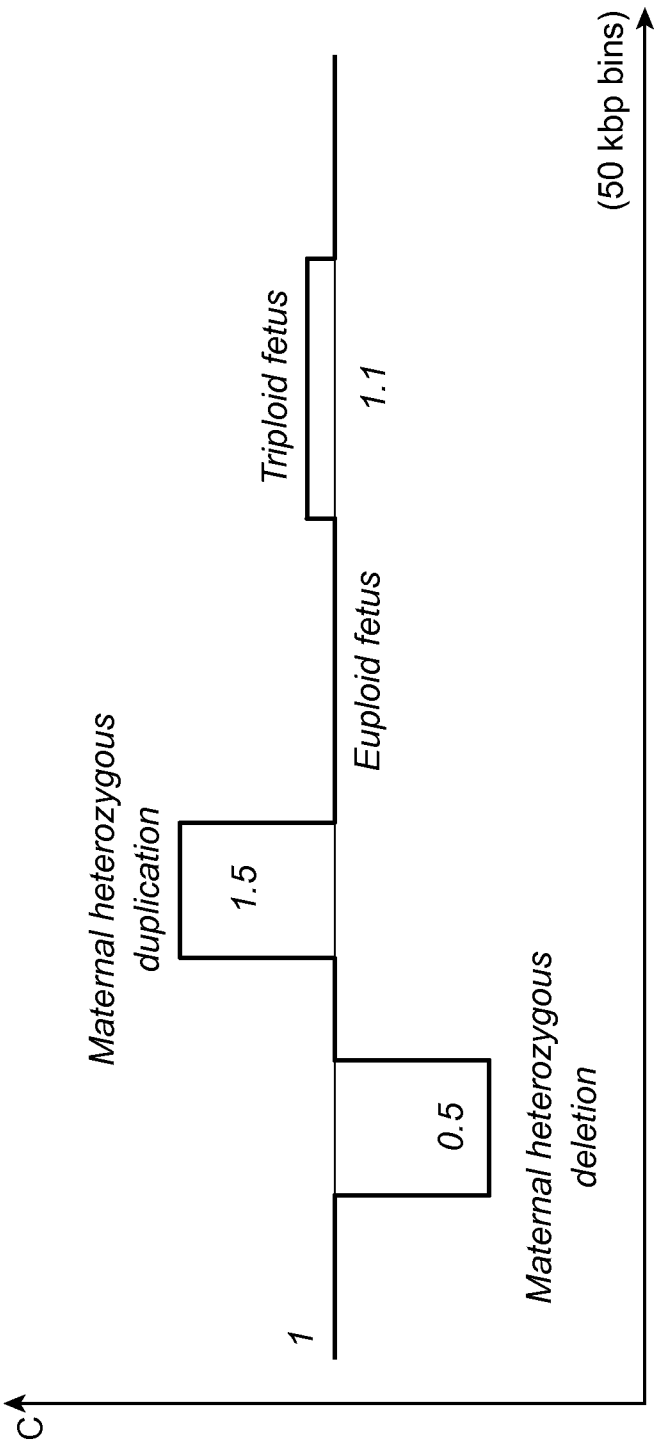

C
(50 kbp bins)
1
Maternal heterozygous deletion
0.5
Euploid fetus
1.5
Maternal heterozygous duplication
Triploid fetus
1.1

FIG. 9

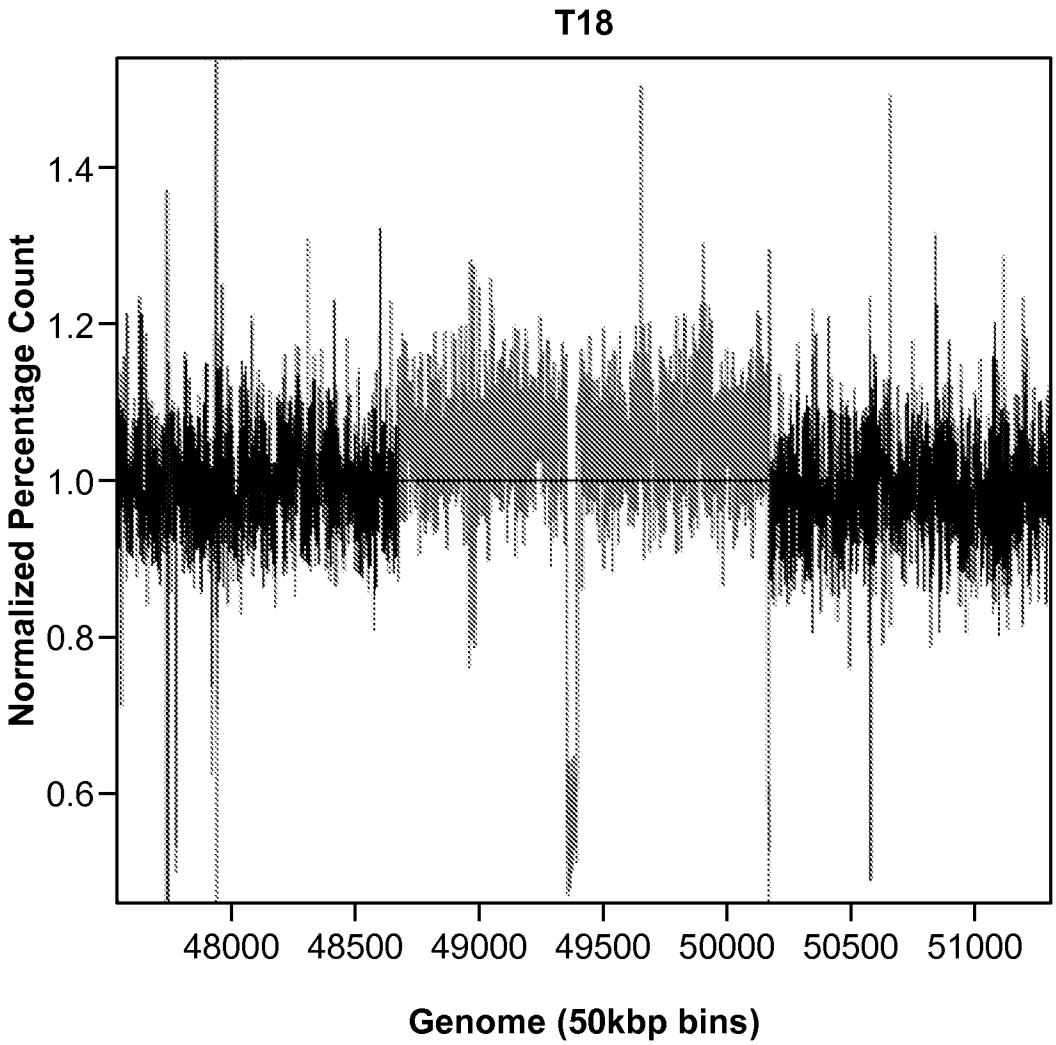

T18
Normalized Percentage Count
1.4
1.2
1.0
0.8
0.6
48000
48500
49000
49500
50000
50500
51000
Genome (50kbp bins)

FIG. 10

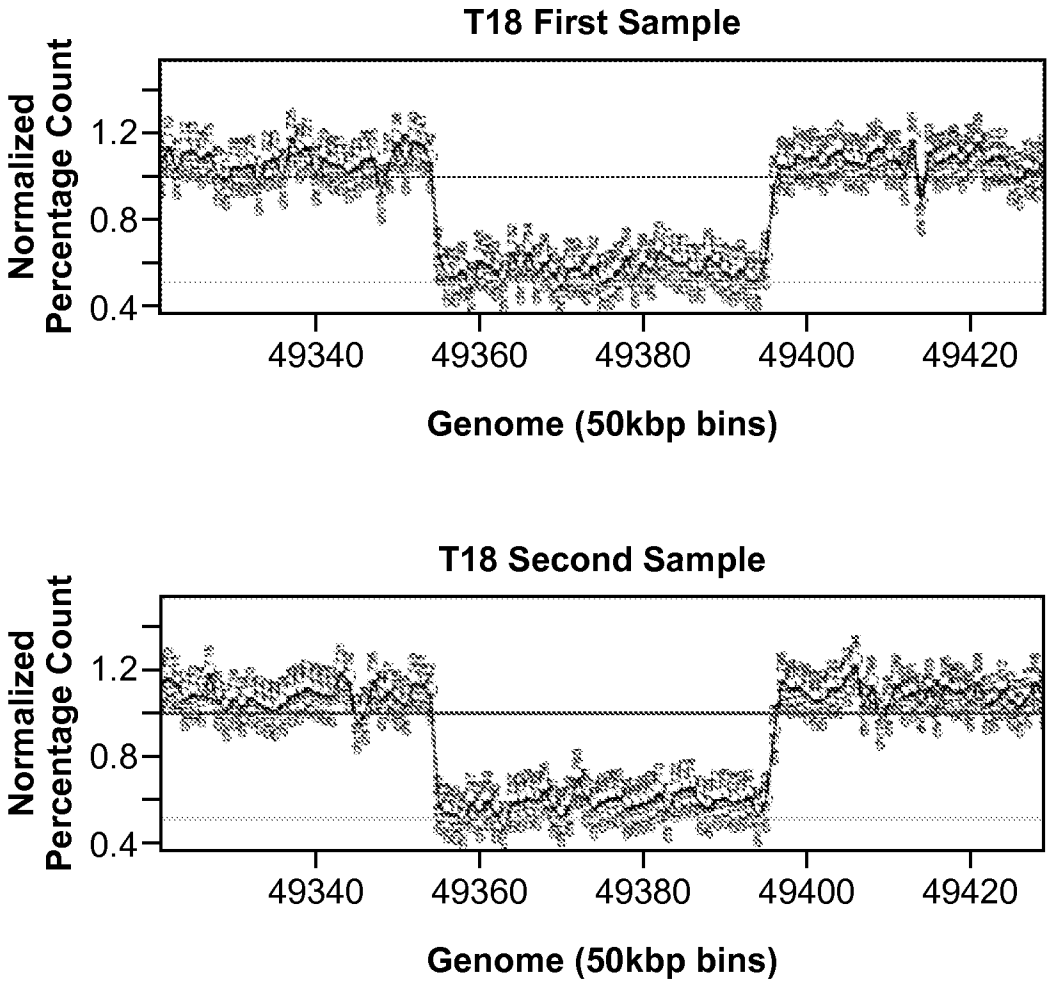

T18 First Sample
Normalized Percentage Count
1.2
0.8
0.4
49340
49360
49380
49400
49420
Genome (50kbp bins)
T18 Second Sample
Normalized Percentage Count
1.2
0.8
0.4
49340
49360
49380
49400
49420
Genome (50kbp bins)

FIG. 11

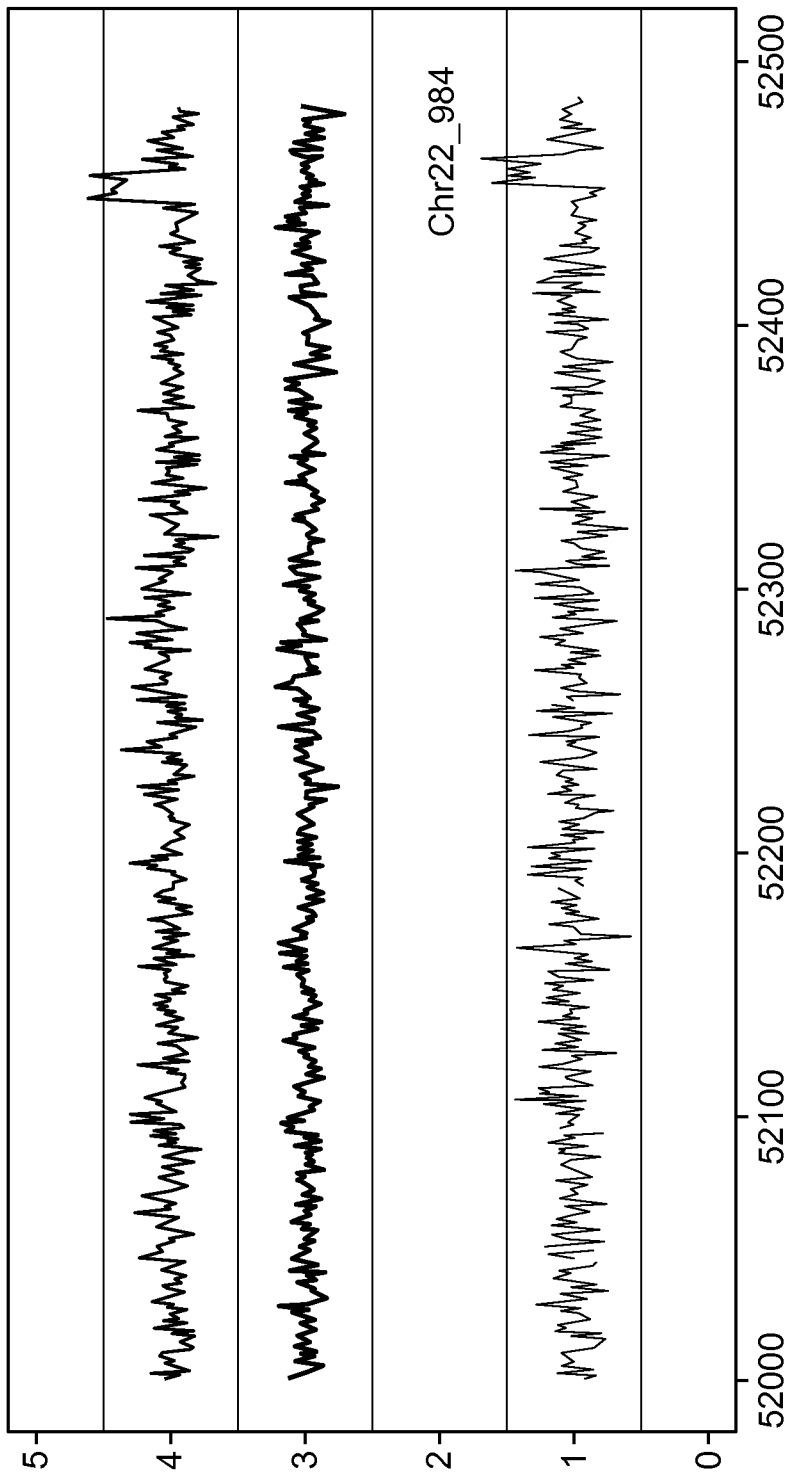

Chr22_984
5
4
3
2
1
0
52000
52100
52200
52300
52400
52500
50 kbp bins

FIG. 12

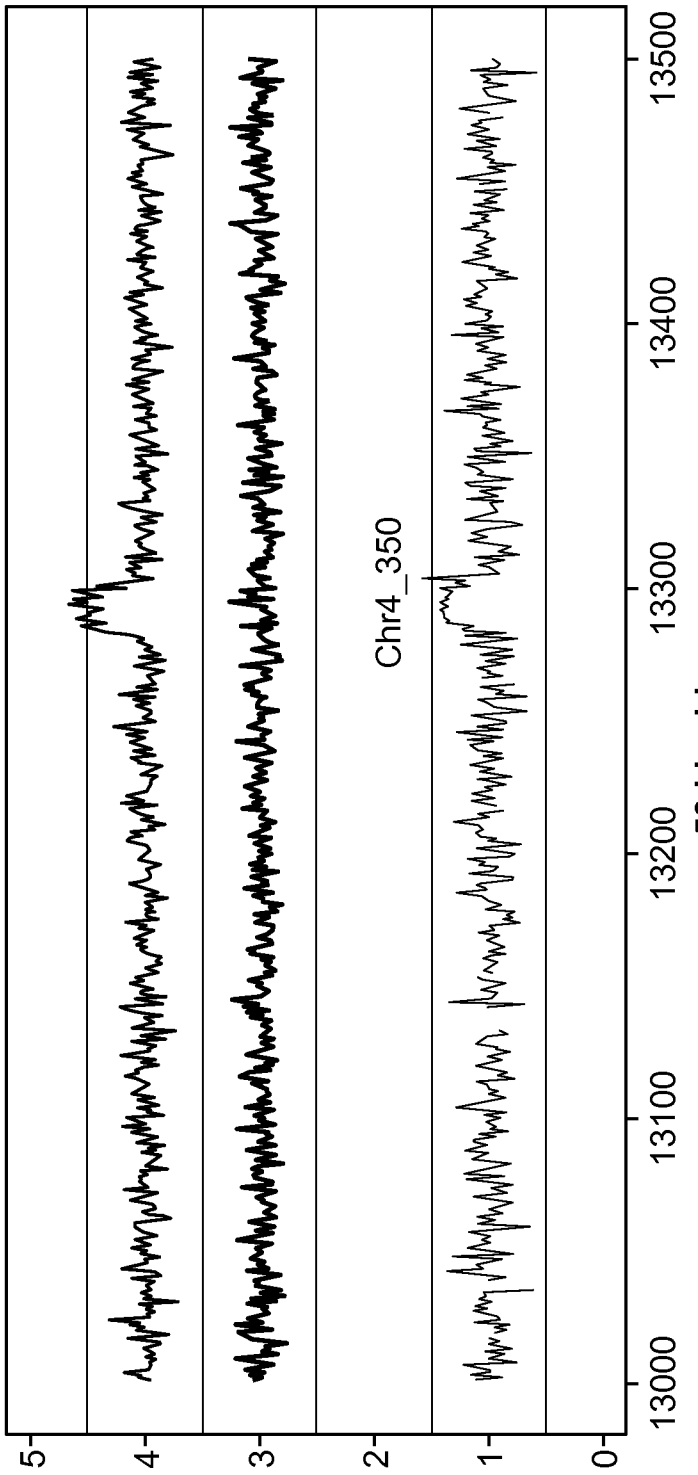

5
4
3
2
1
0
13000
13100
13200
13300
13400
13500
Chr4_350
50 kbp bins

FIG. 13

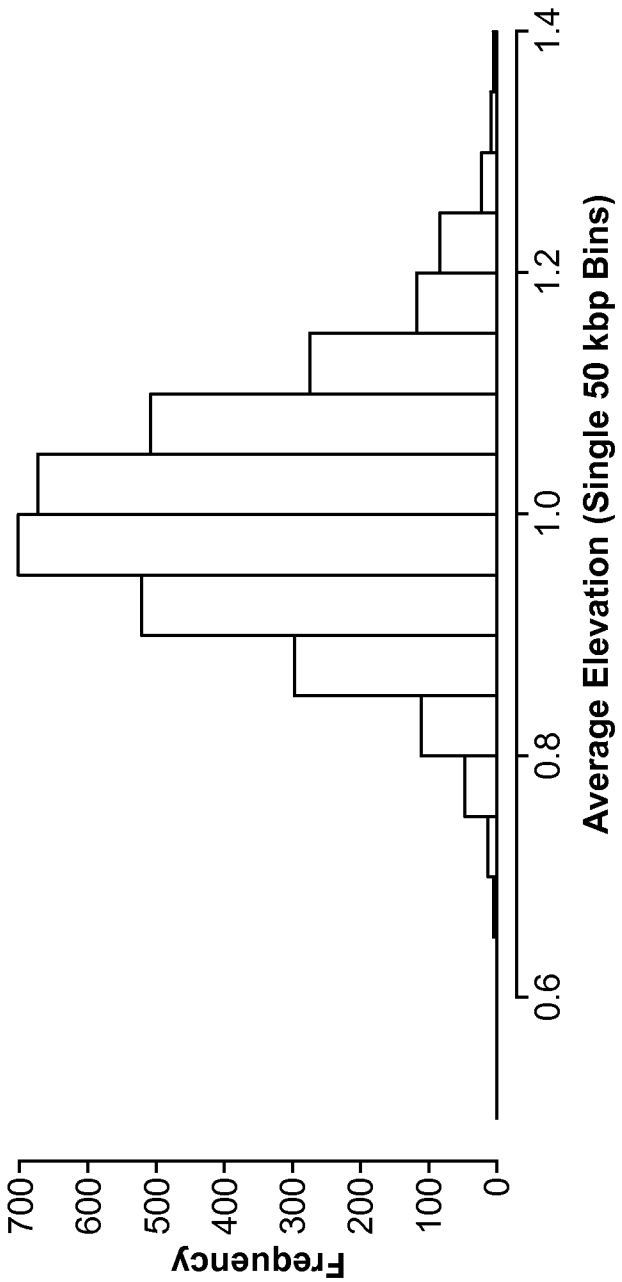

Frequency
0
100
200
300
400
500
600
700
Average Elevation (Single 50 kbp Bins)
0.6
0.8
1.0
1.2
1.4

FIG. 14

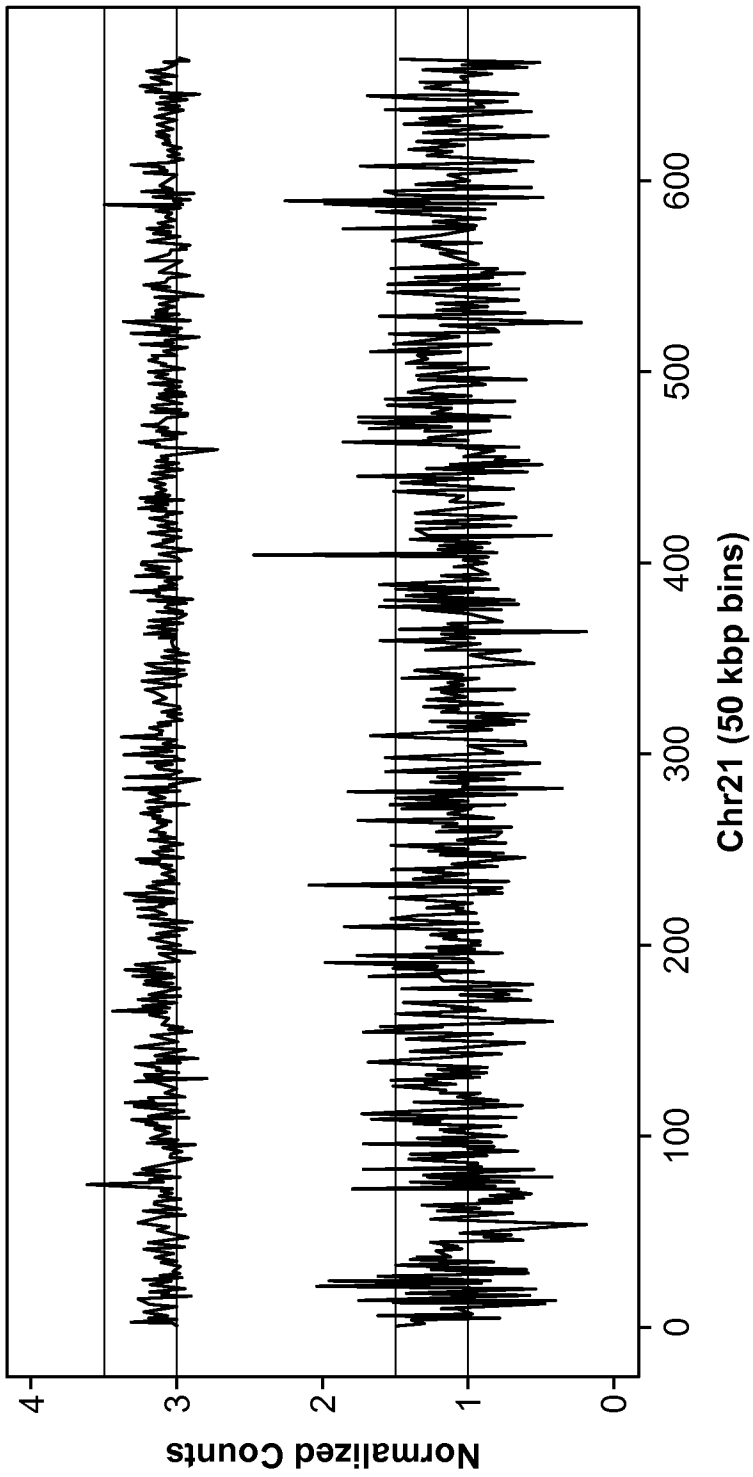

Normalized Counts
0
1
2
3
4
Chr21 (50 kbp bins)
0
100
200
300
400
500
600

FIG. 15

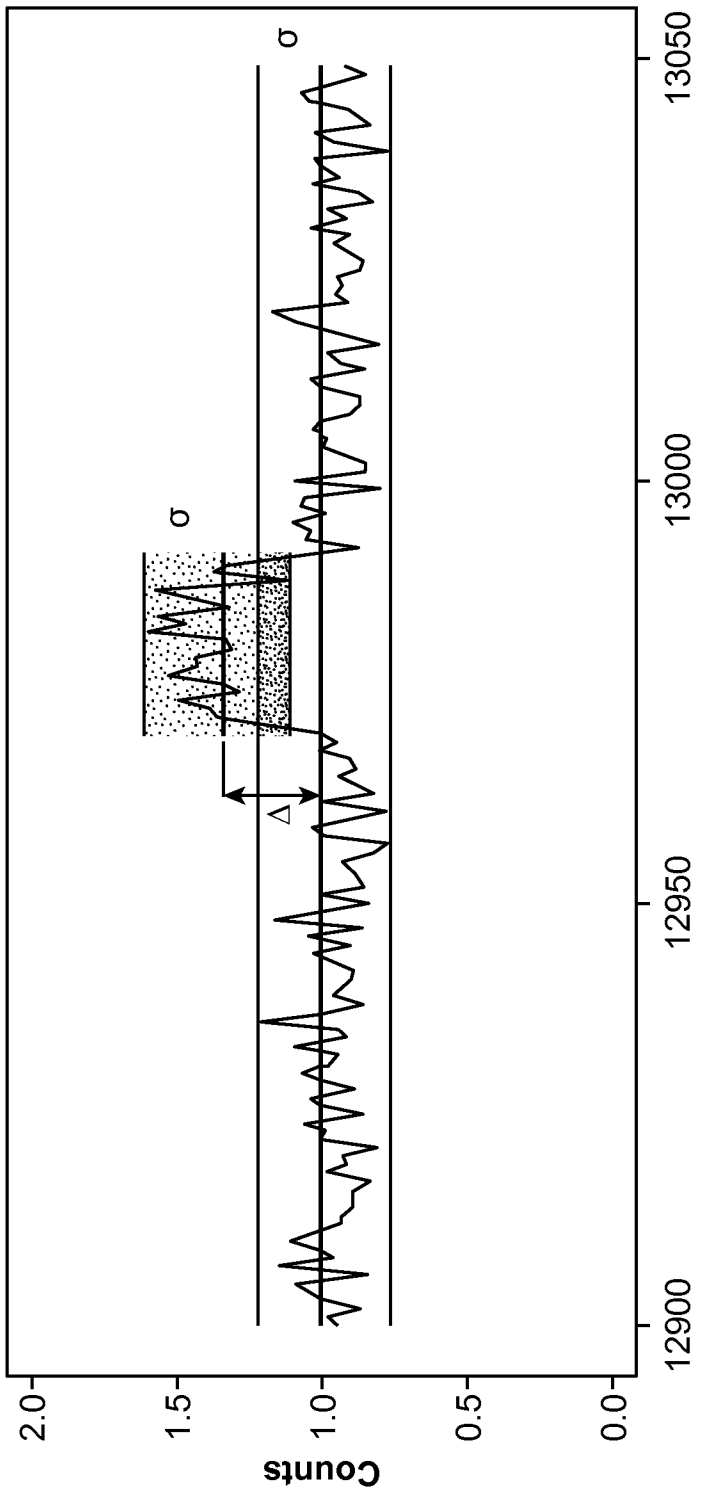

σ
σ
Δ
Counts
2.0
1.5
1.0
0.5
0.0
12900
12950
13000
13050
Chr4 (50 kbp bins)

FIG. 16

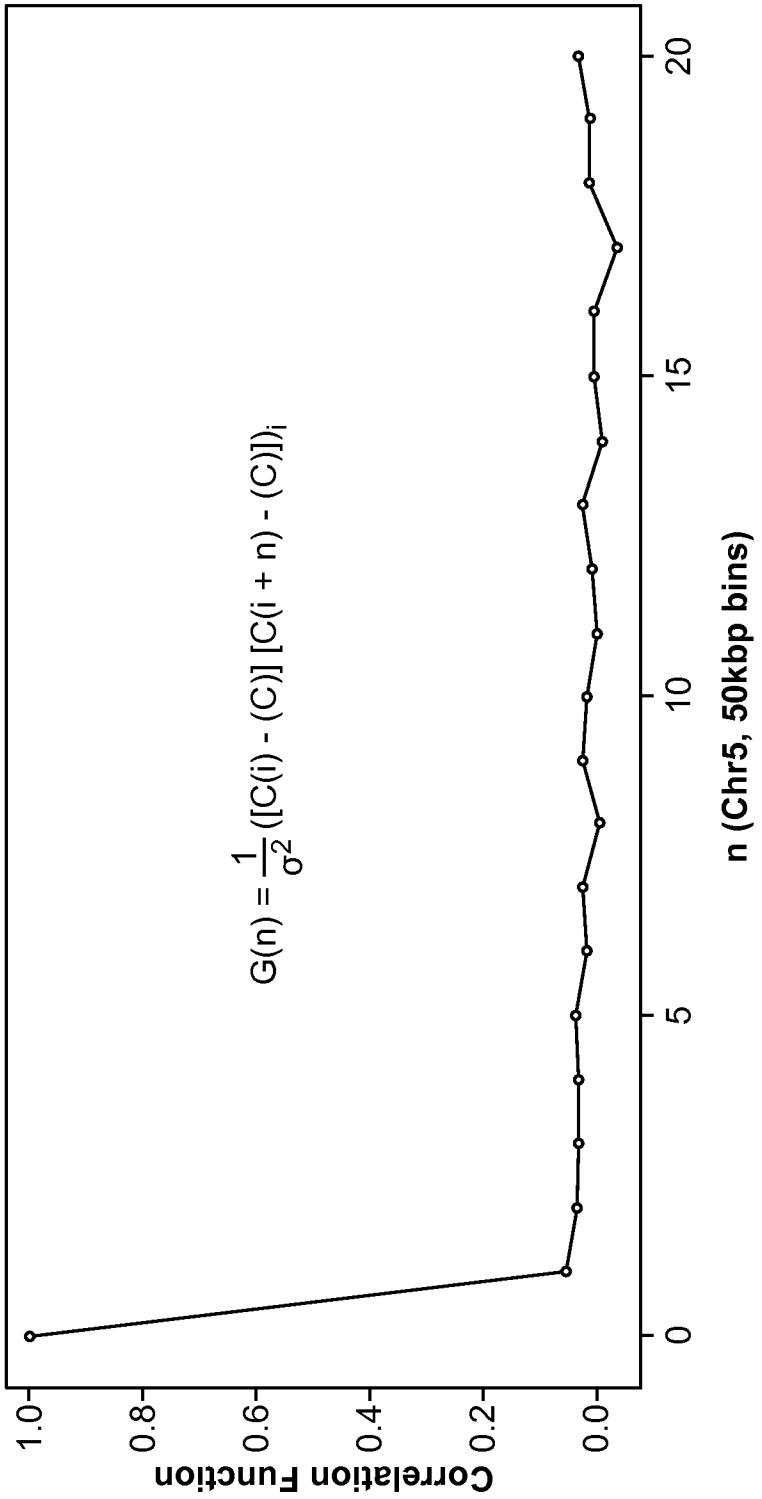

$G(n) = \frac{1}{\sigma^2}([C(i) - (C)][C(i+n) - (C)])_i$
Correlation Function
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
n (Chr5, 50kbp bins)

FIG. 17

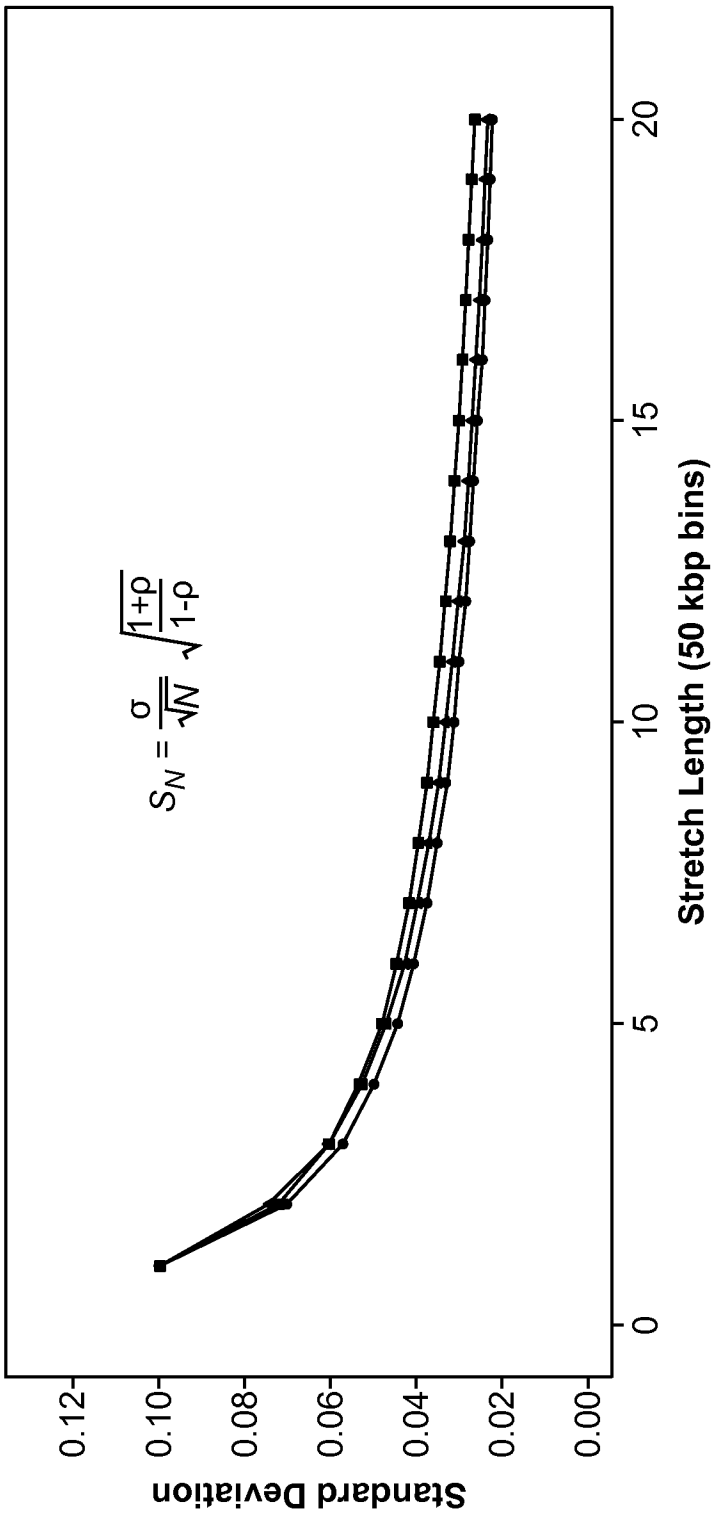

$S_N = \frac{\sigma}{\sqrt{N}} \sqrt{\frac{1+\rho}{1-\rho}}$
Standard Deviation
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0
5
10
15
20
Stretch Length (50 kbp bins)

FIG. 18

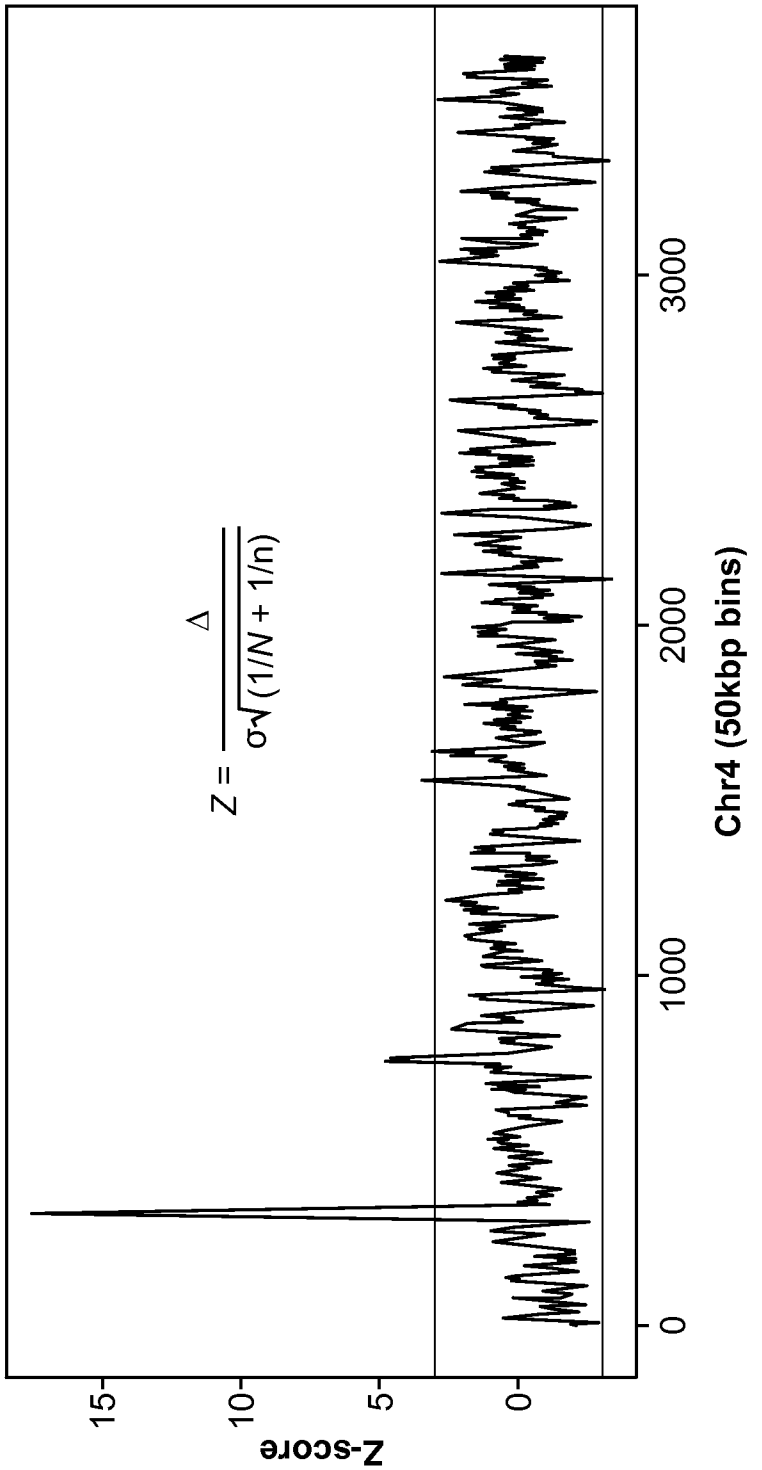

Z-score
15
10
5
0
0
1000
2000
3000
Chr4 (50kbp bins)
$Z = \frac{\Delta}{\sigma\sqrt{(1/N + 1/n)}}$

FIG. 19

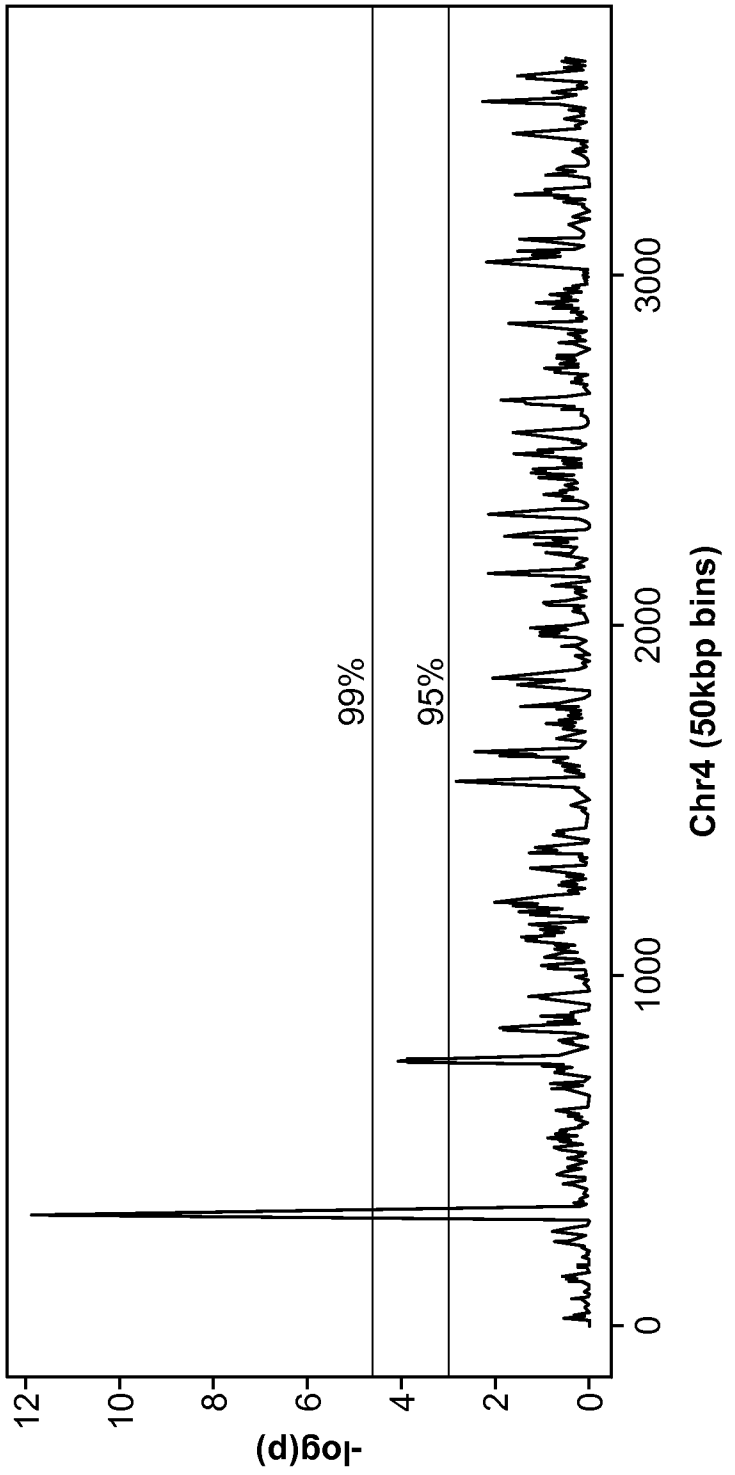


FIG. 20

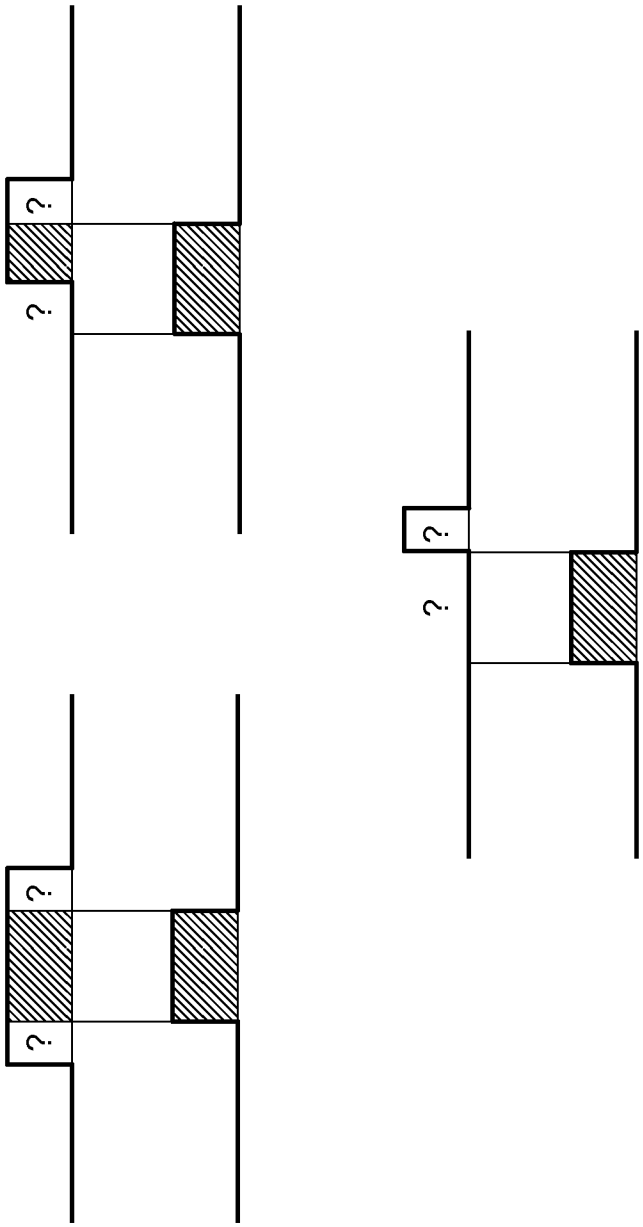


FIG. 21

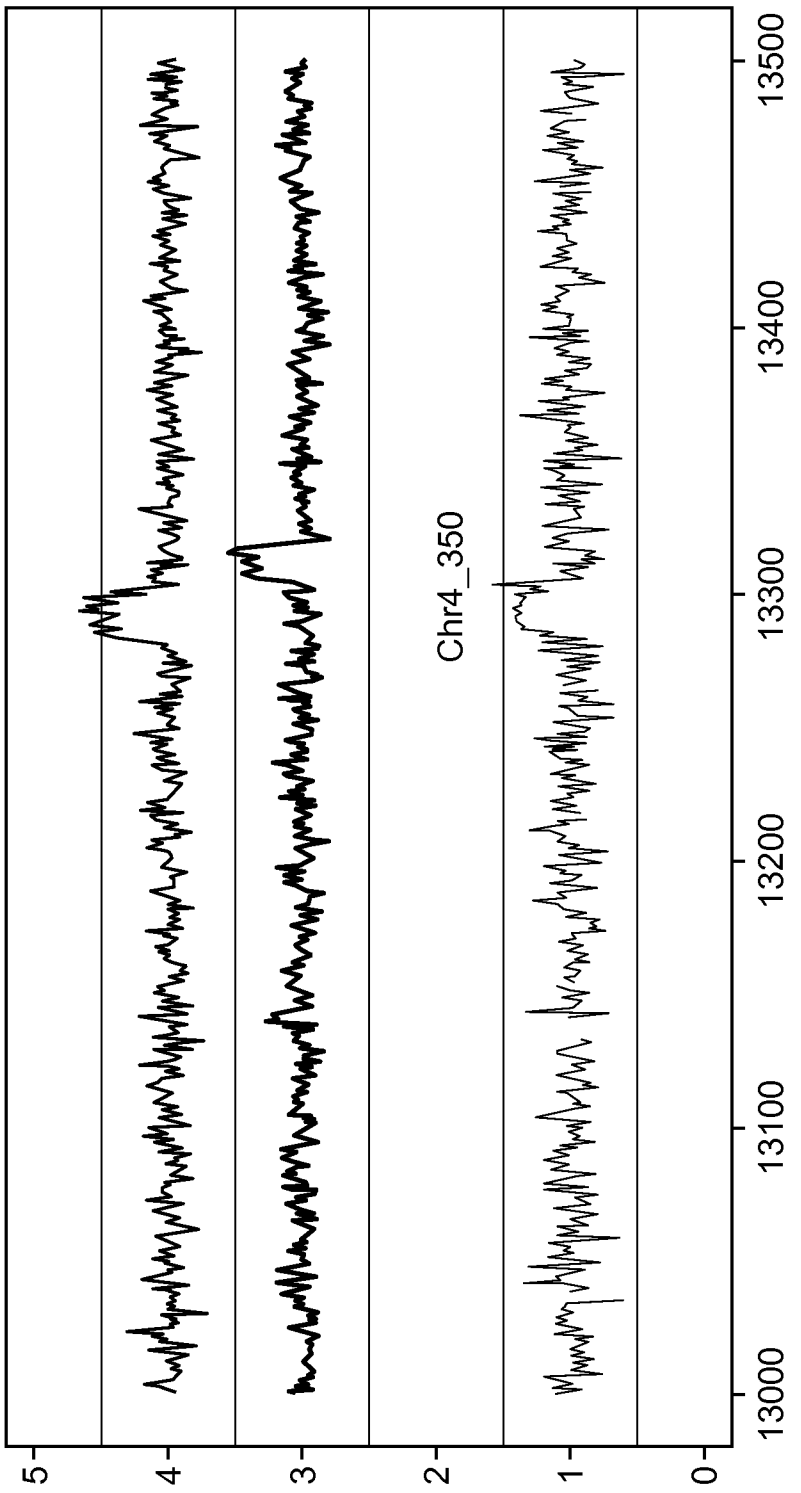

5
4
3
2
1
0
Chr4_350
13000
13100
13200
13300
13400
13500
Chr4 (50kbp bins)

FIG. 22

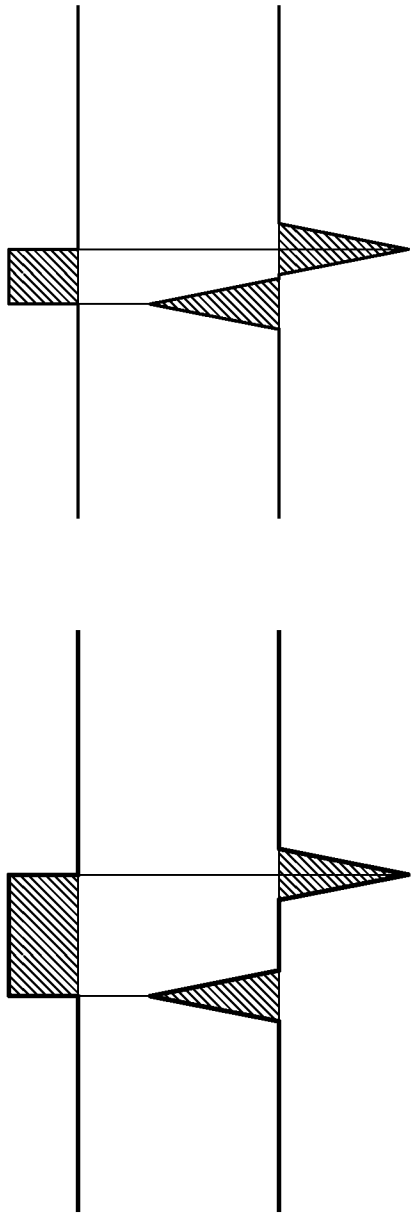


FIG. 23

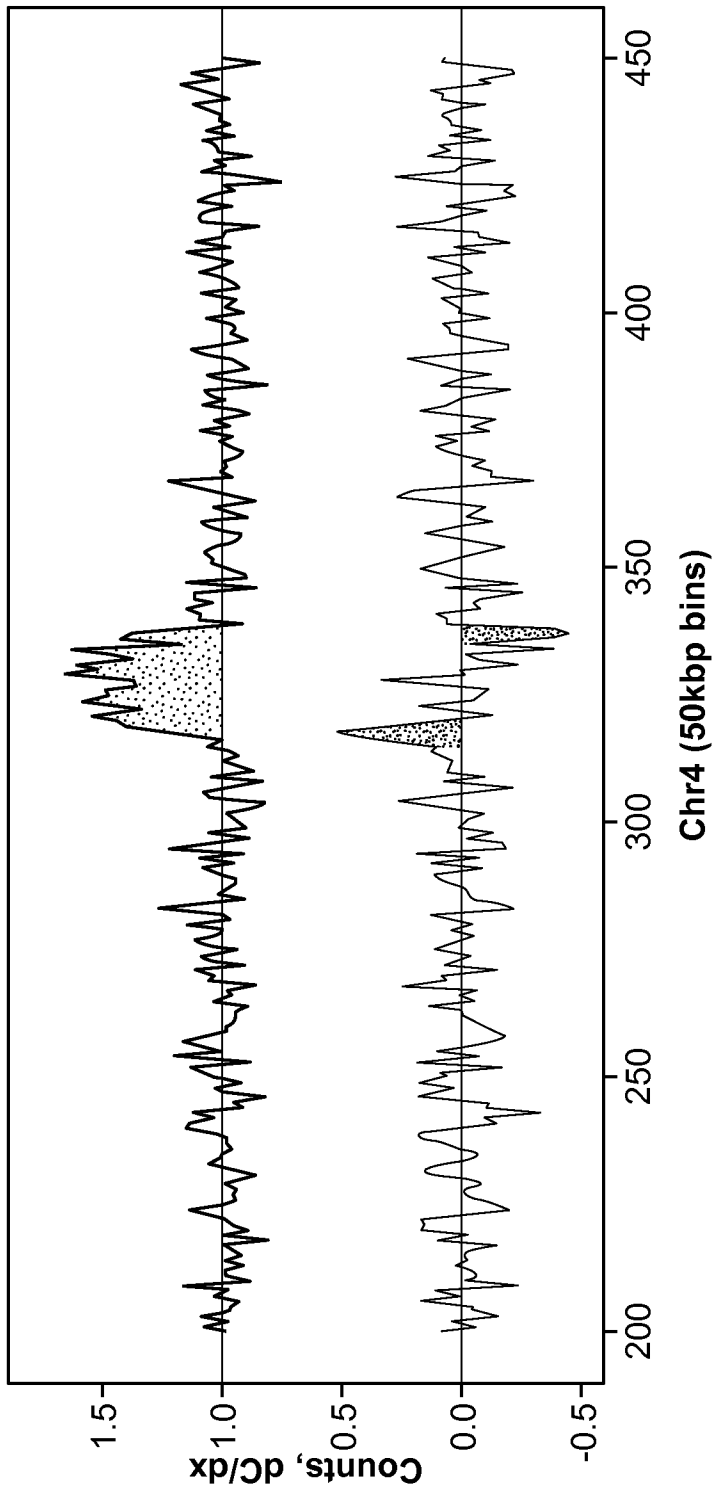

Counts, dC/dx
1.5
1.0
0.5
0.0
-0.5
200
250
300
350
400
450
Chr4 (50kbp bins)

FIG. 24

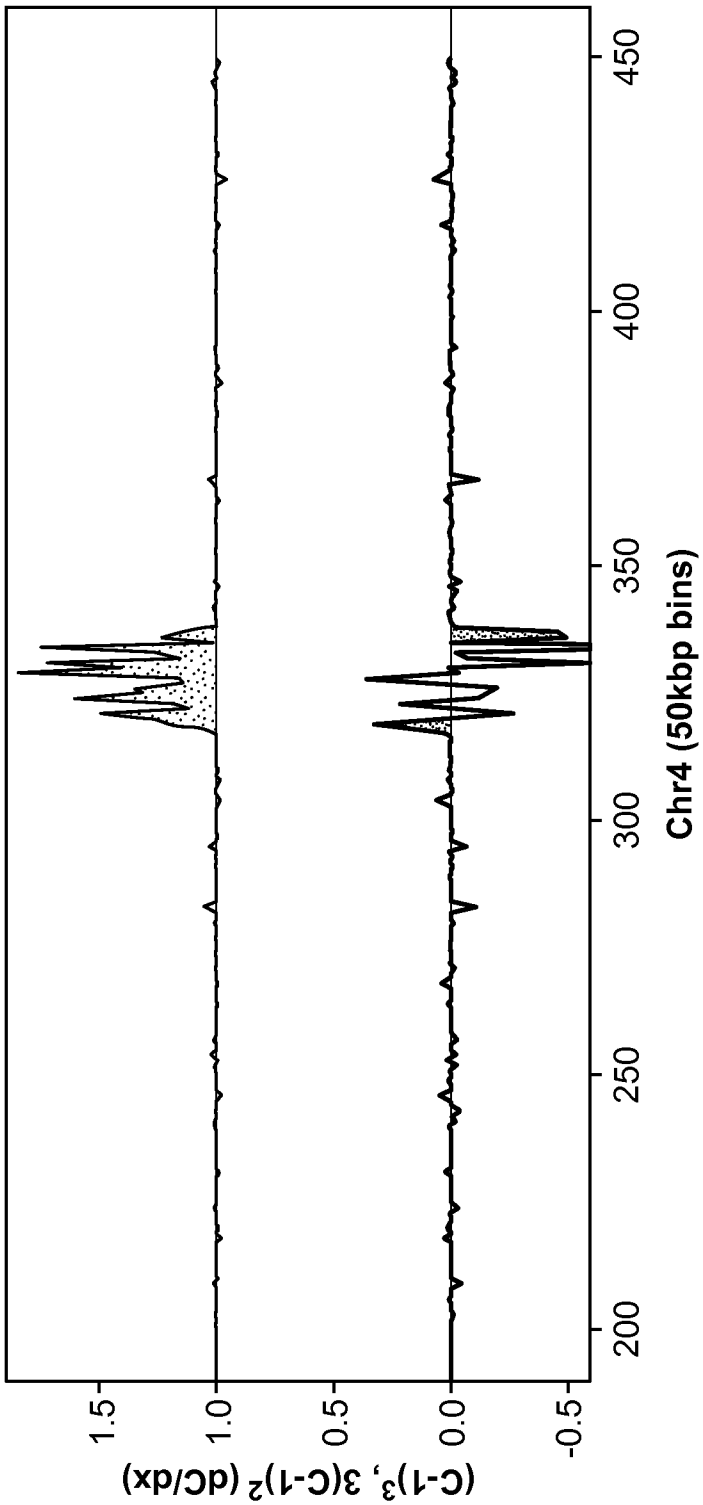

(C-1)3, 3(C-1)2 (dC/dx)
1.5
1.0
0.5
0.0
-0.5
200
250
300
350
400
450
Chr4 (50kbp bins)

FIG. 25

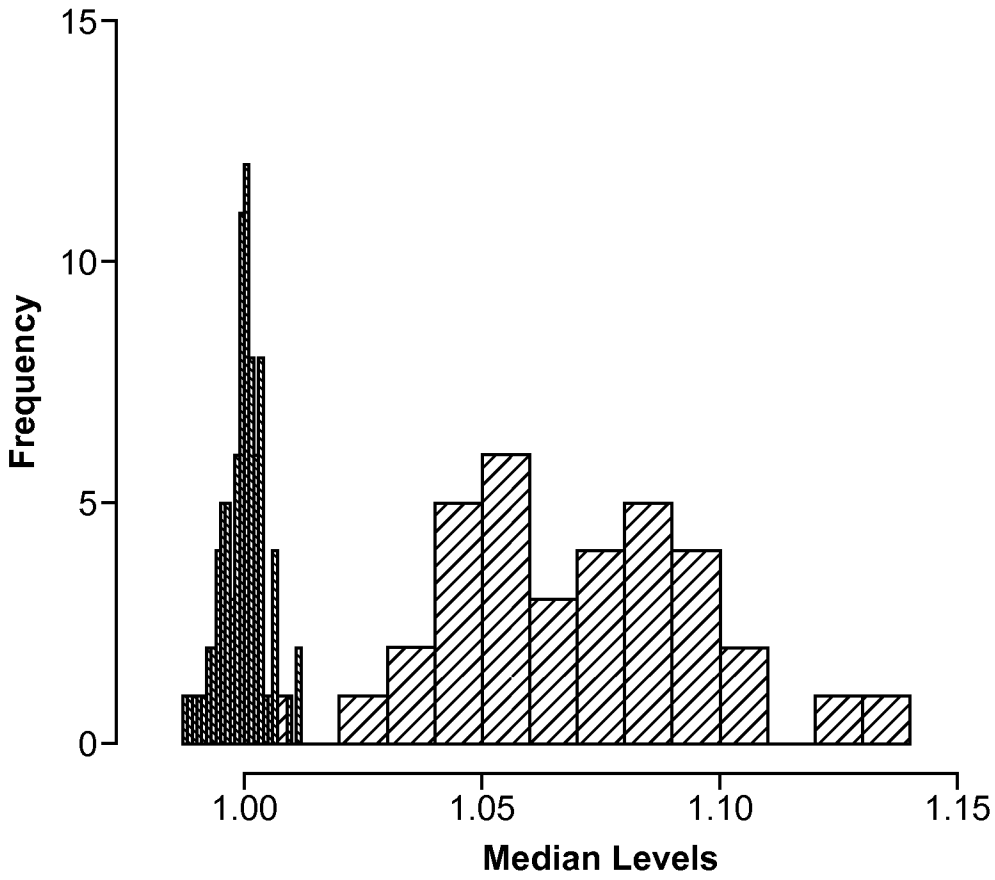


FIG. 26

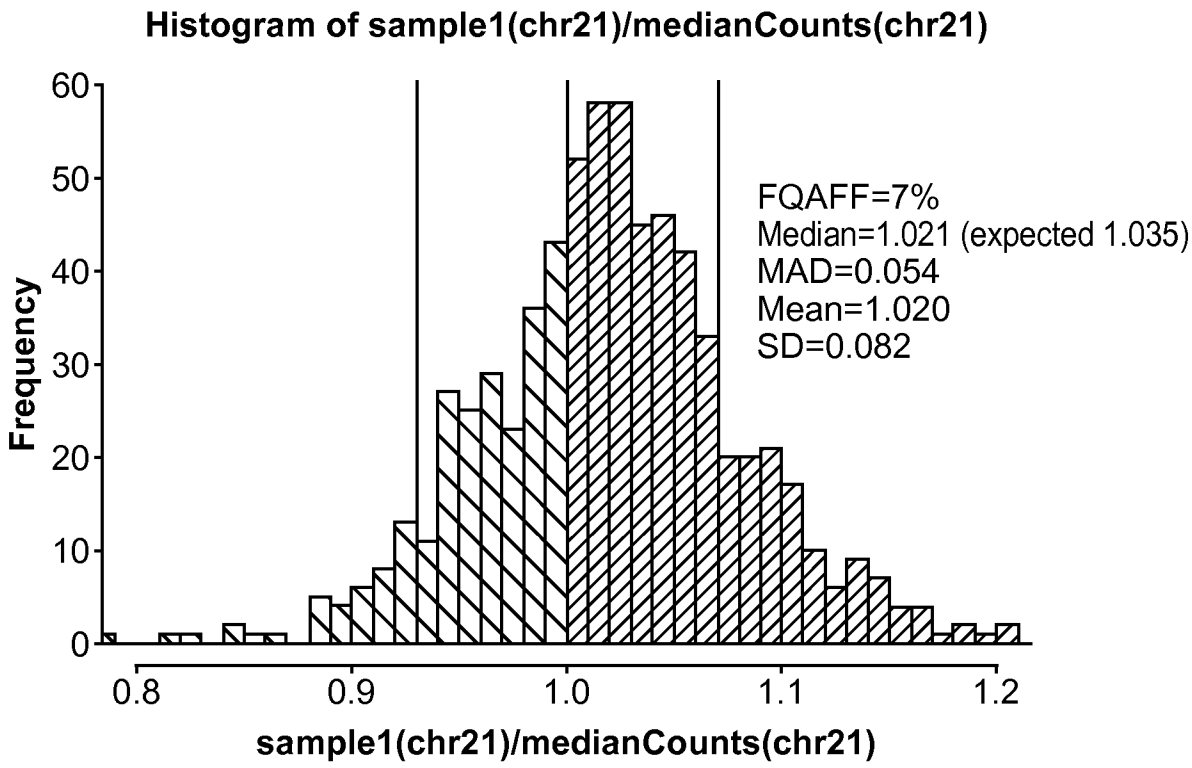

Histogram of sample1(chr21)/medianCounts(chr21)
Frequency
60
50
40
30
20
10
0
0.8
0.9
1.0
1.1
1.2
sample1(chr21)/medianCounts(chr21)
FQAFF=7%
Median=1.021 (expected 1.035)
MAD=0.054
Mean=1.020
SD=0.082

FIG. 27

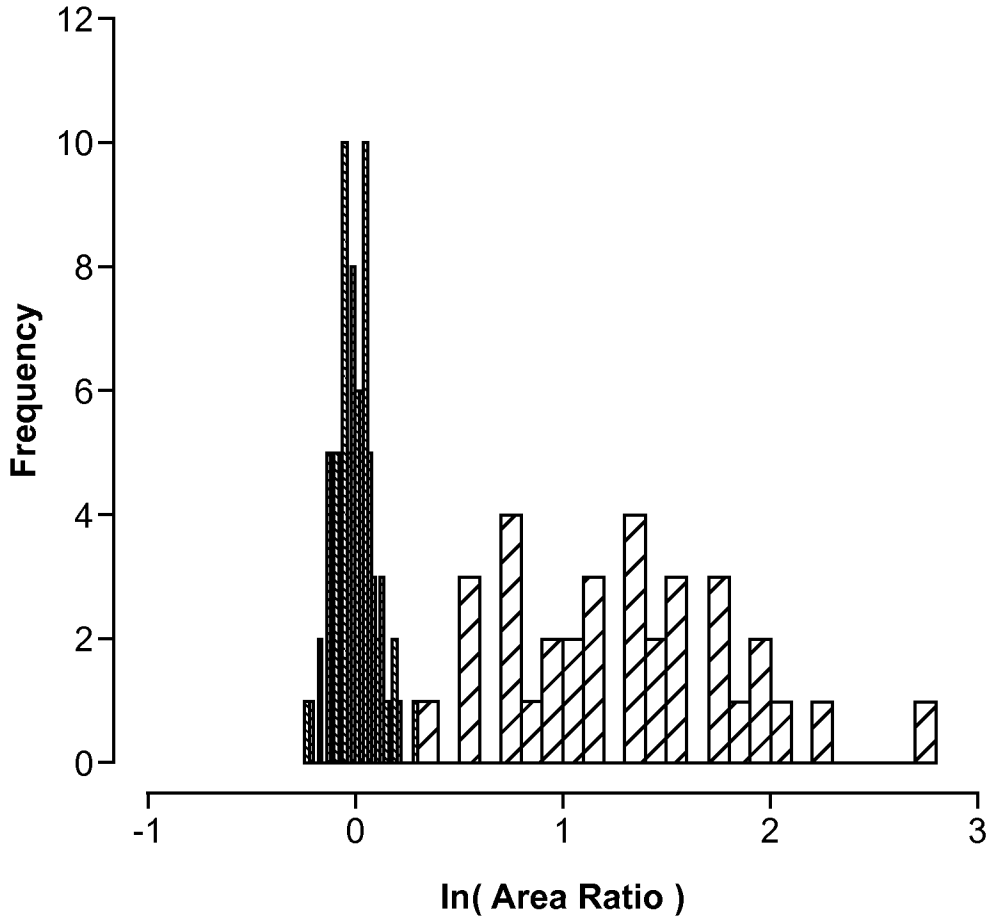


FIG. 28

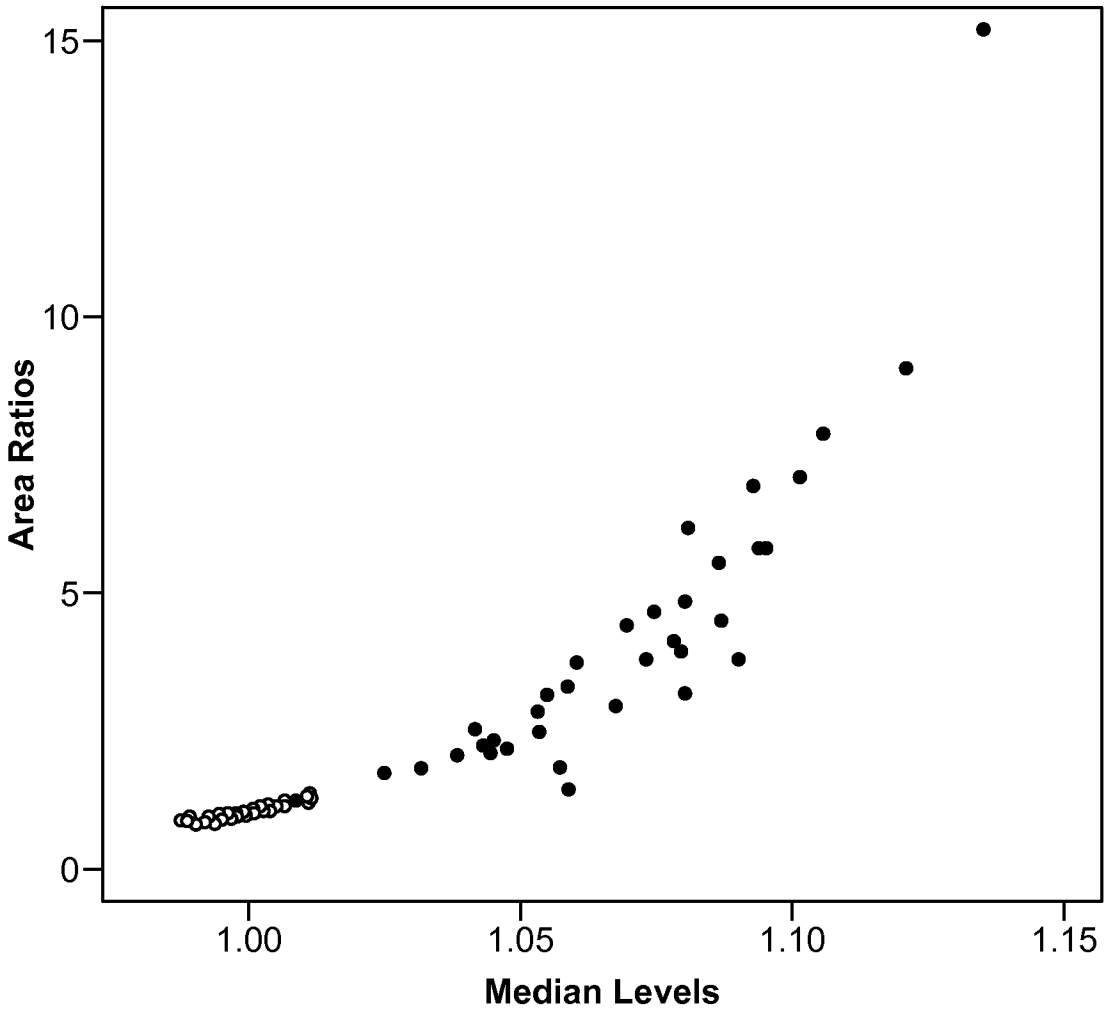


FIG. 29

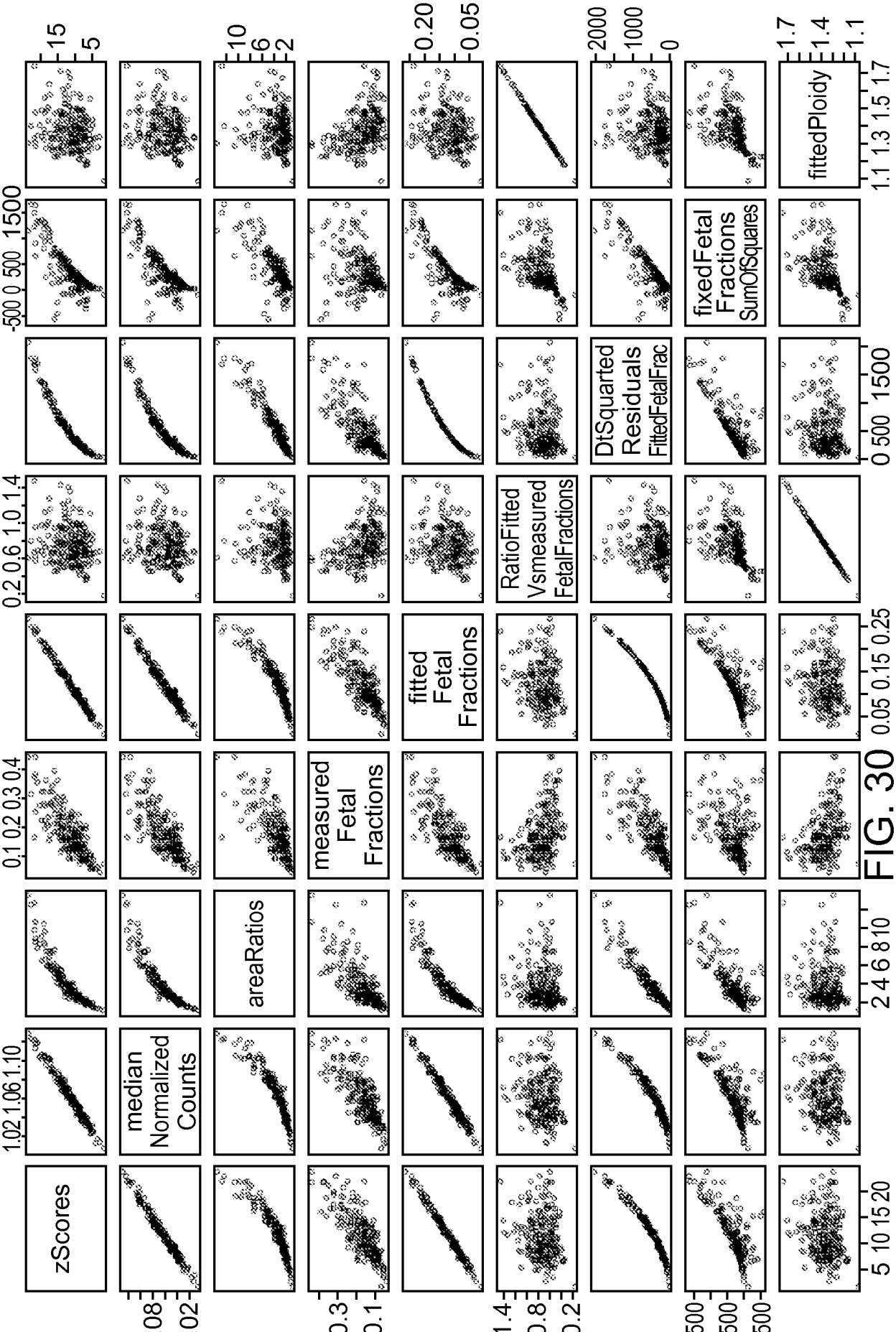

zScores
median Normalized Counts
areaRatios
measured Fetal Fractions
fitted Fetal Fractions
RatioFitted Vsmeasured FetalFractions
DtSquarted Residuals FittedFetalFrac
fixedFetal Fractions SumOfSquares
fittedPloidy

FIG. 30

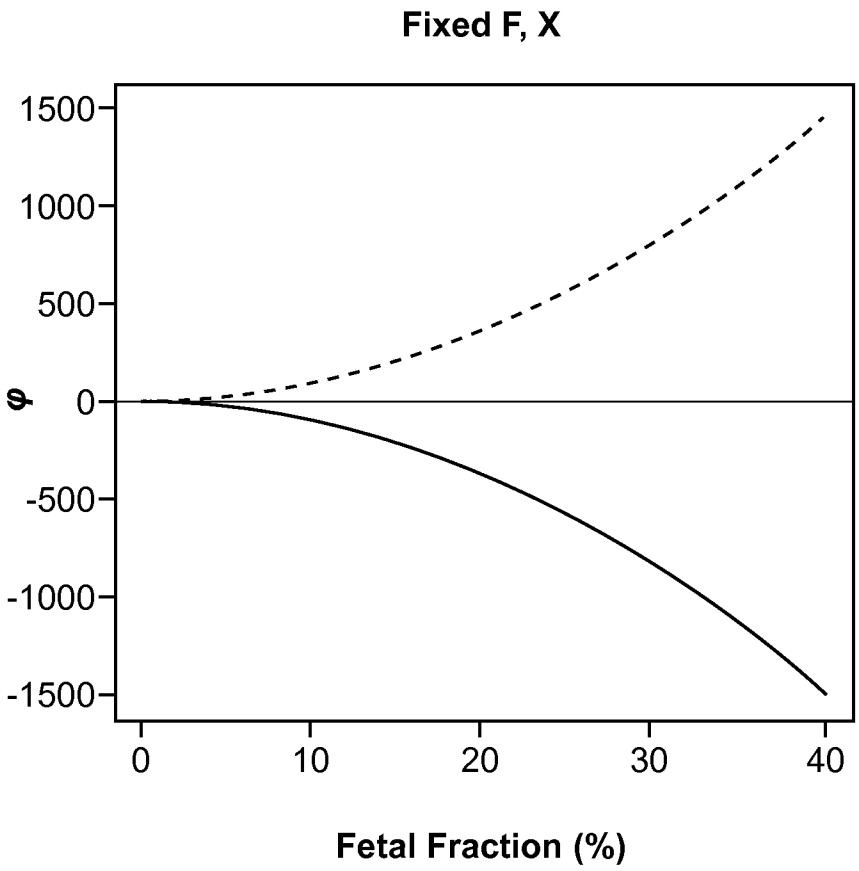

Fixed F, X
1500
1000
500
0
-500
-1000
-1500
S
0
10
20
30
40
Fetal Fraction (%)

FIG. 31

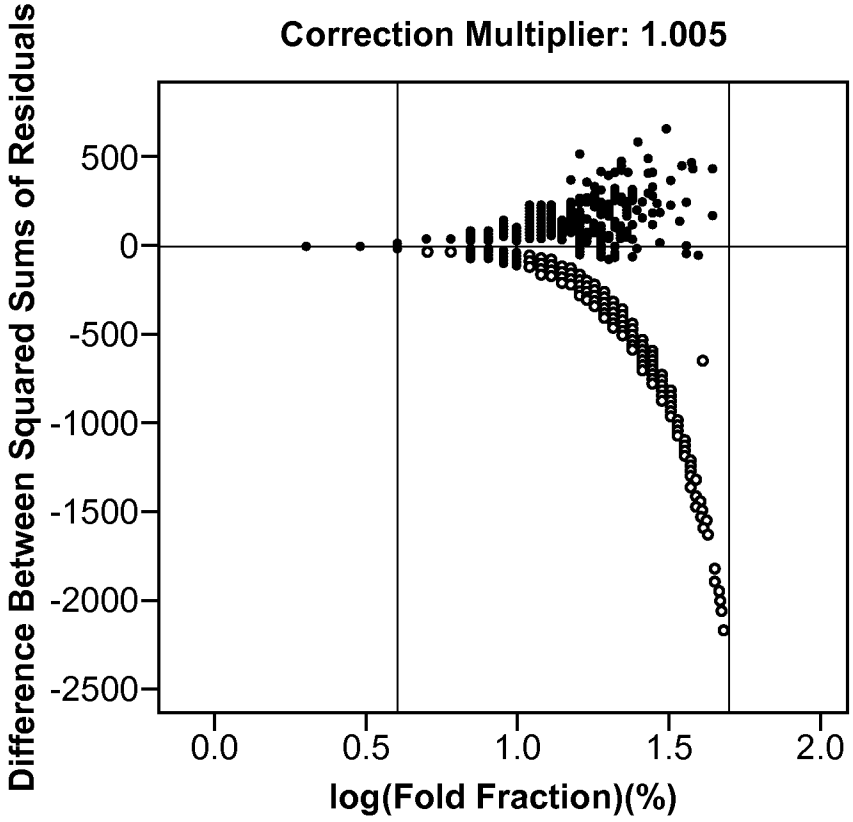

Correction Multiplier: 1.005
Difference Between Squared Sums of Residuals
500
0
-500
-1000
-1500
-2000
-2500
0.0
0.5
1.0
1.5
2.0
log(Fold Fraction)(%)

FIG. 32

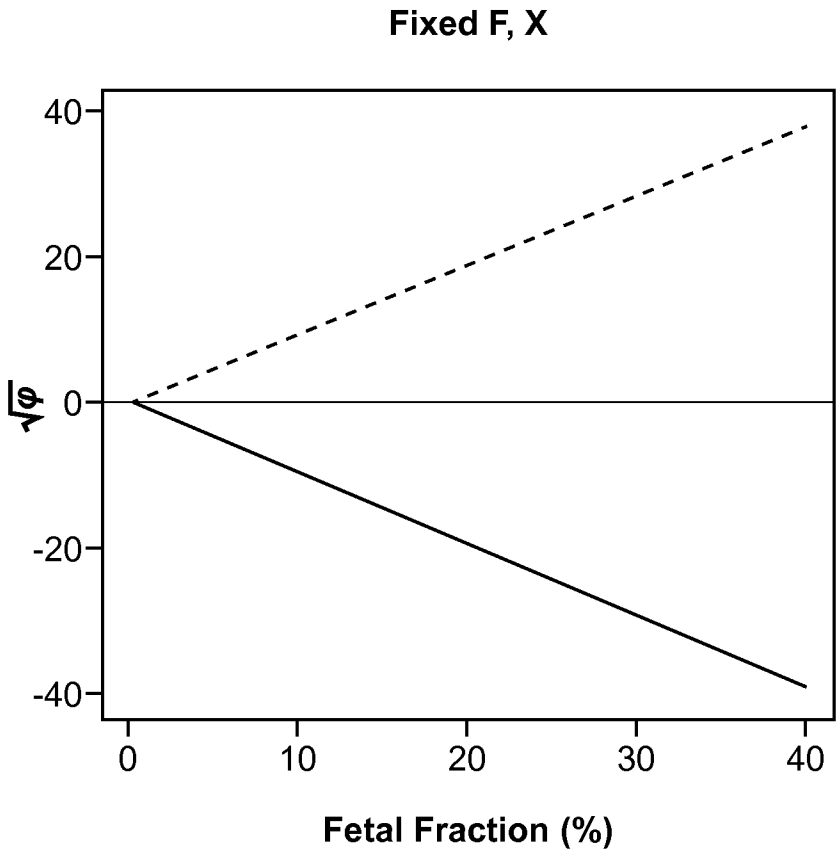


FIG. 33

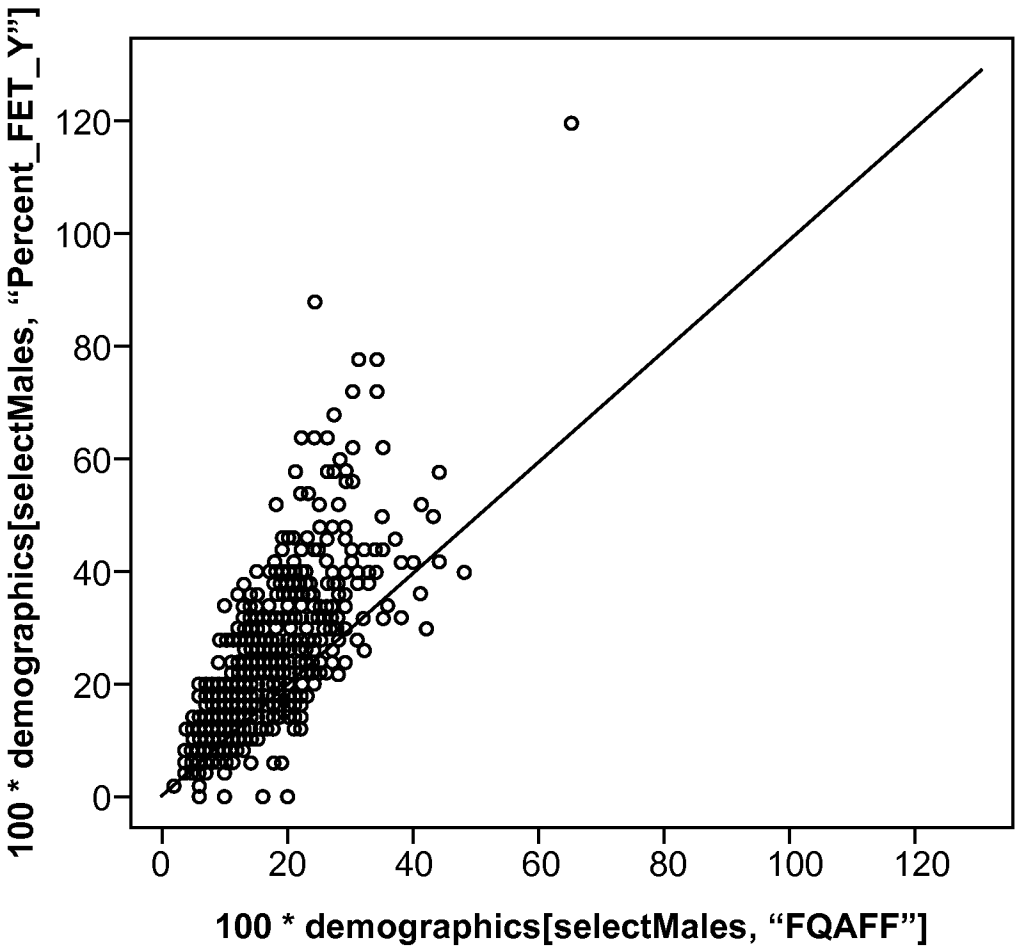

100 * demographics[selectMales, "Percent_FET_Y"]
120
100
80
60
40
20
0
0
20
40
60
80
100
120
100 * demographics[selectMales, "FQAFF"]

FIG. 34

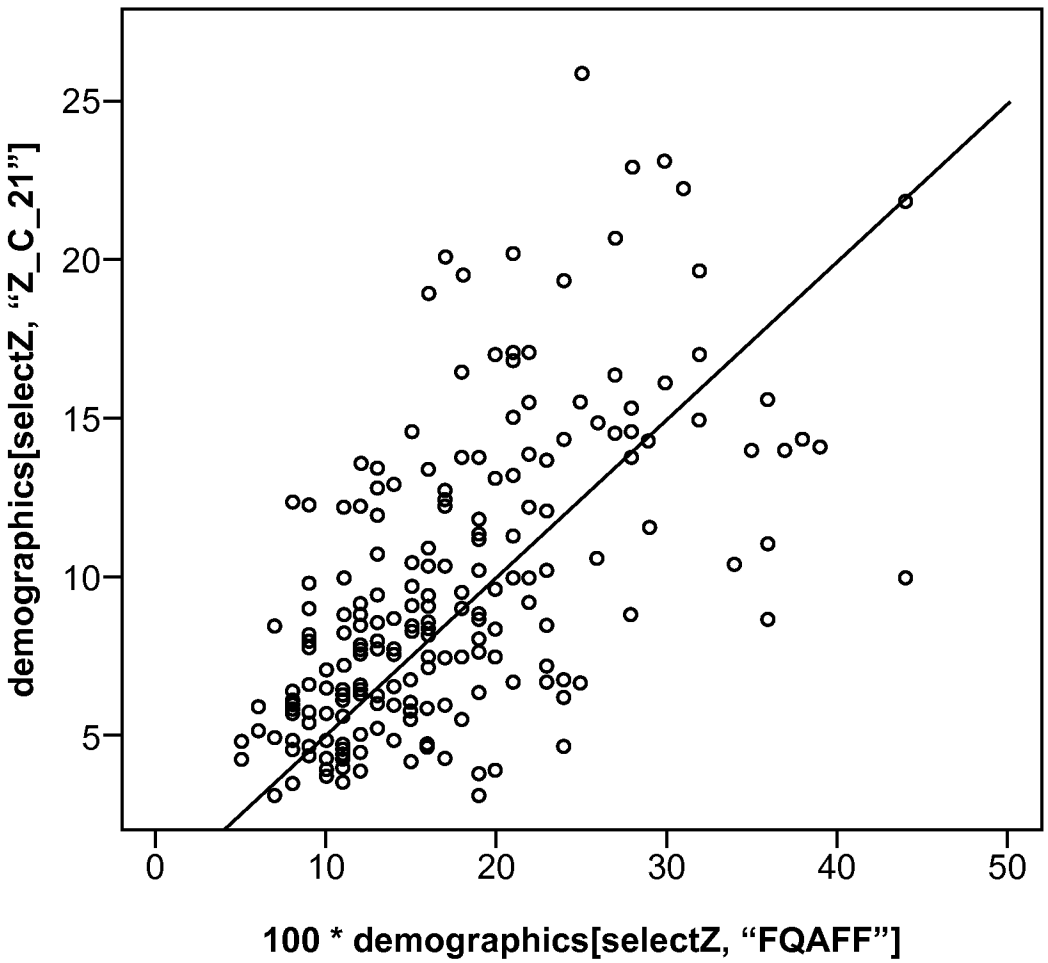

demographics[selectZ, "Z_C_21"]
25
20
15
10
5
0
10
20
30
40
50
100 * demographics[selectZ, "FQAFF"]

FIG. 35

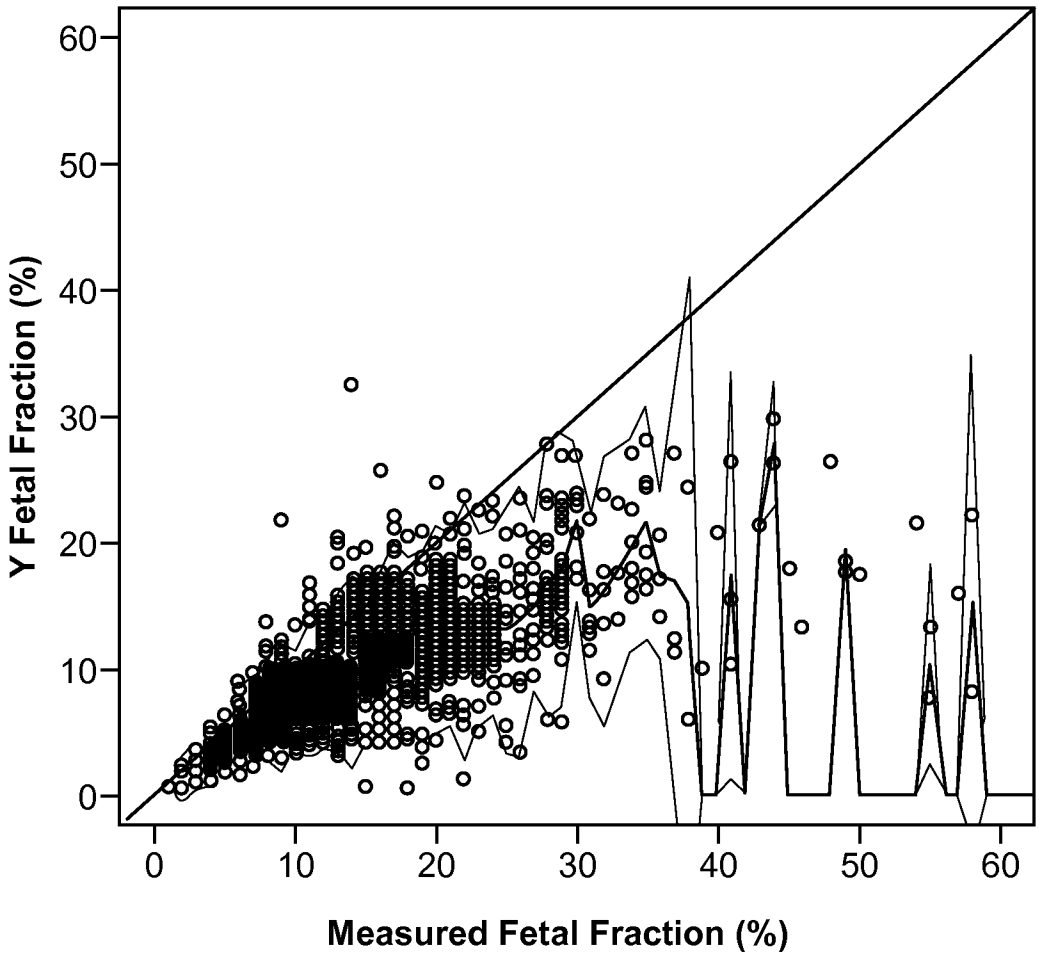
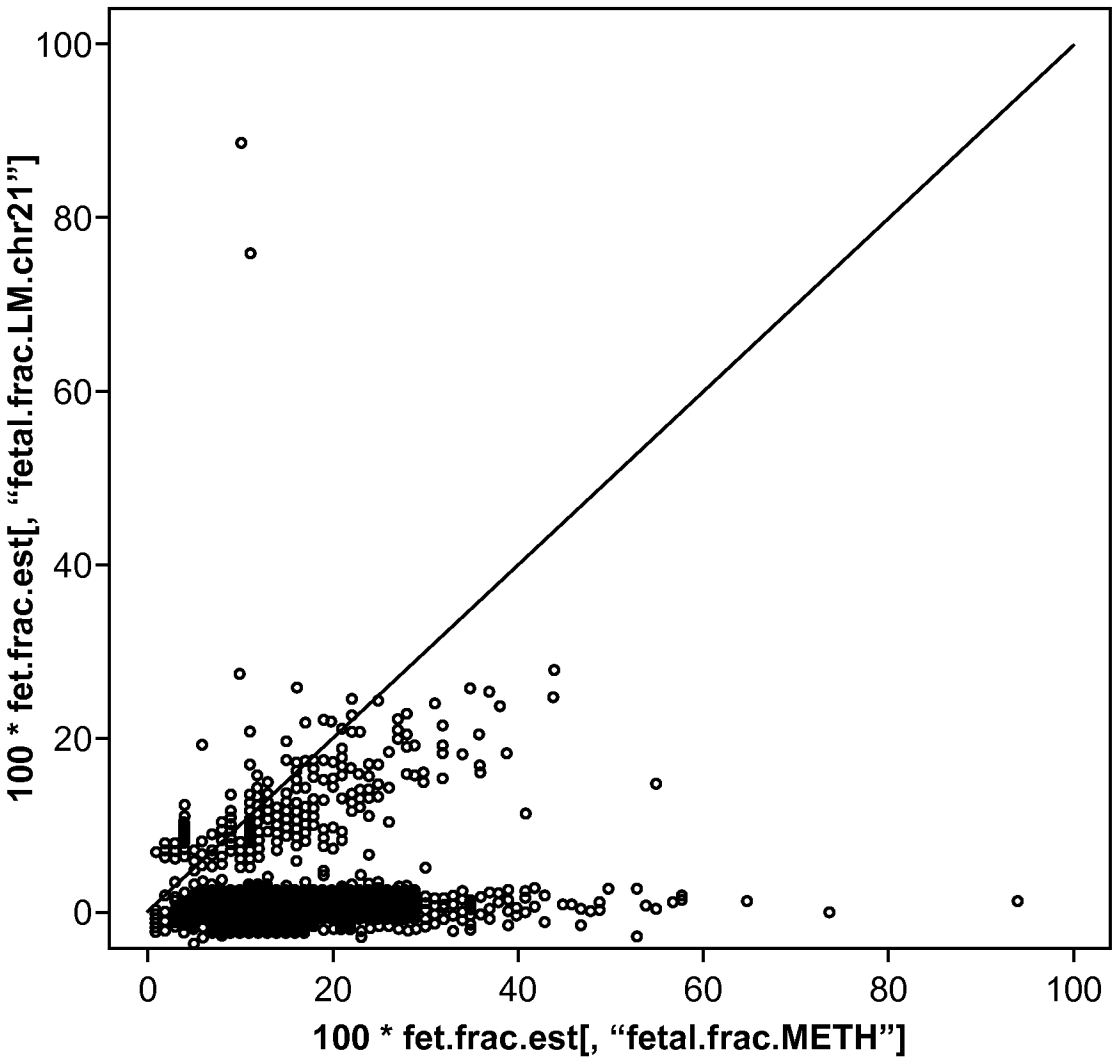


FIG. 36



Fetal fraction estimates based on
Chr 21 vs. measured fetal fractions.

FIG. 37

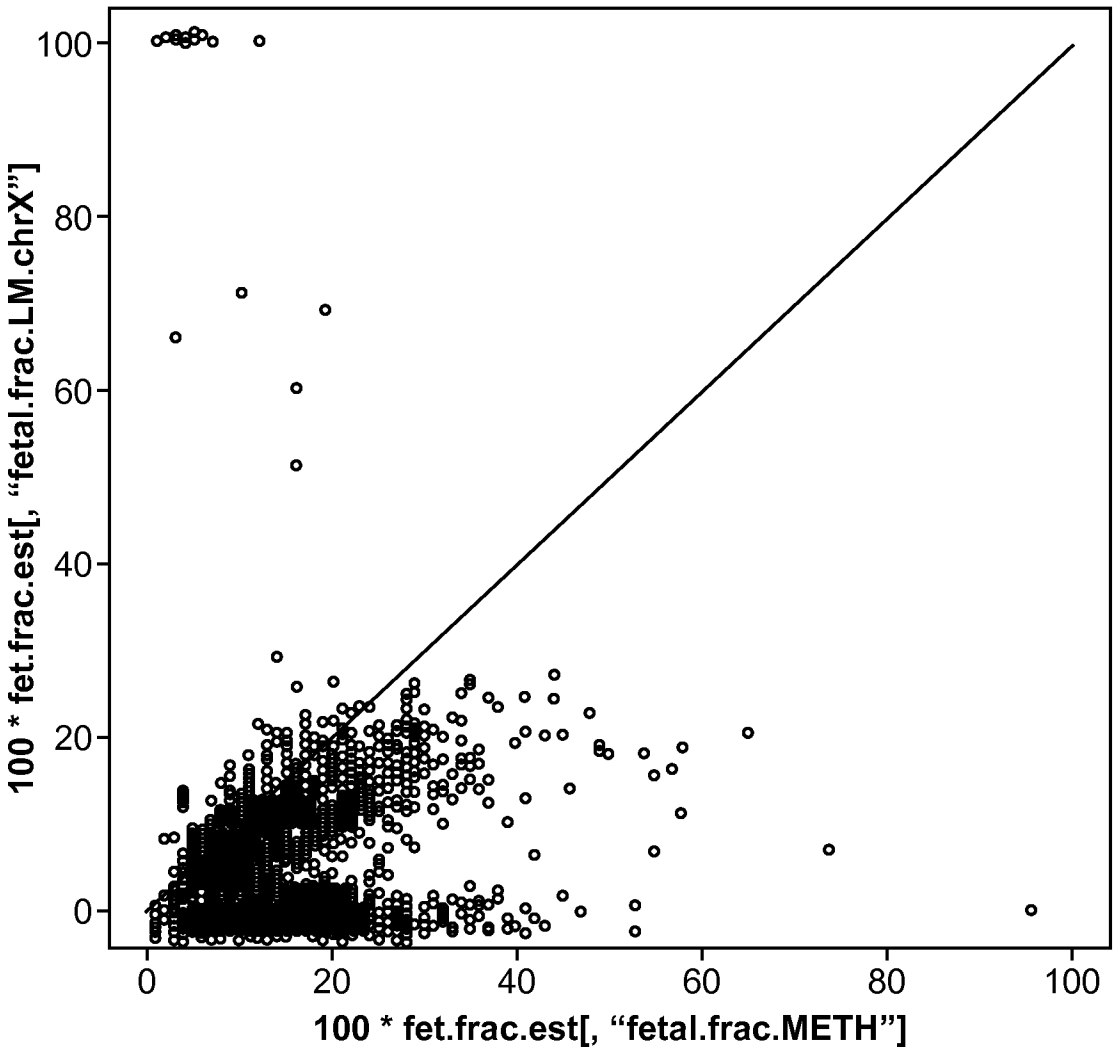

100
80
60
40
20
0
100 * fet.frac.est[, "fetal.frac.LM.chrX"]
0
20
40
60
80
100
100 * fet.frac.est[, "fetal.frac.METH"]

FIG. 38

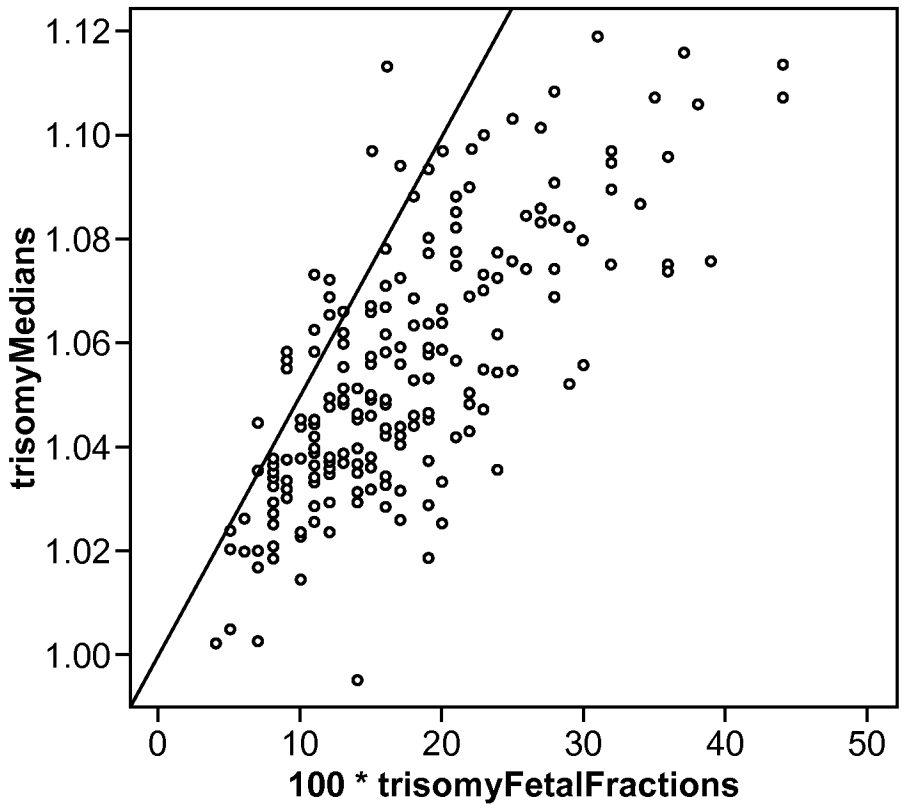

1.12
1.10
1.08
1.06
1.04
1.02
1.00
trisomyMedians
0
10
20
30
40
50
100 * trisomyFetalFractions

FIG. 39

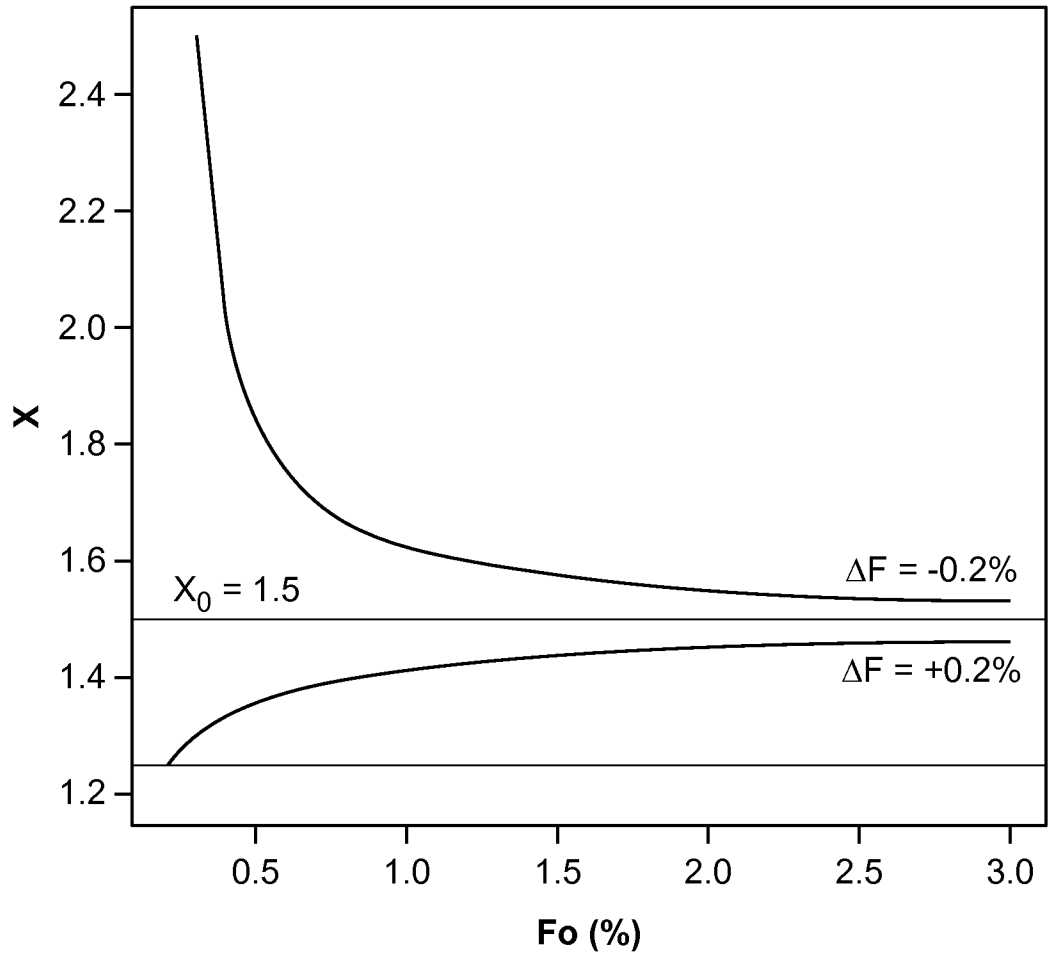


FIG. 40

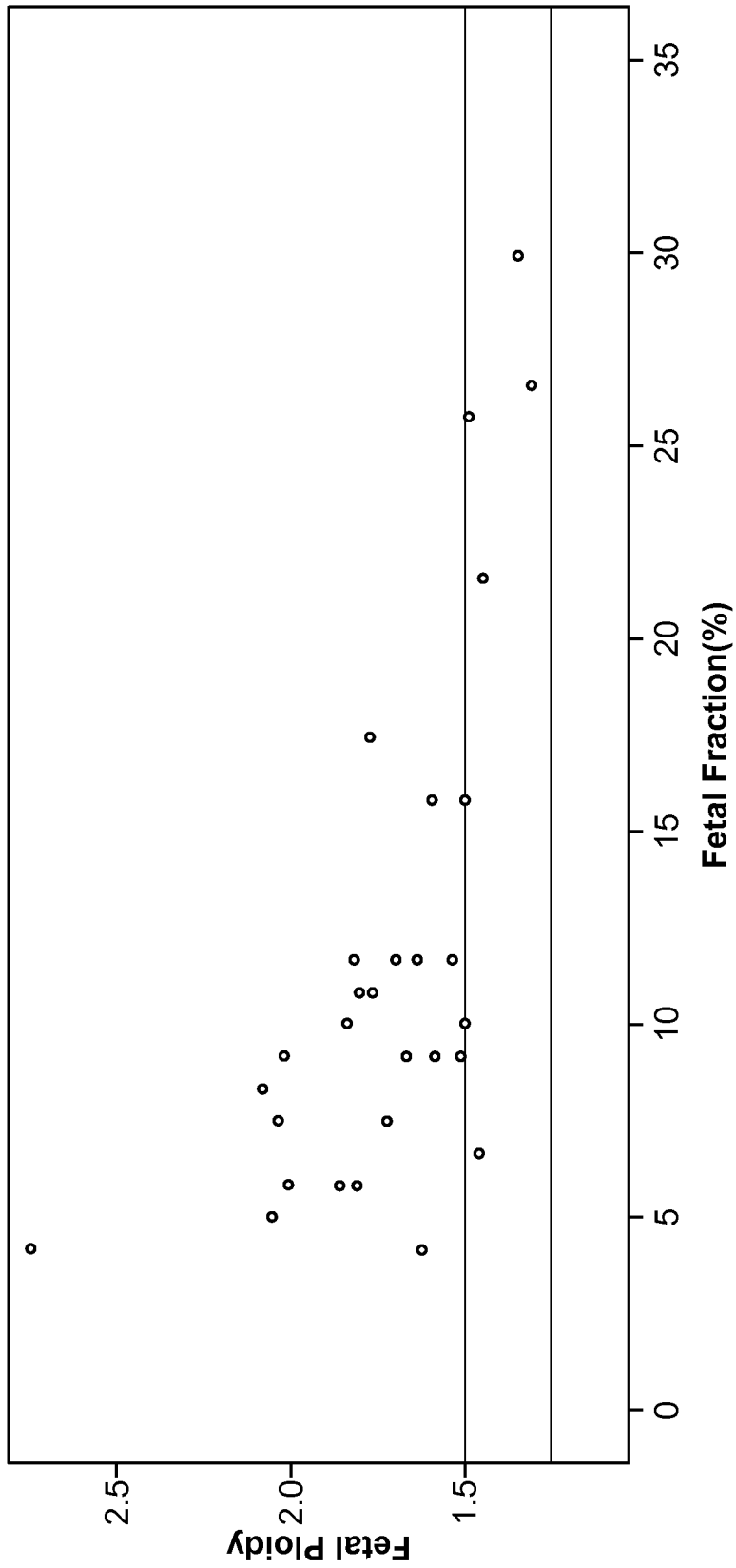


FIG. 41

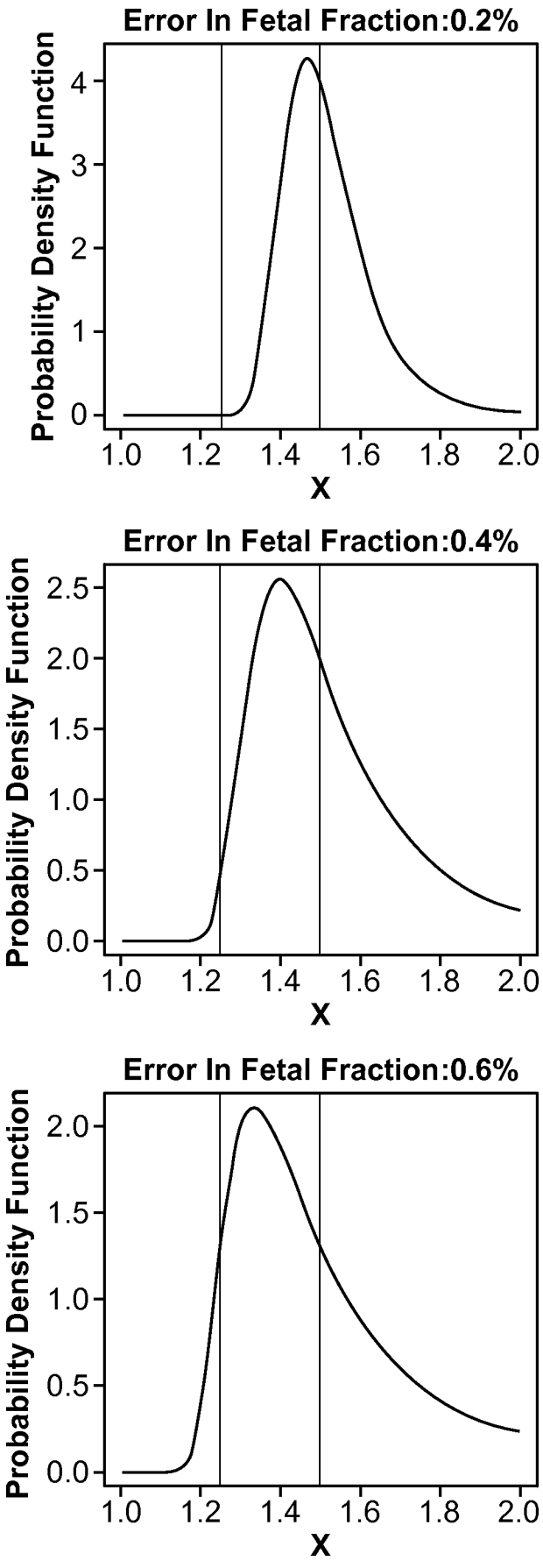

Error In Fetal Fraction:0.2%
Probability Density Function
0
1
2
3
4
1.0
1.2
1.4
1.6
1.8
2.0
X
Error In Fetal Fraction:0.4%
Probability Density Function
0.0
0.5
1.0
1.5
2.0
2.5
1.0
1.2
1.4
1.6
1.8
2.0
X
Error In Fetal Fraction:0.6%
Probability Density Function
0.0
0.5
1.0
1.5
2.0
1.0
1.2
1.4
1.6
1.8
2.0
X

FIG. 42

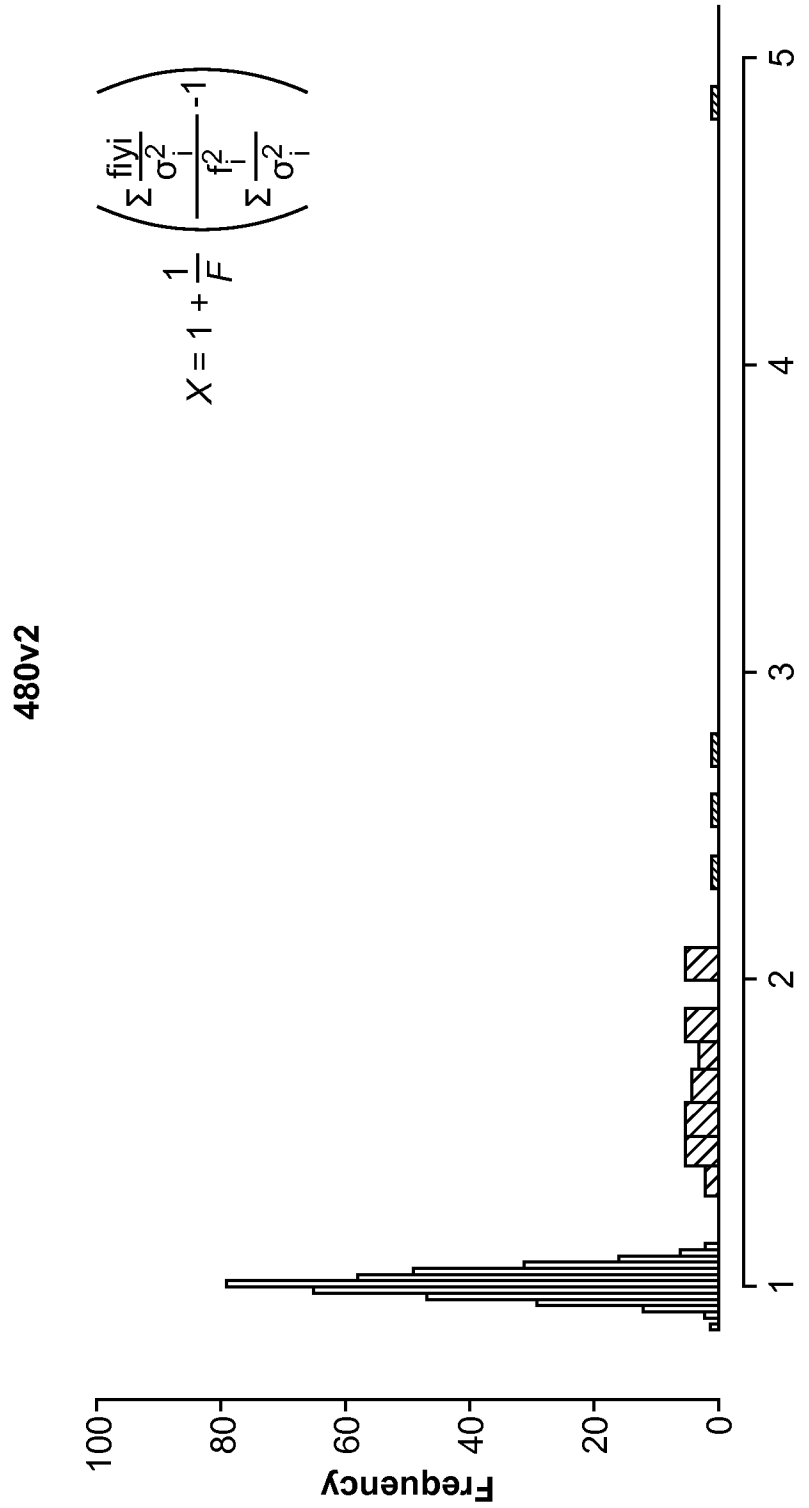

480v2
$X = 1 + \frac{1}{F}\left(\frac{\Sigma \frac{fiyi}{\sigma_i^2}}{\Sigma \frac{f_i^2}{\sigma_i^2}} - 1\right)$
Frequency
100
80
60
40
20
0
1
2
3
4
5
Ploidy

FIG. 43

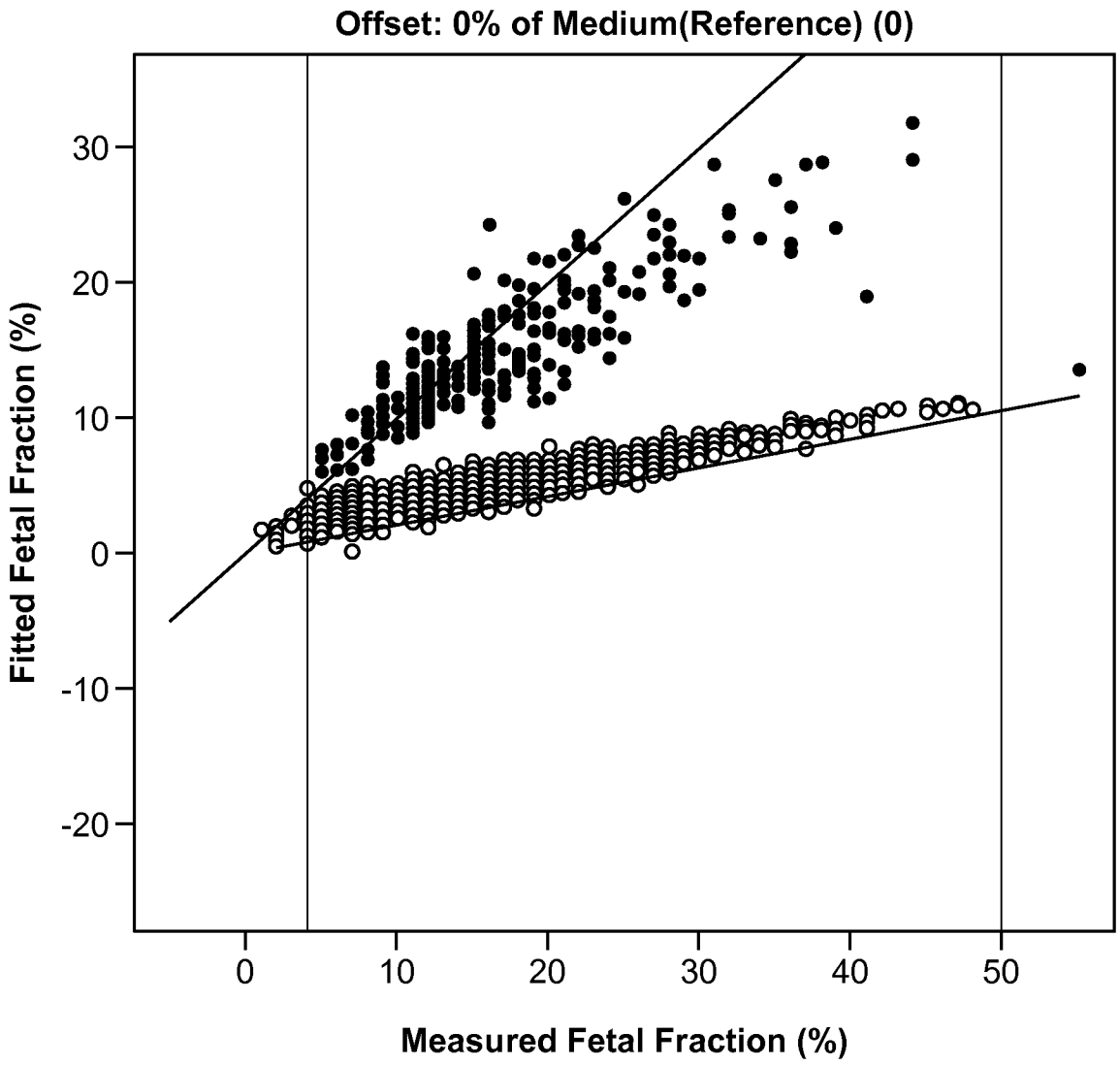

Offset: 0% of Medium(Reference) (0)
Fitted Fetal Fraction (%)
30
20
10
0
-10
-20
0
10
20
30
40
50
Measured Fetal Fraction (%)

FIG. 44

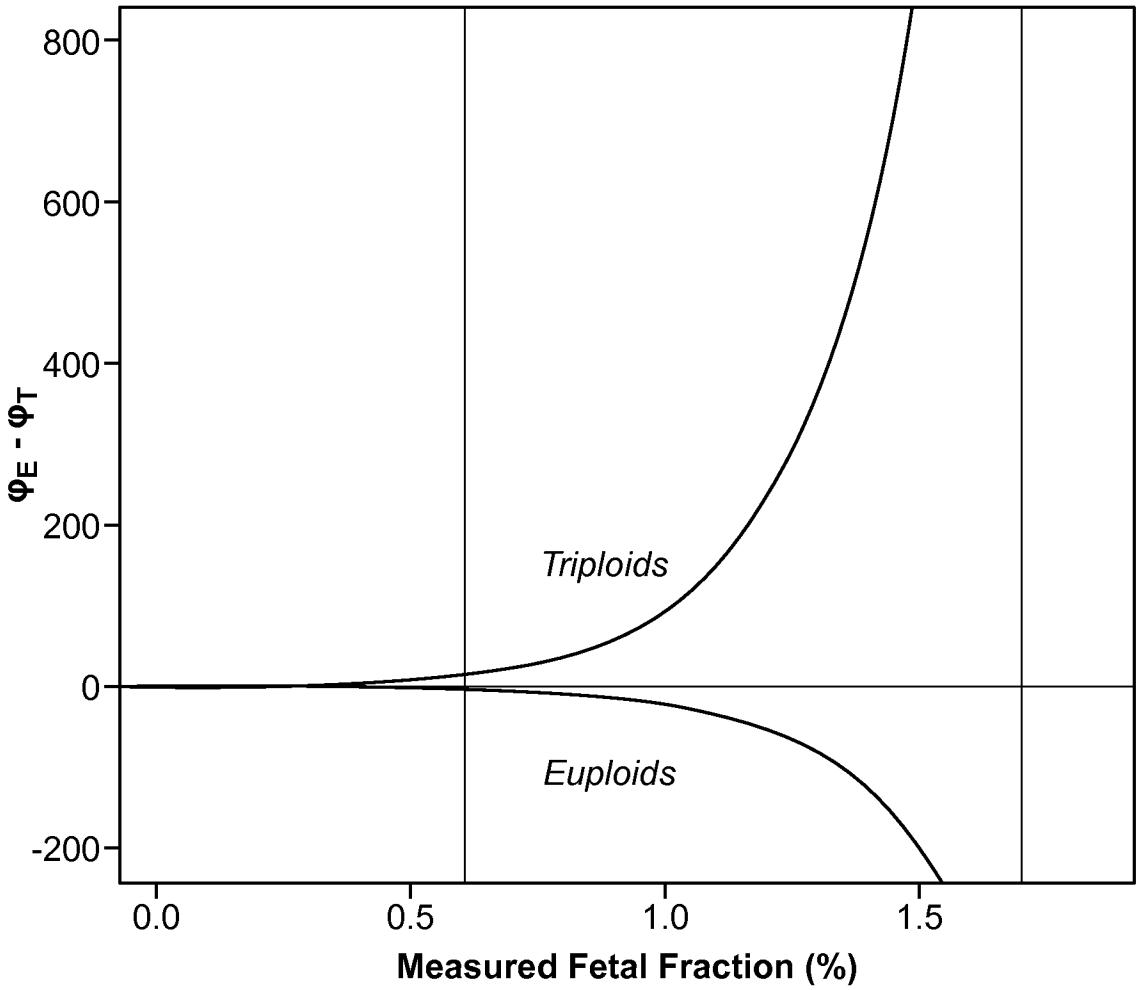

800
600
400
200
0
-200
$\varphi_E - \varphi_T$
Triploids
Euploids
0.0
0.5
1.0
1.5
Measured Fetal Fraction (%)

FIG. 45

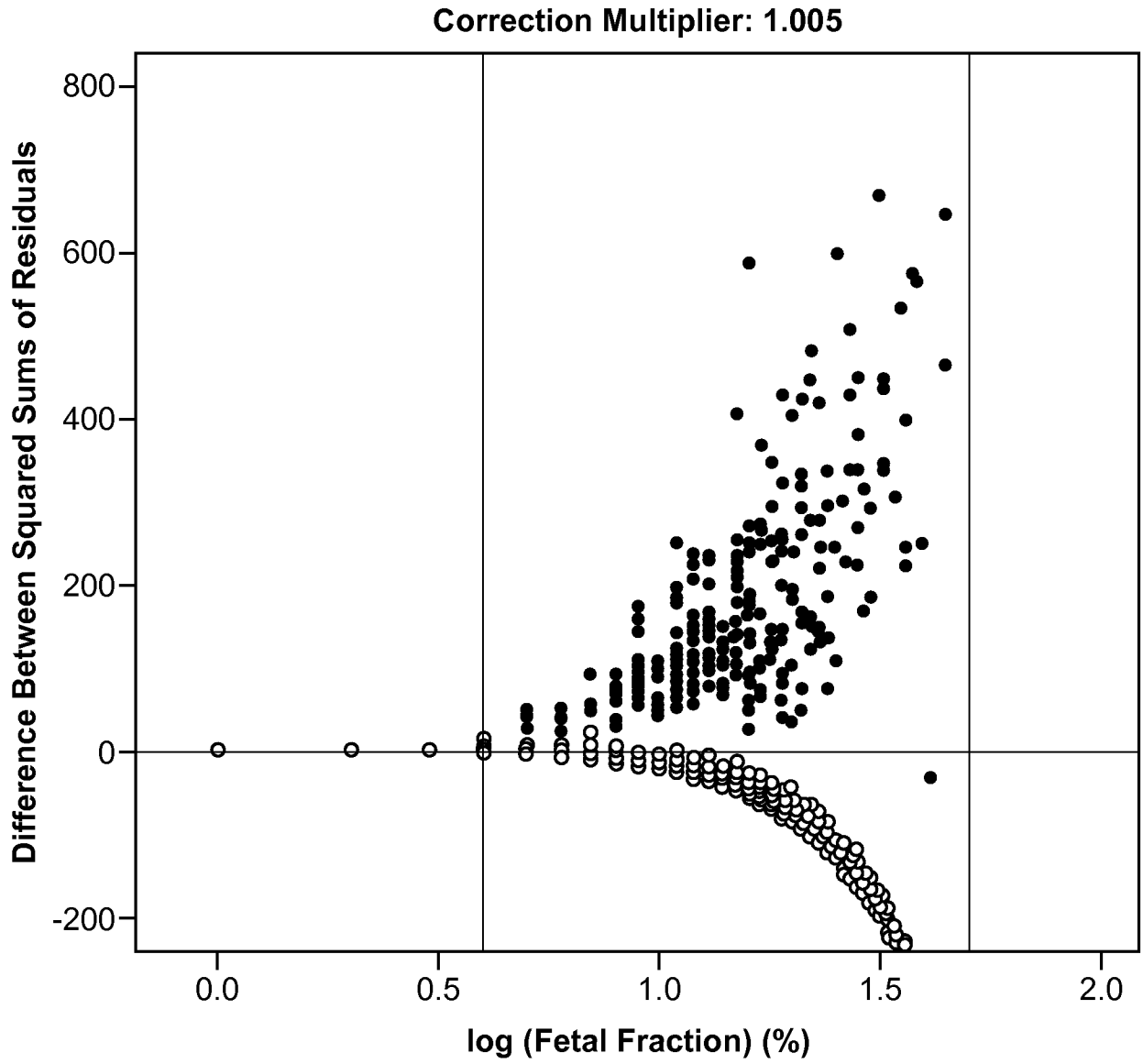

Correction Multiplier: 1.005
Difference Between Squared Sums of Residuals
800
600
400
200
0
-200
0.0
0.5
1.0
1.5
2.0
log (Fetal Fraction) (%)

FIG. 46

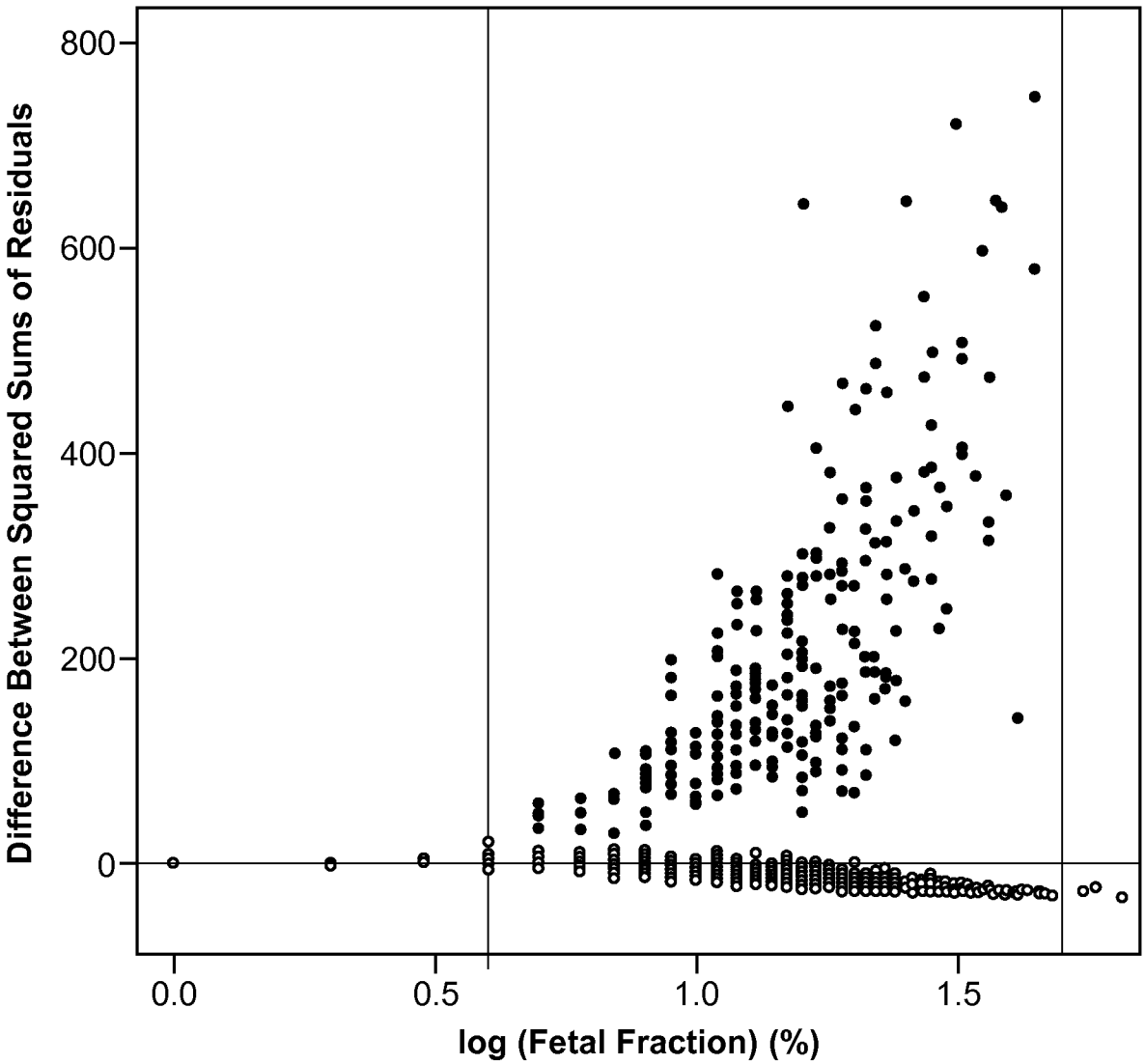

800
600
400
200
0
Difference Between Squared Sums of Residuals
0.0
0.5
1.0
1.5
log (Fetal Fraction) (%)

FIG. 47

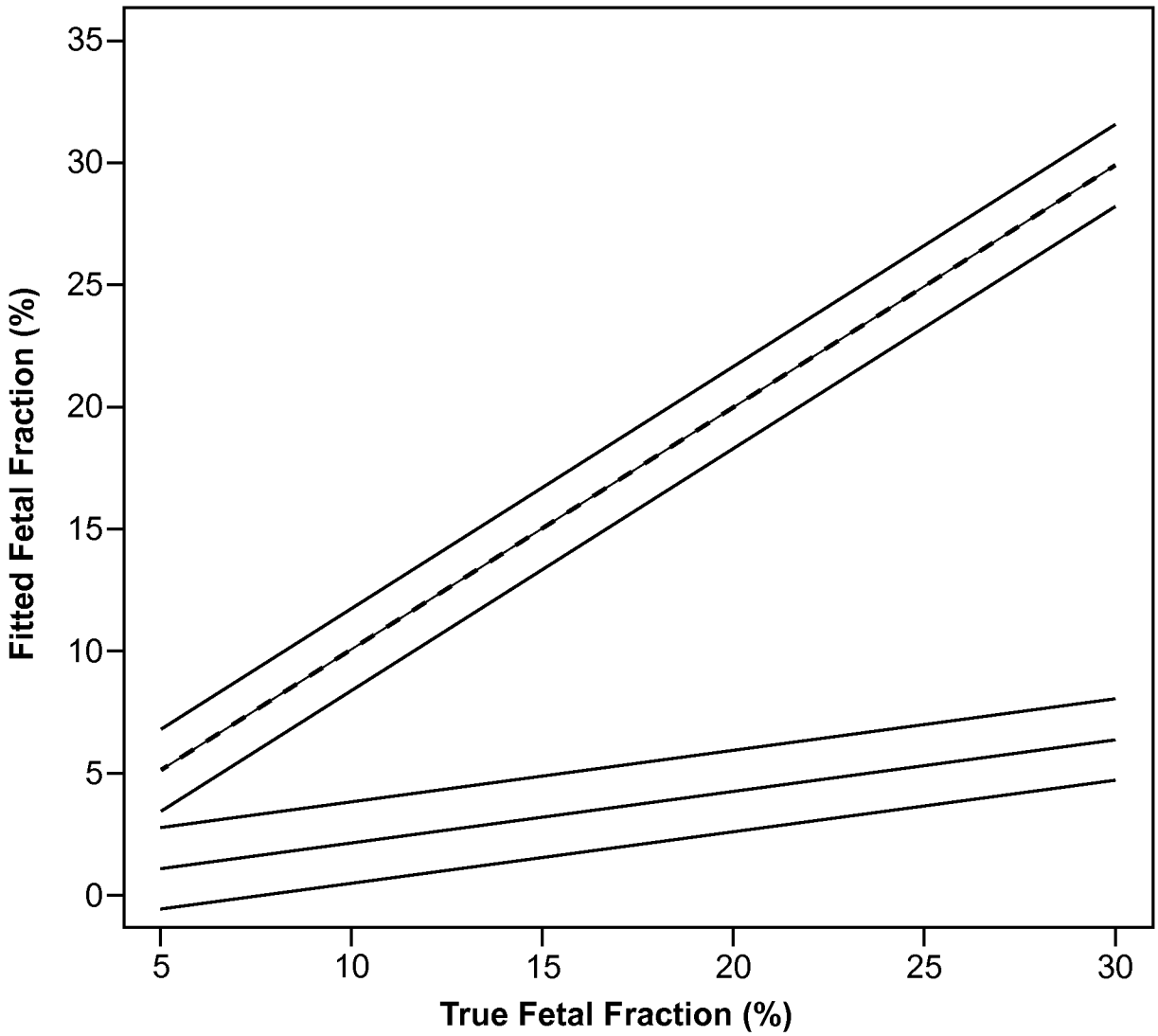

35
30
25
20
15
10
5
0
Fitted Fetal Fraction (%)
5
10
15
20
25
30
True Fetal Fraction (%)

FIG. 48

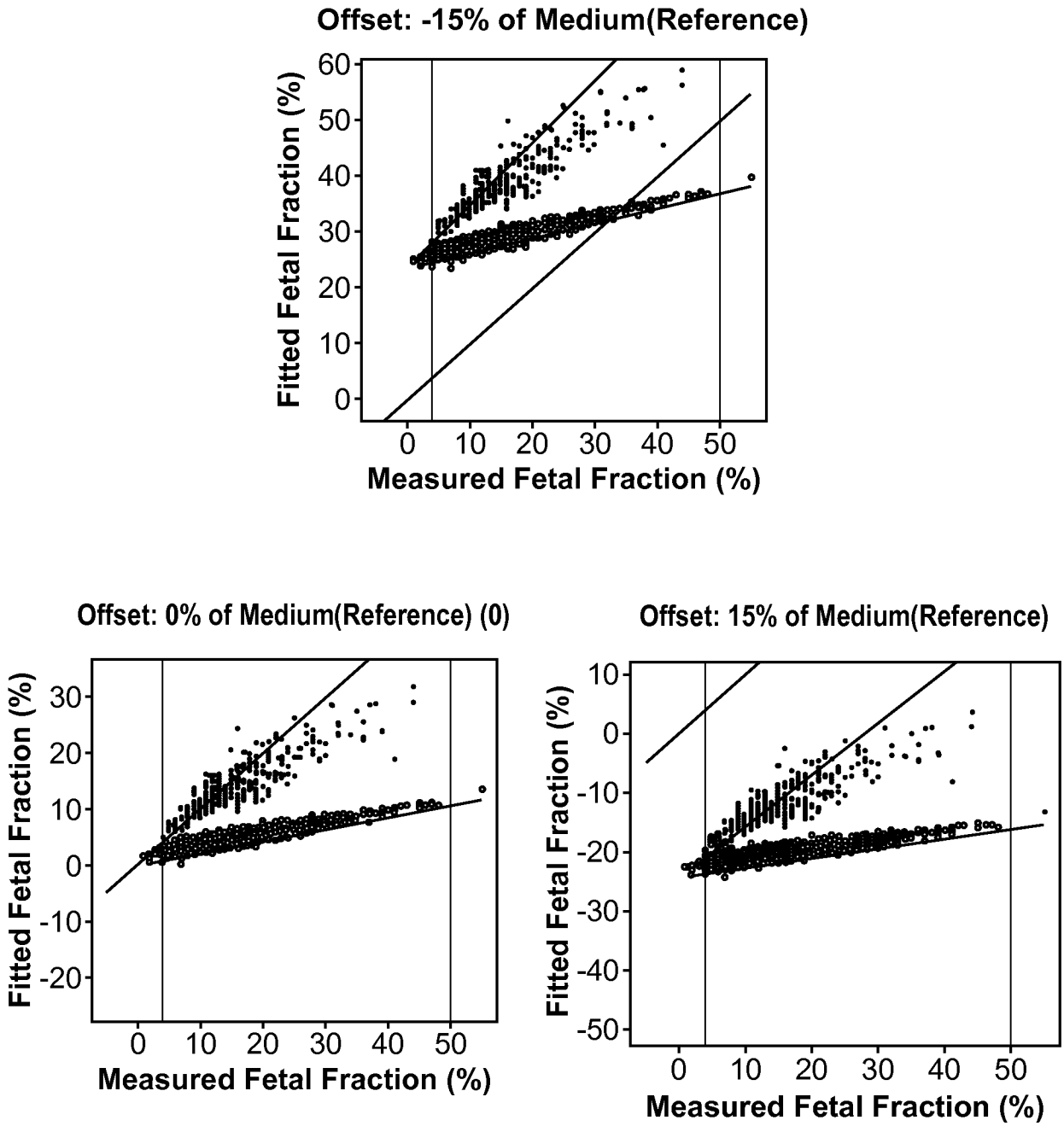

Offset: -15% of Medium(Reference)
Fitted Fetal Fraction (%)
Measured Fetal Fraction (%)
Offset: 0% of Medium(Reference) (0)
Fitted Fetal Fraction (%)
Measured Fetal Fraction (%)
Offset: 15% of Medium(Reference)
Fitted Fetal Fraction (%)
Measured Fetal Fraction (%)

FIG. 49

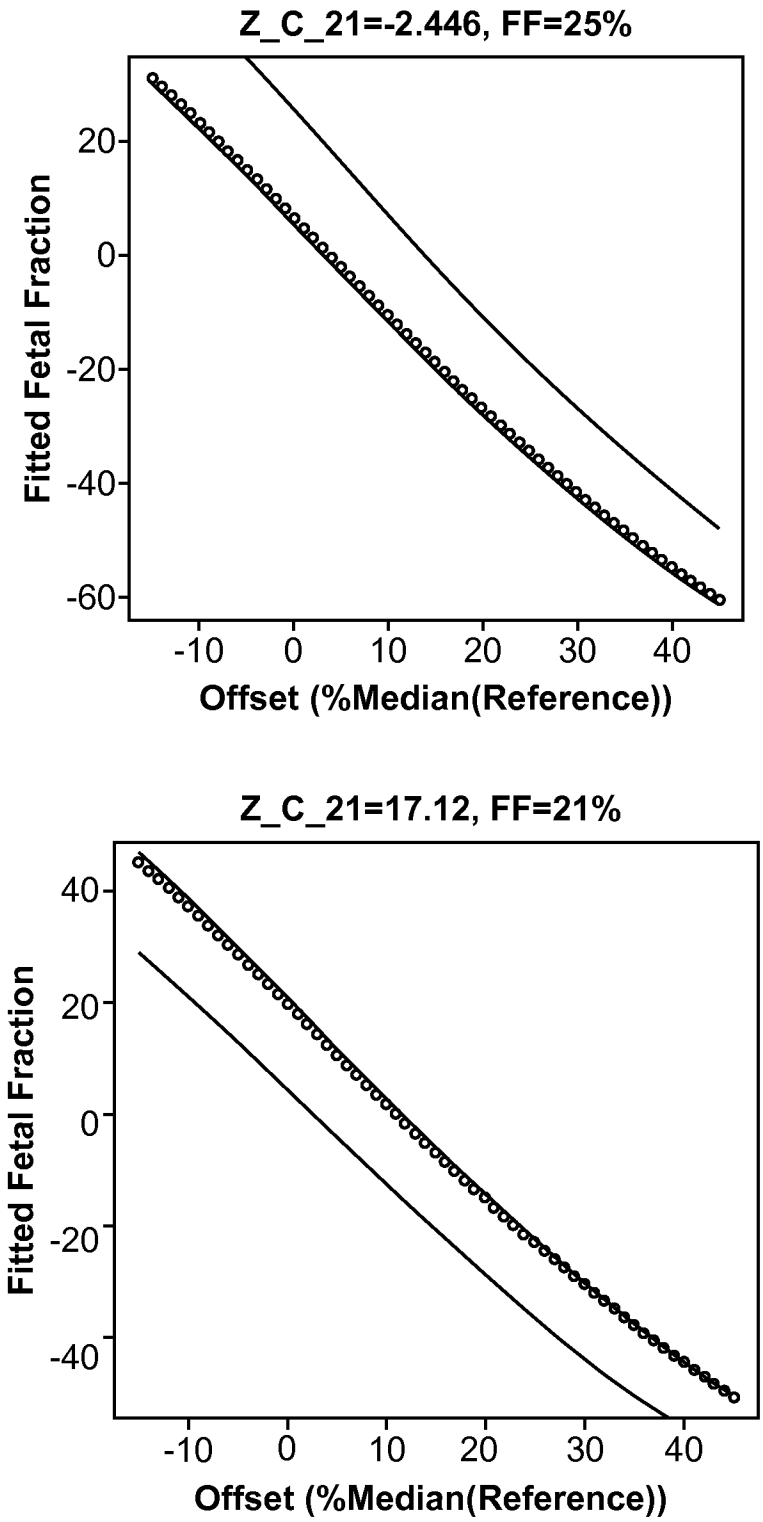

Z_C_21=-2.446, FF=25%
Fitted Fetal Fraction
20
0
-20
-40
-60
-10
0
10
20
30
40
Offset (%Median(Reference))
Z_C_21=17.12, FF=21%
Fitted Fetal Fraction
40
20
0
-20
-40
-10
0
10
20
30
40
Offset (%Median(Reference))

FIG. 50

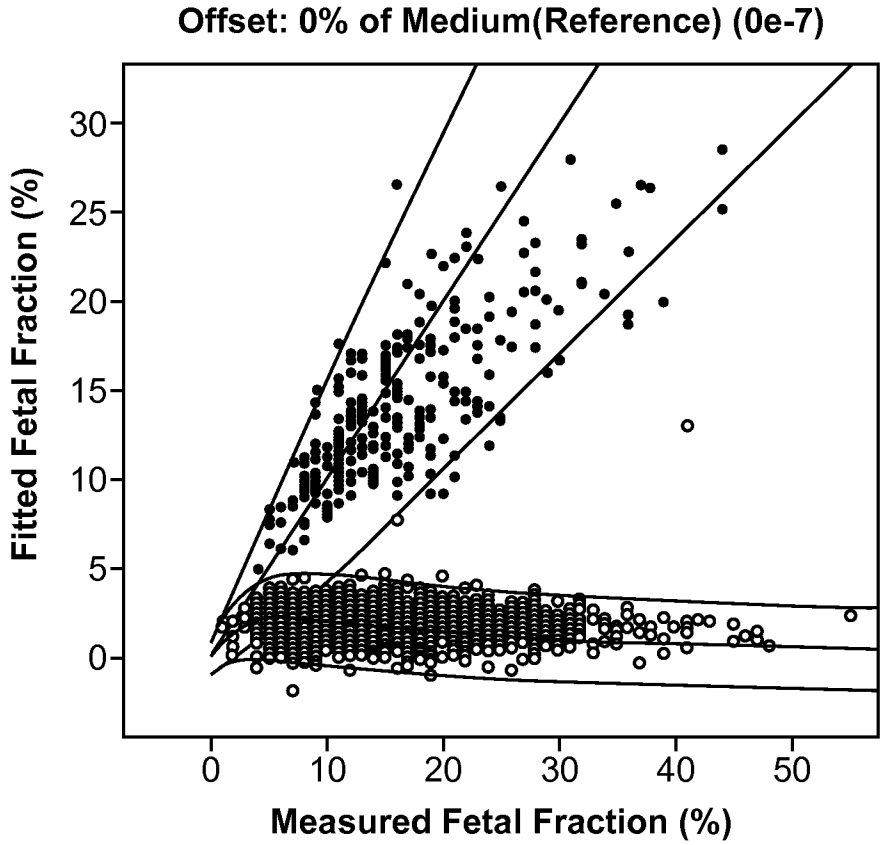

Offset: 0% of Medium(Reference) (0e-7)
Fitted Fetal Fraction (%)
30
25
20
15
10
5
0
0
10
20
30
40
50
Measured Fetal Fraction (%)

FIG. 51

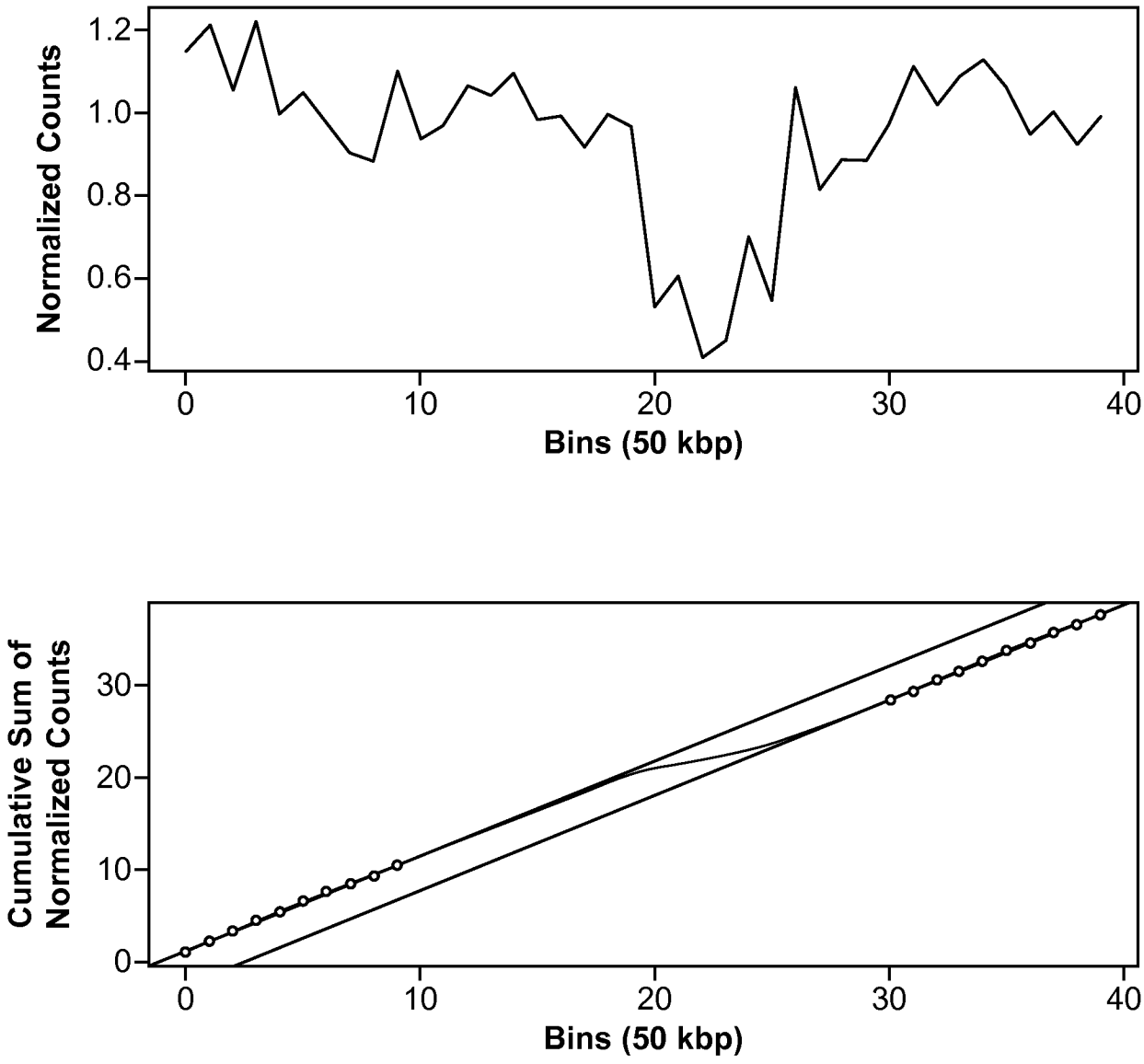

Normalized Counts
1.2
1.0
0.8
0.6
0.4
0
10
20
30
40
Bins (50 kbp)
Cumulative Sum of Normalized Counts
30
20
10
0
0
10
20
30
40
Bins (50 kbp)

FIG. 52

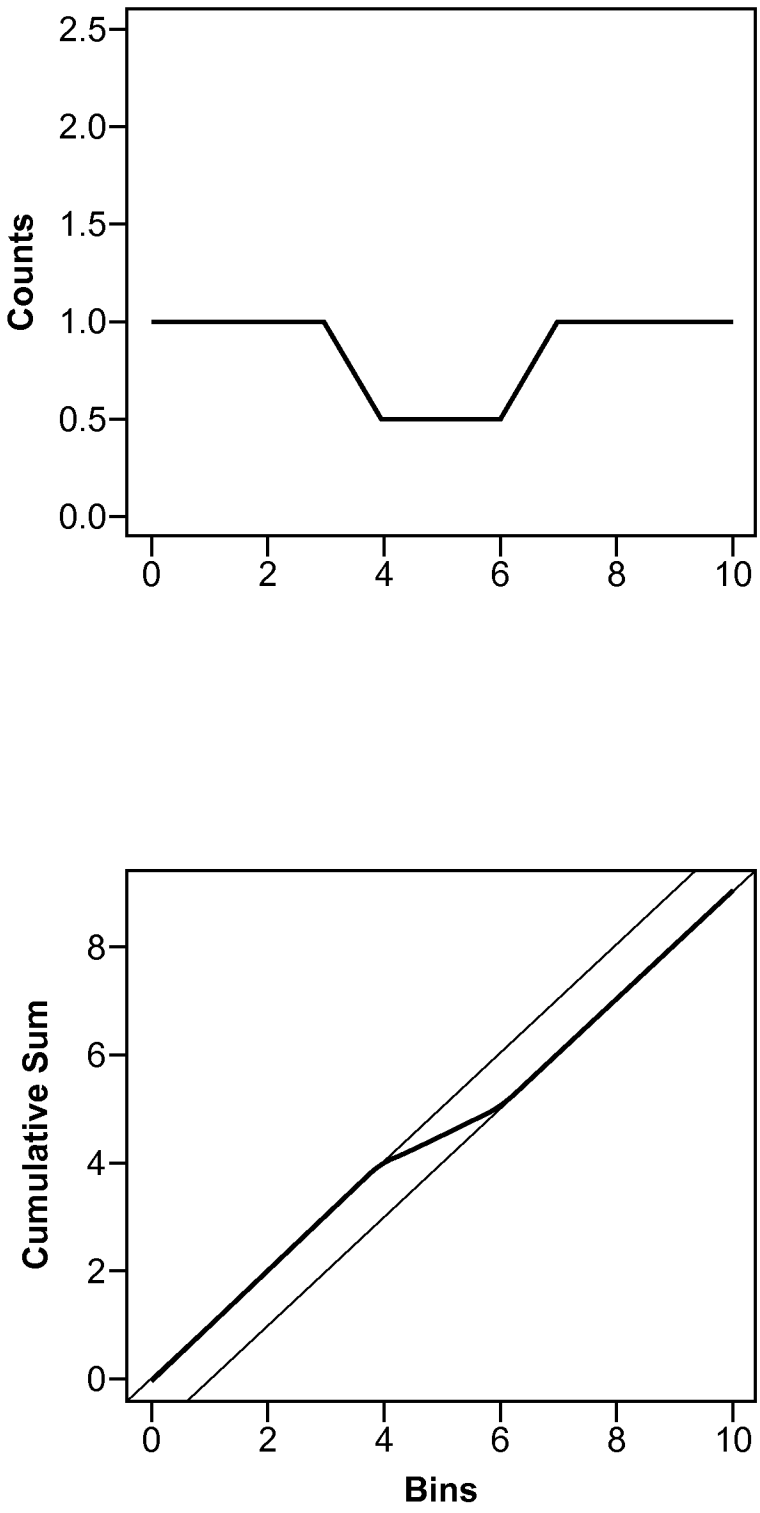

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
8
6
4
2
0
0
2
4
6
8
10
Bins

FIG. 53

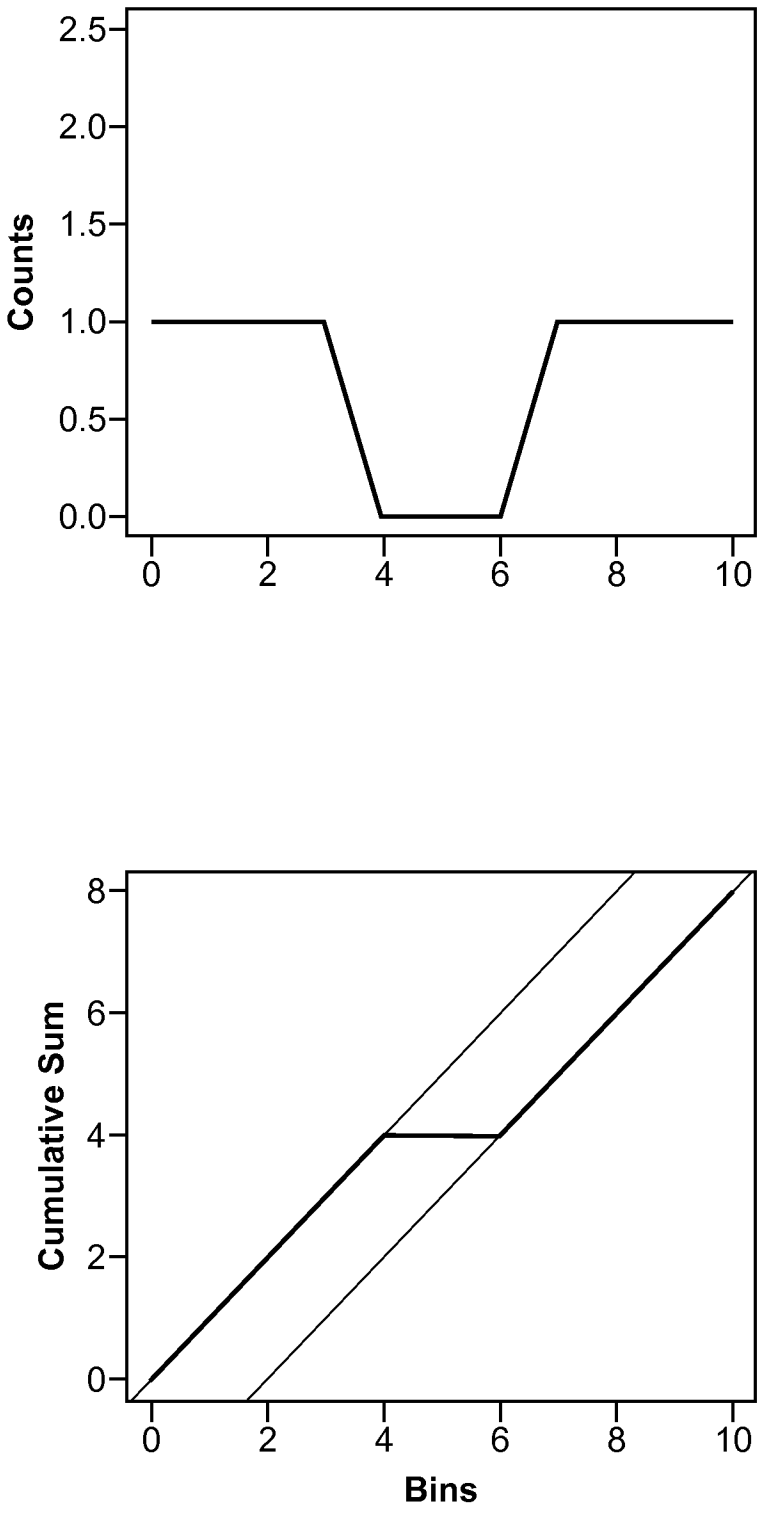

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
8
6
4
2
0
0
2
4
6
8
10
Bins

FIG. 54

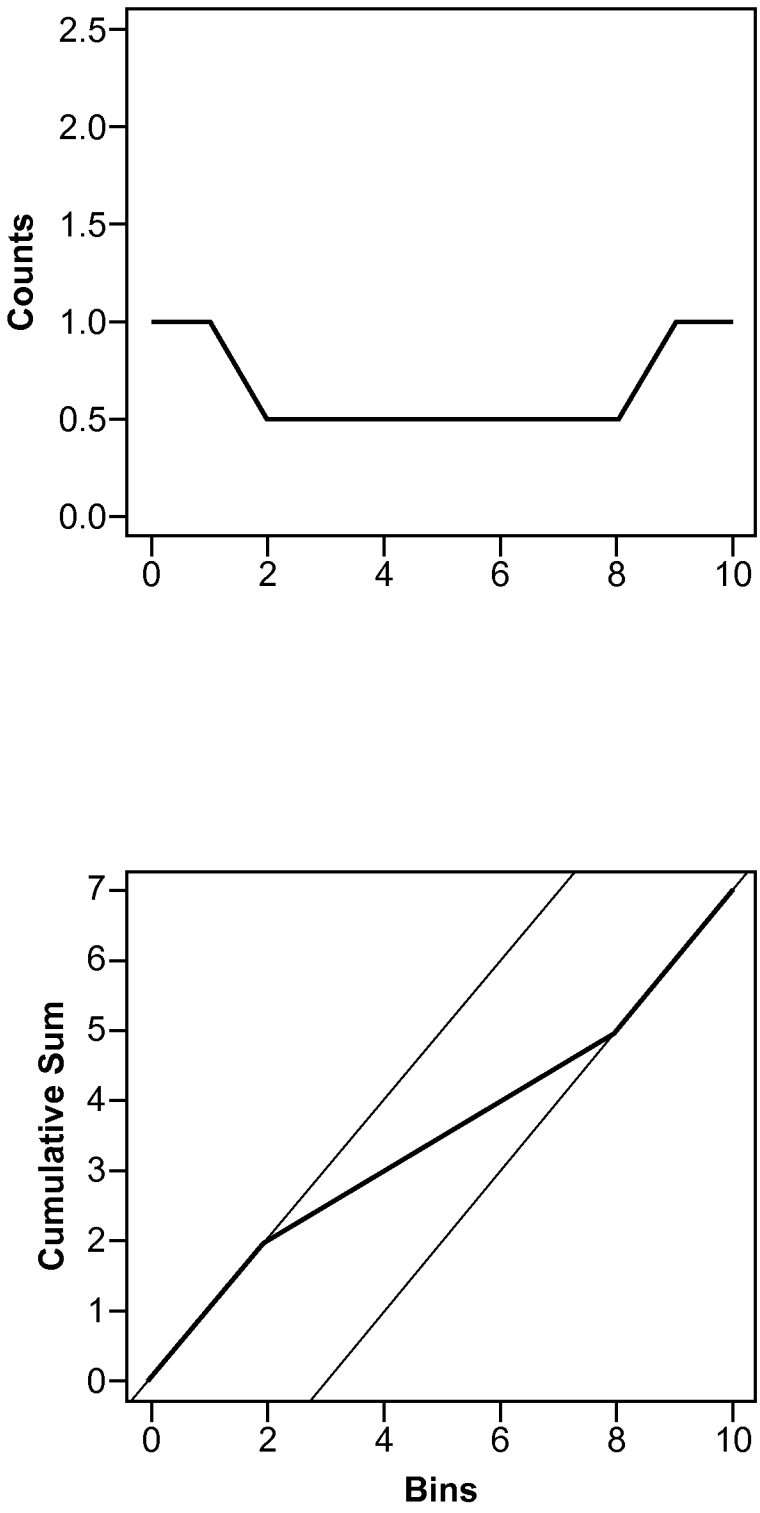

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
7
6
5
4
3
2
1
0
0
2
4
6
8
10
Bins

FIG. 55

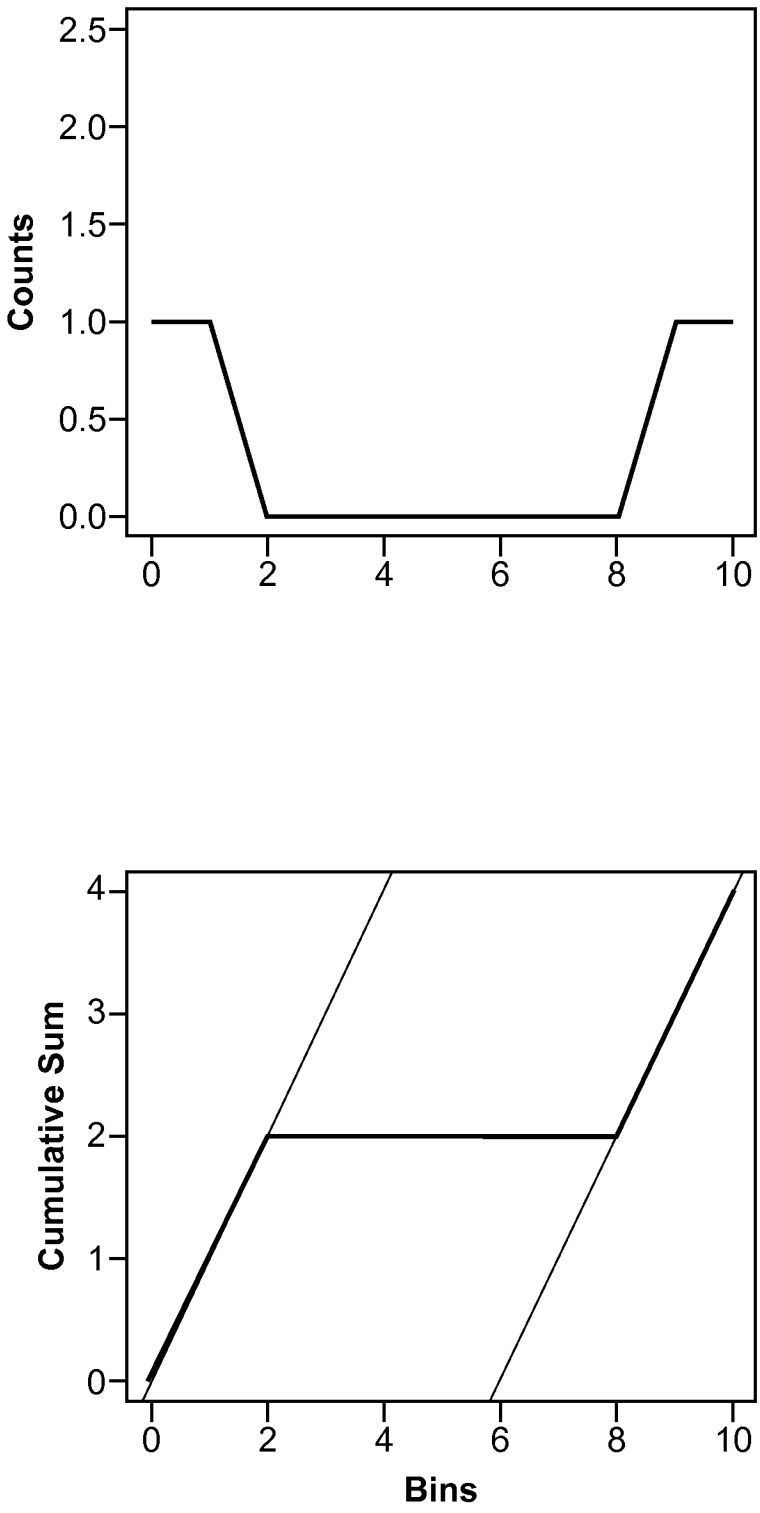

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
4
3
2
1
0
0
2
4
6
8
10
Bins

FIG. 56

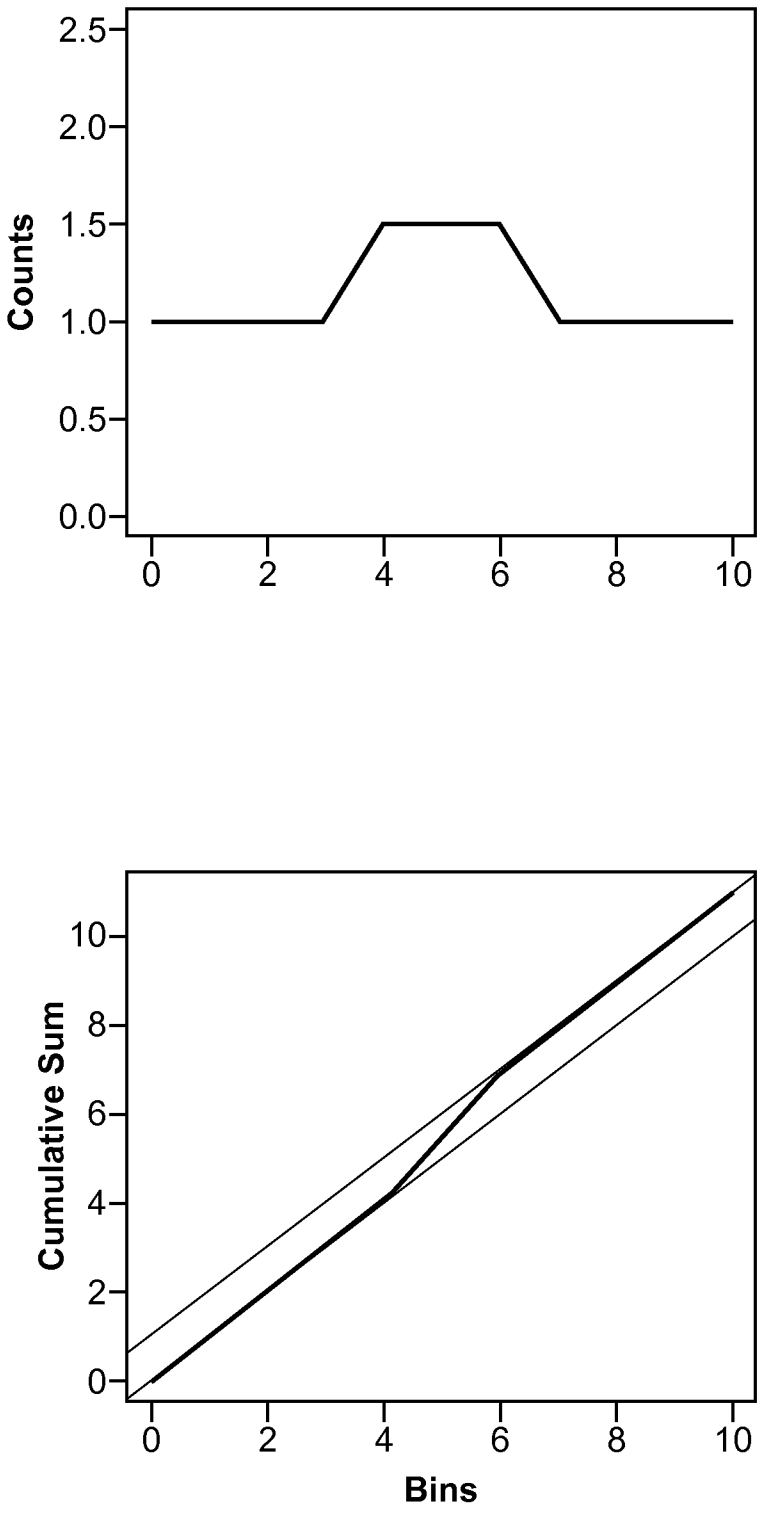

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
10
8
6
4
2
0
0
2
4
6
8
10
Bins

FIG. 57

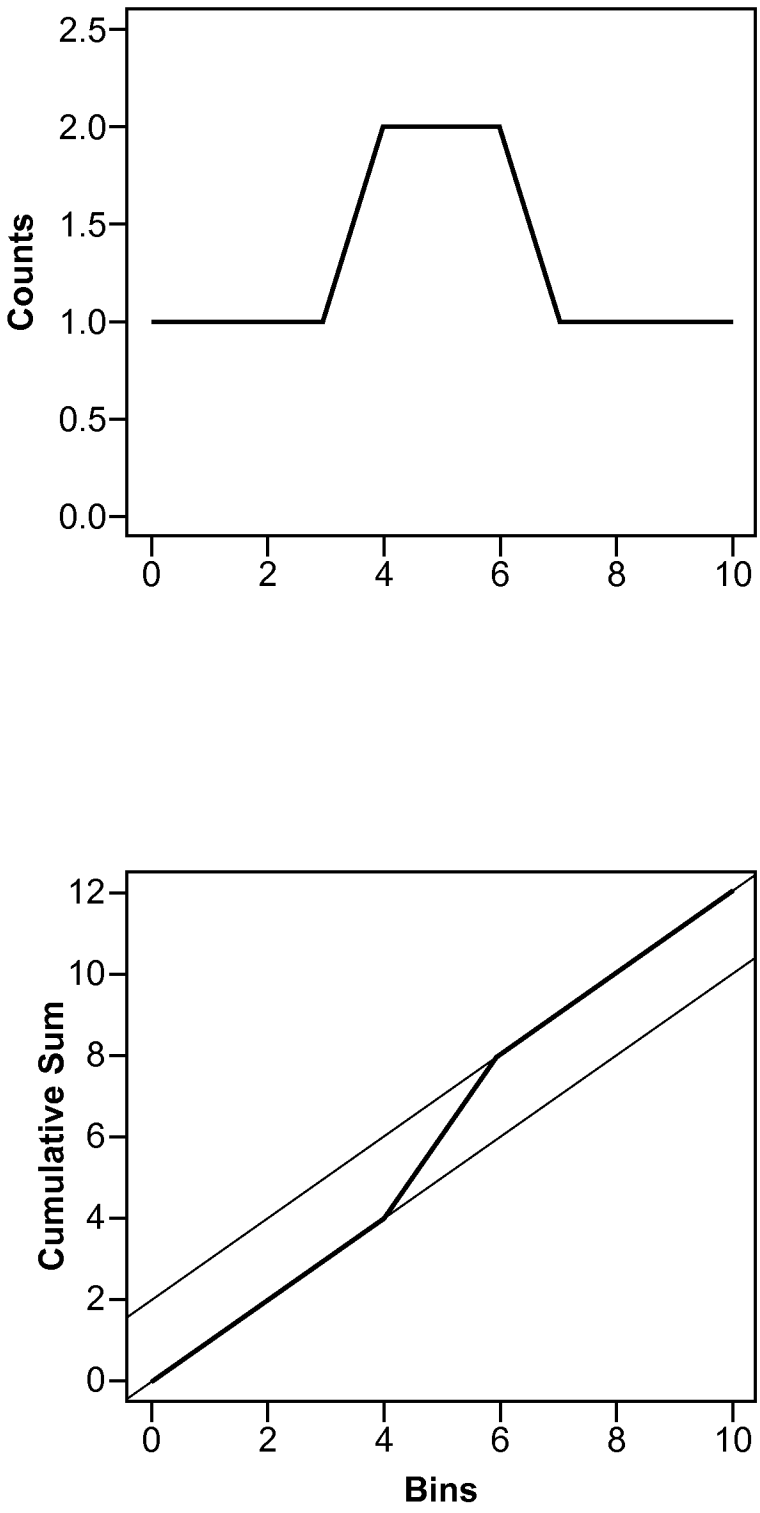

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
12
10
8
6
4
2
0
0
2
4
6
8
10
Bins

FIG. 58

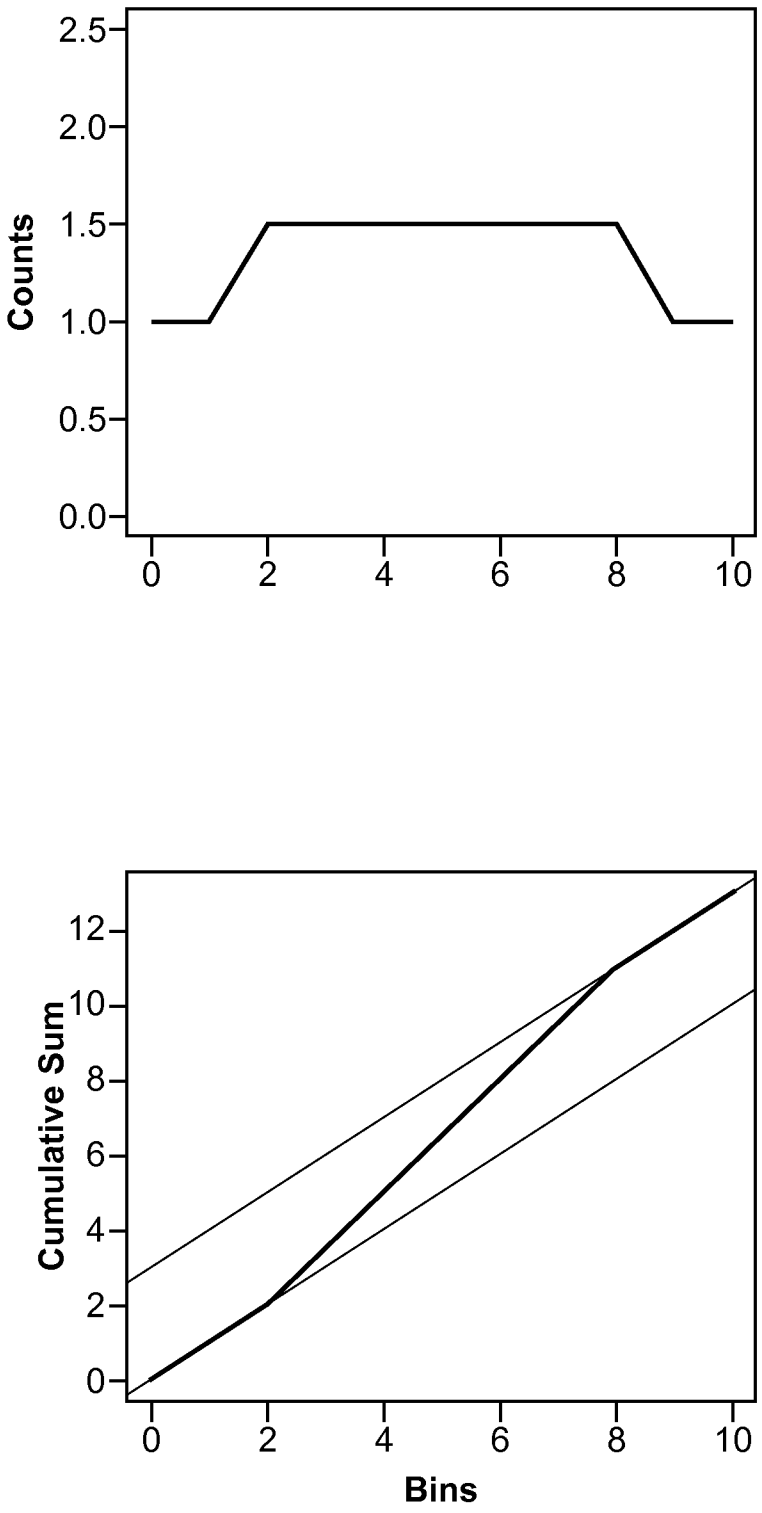

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
12
10
8
6
4
2
0
0
2
4
6
8
10
Bins

FIG. 59

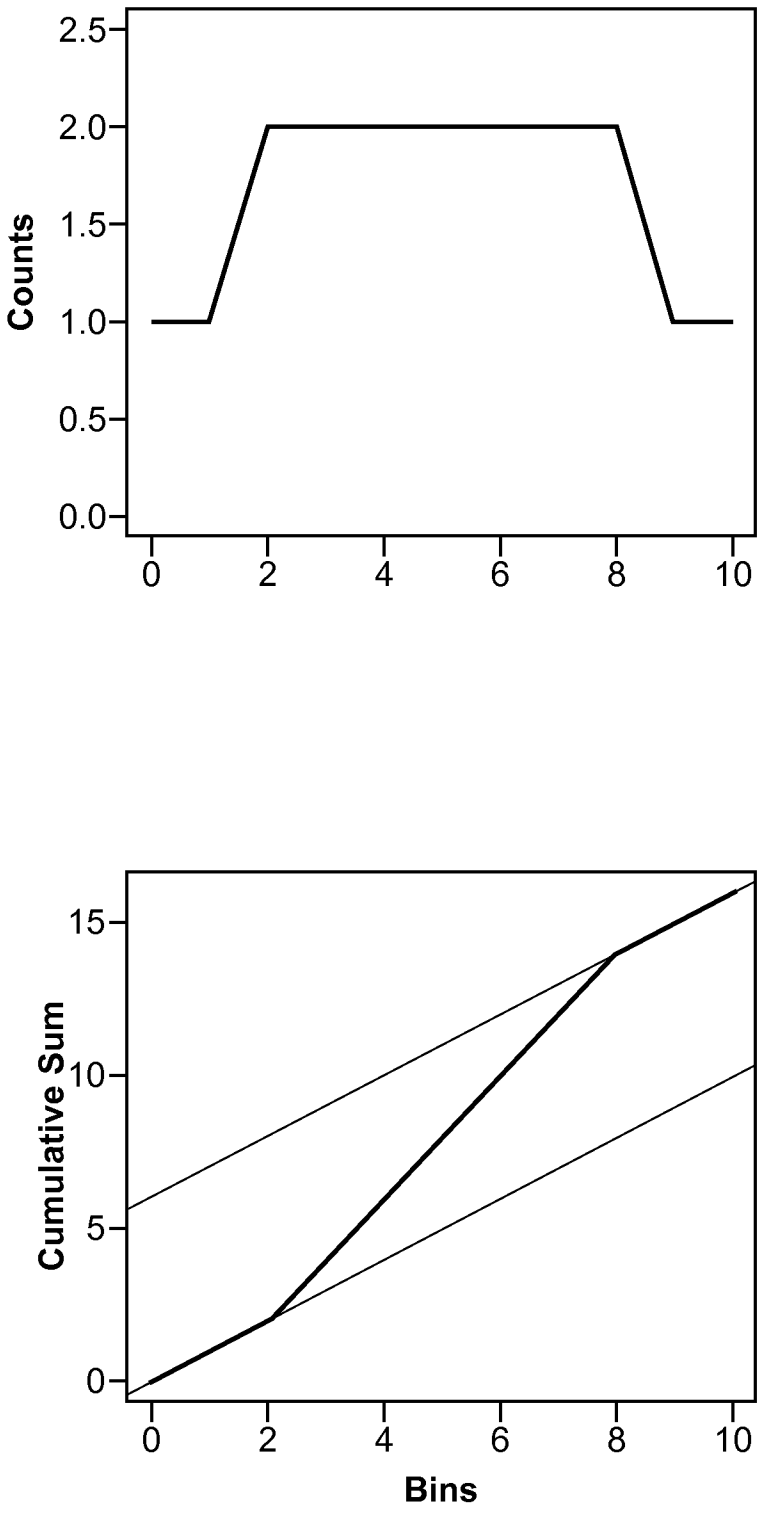

Counts
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Cumulative Sum
15
10
5
0
0
2
4
6
8
10
Bins

FIG. 60

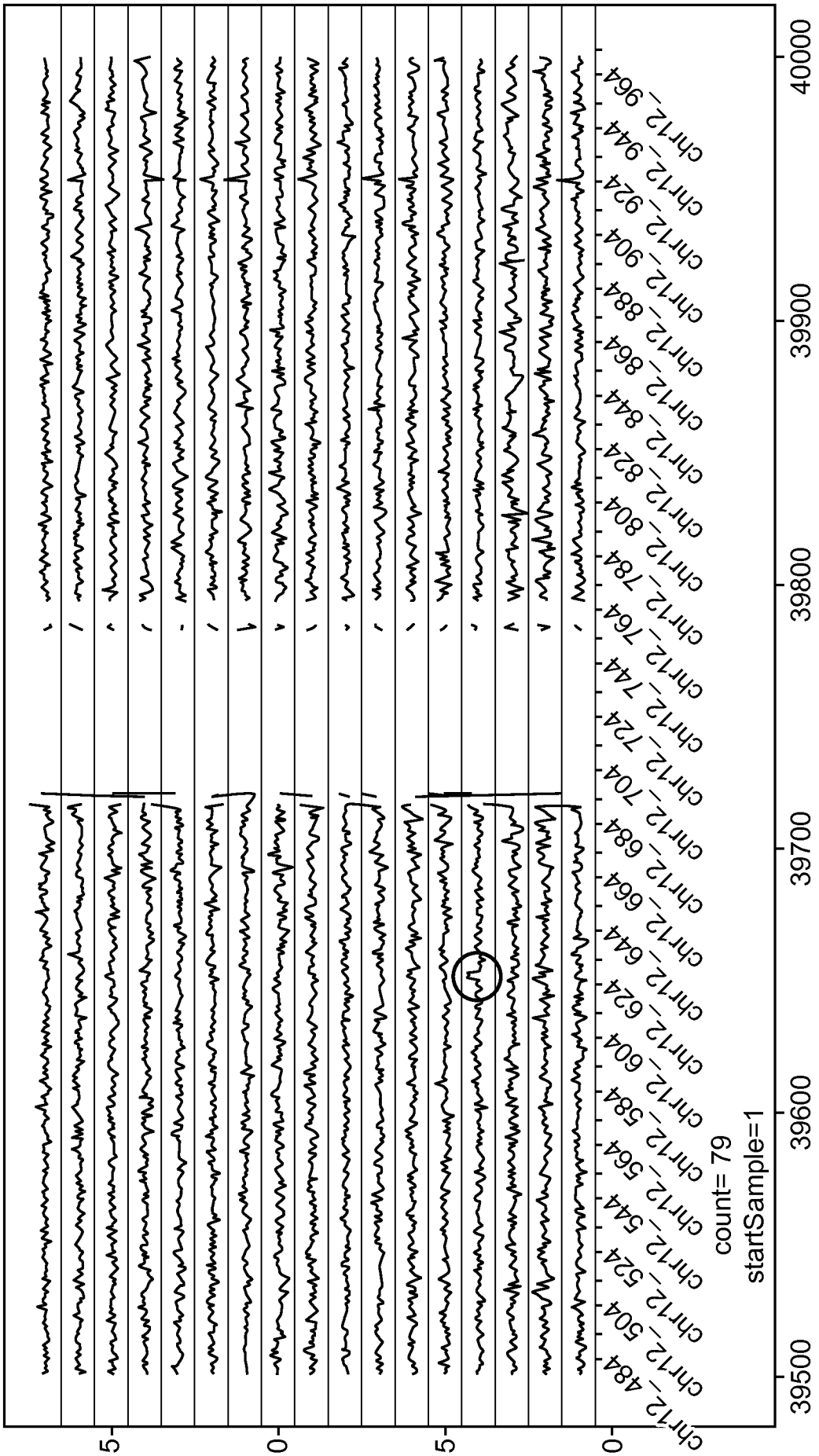

15
10
5
0
39500
39600
39700
39800
39900
40000
count= 79
startSample=1
chr12_48a
chr12_50a
chr12_52a
chr12_54a
chr12_56a
chr12_58a
chr12_60a
chr12_62a
chr12_64a
chr12_66a
chr12_68a
chr12_70a
chr12_72a
chr12_74a
chr12_76a
chr12_78a
chr12_80a
chr12_82a
chr12_84a
chr12_86a
chr12_88a
chr12_90a
chr12_92a
chr12_94a
chr12_96a

FIG. 61A

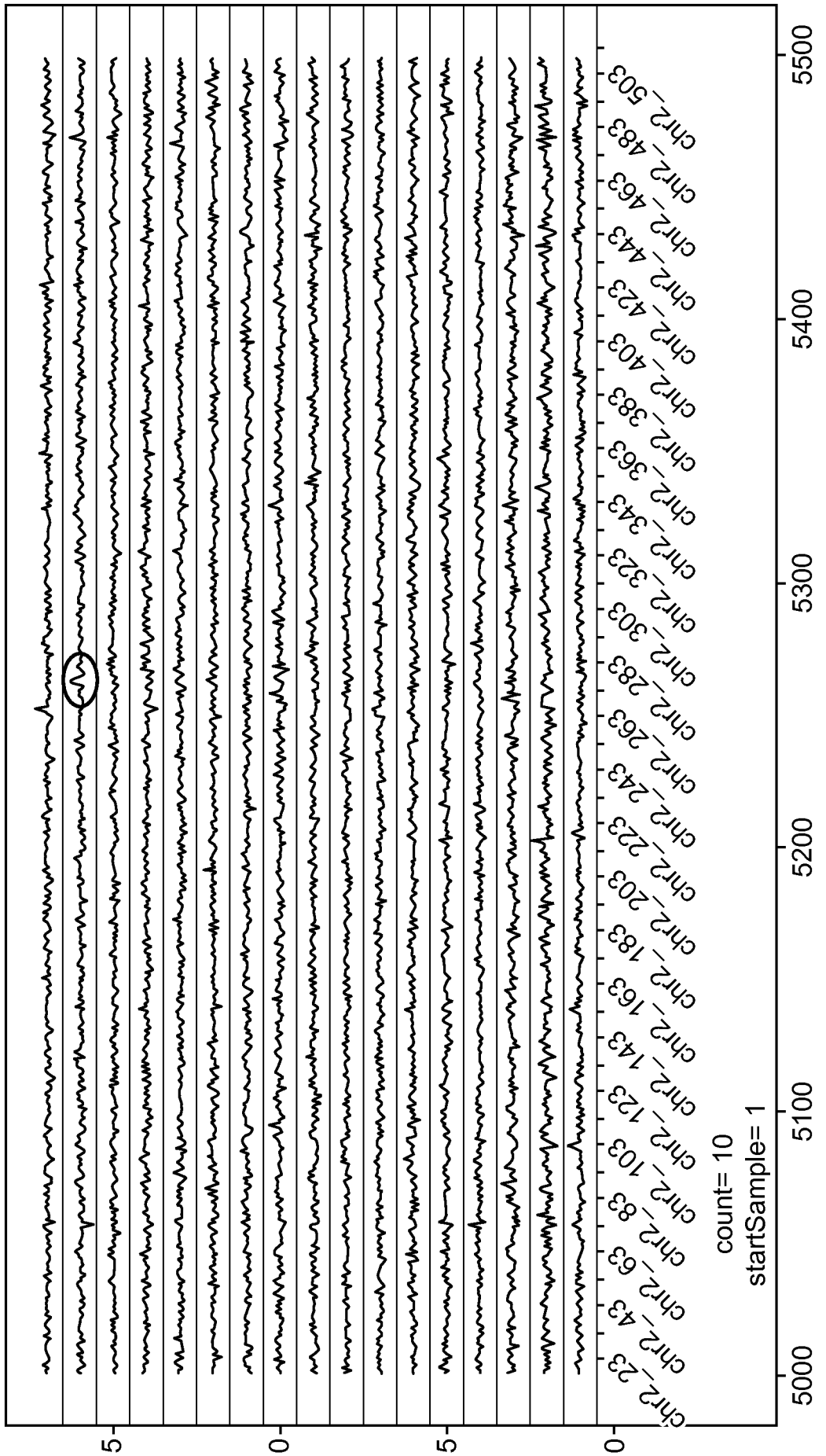

0
5
10
15
5000
5100
5200
5300
5400
5500
count= 10
startSample= 1
chr2_23
chr2_43
chr2_63
chr2_83
chr2_103
chr2_123
chr2_143
chr2_163
chr2_183
chr2_203
chr2_223
chr2_243
chr2_263
chr2_283
chr2_303
chr2_323
chr2_343
chr2_363
chr2_383
chr2_403
chr2_423
chr2_443
chr2_463
chr2_483
chr2_503

FIG. 61B

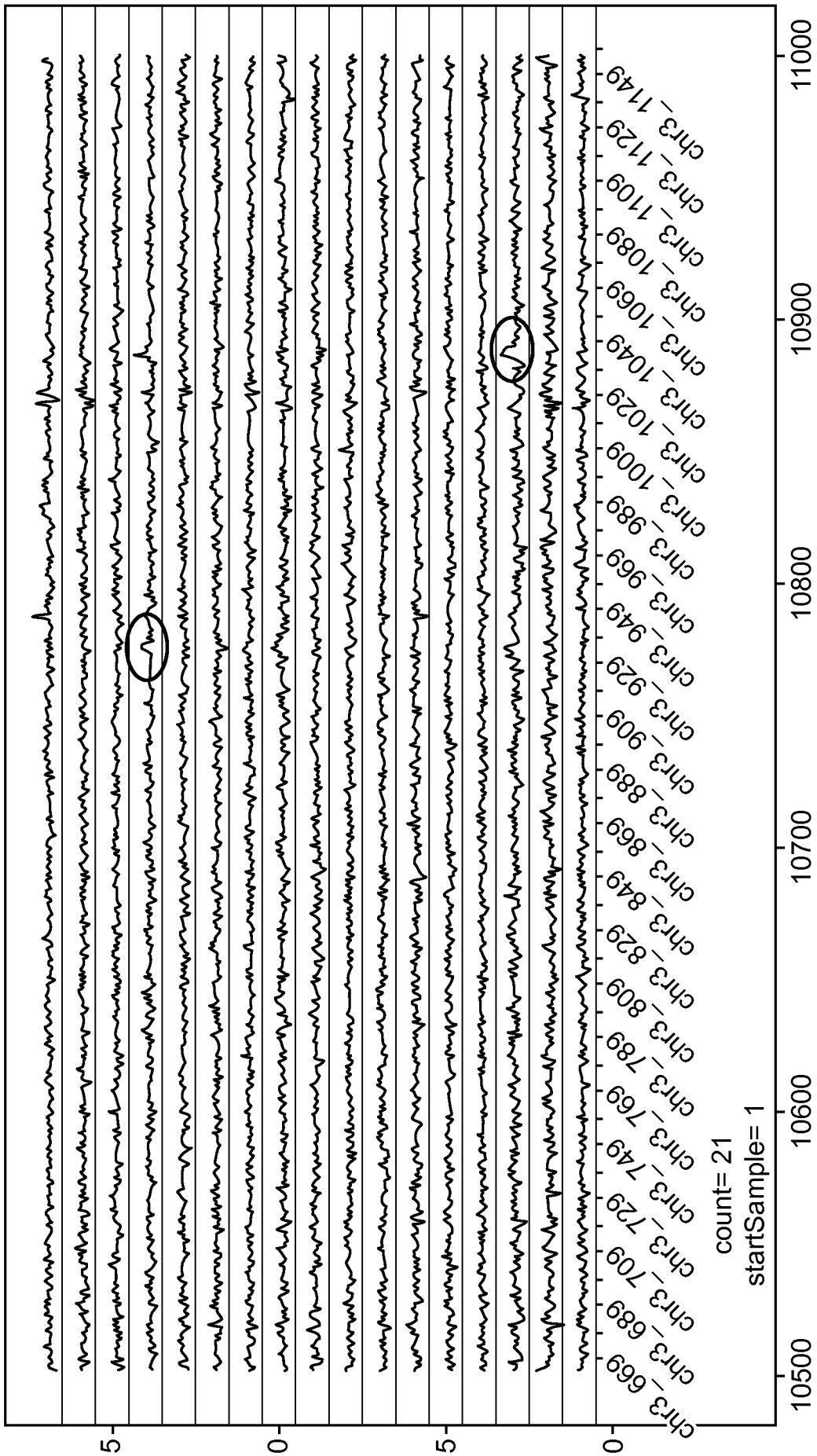

15
10
5
0
10500
10600
10700
10800
10900
11000
count= 21
startSample= 1
chr3_669
chr3_689
chr3_709
chr3_729
chr3_749
chr3_769
chr3_789
chr3_809
chr3_829
chr3_849
chr3_869
chr3_889
chr3_909
chr3_929
chr3_949
chr3_969
chr3_989
chr3_1009
chr3_1029
chr3_1049
chr3_1069
chr3_1089
chr3_1109
chr3_1129
chr3_1149

FIG. 61C

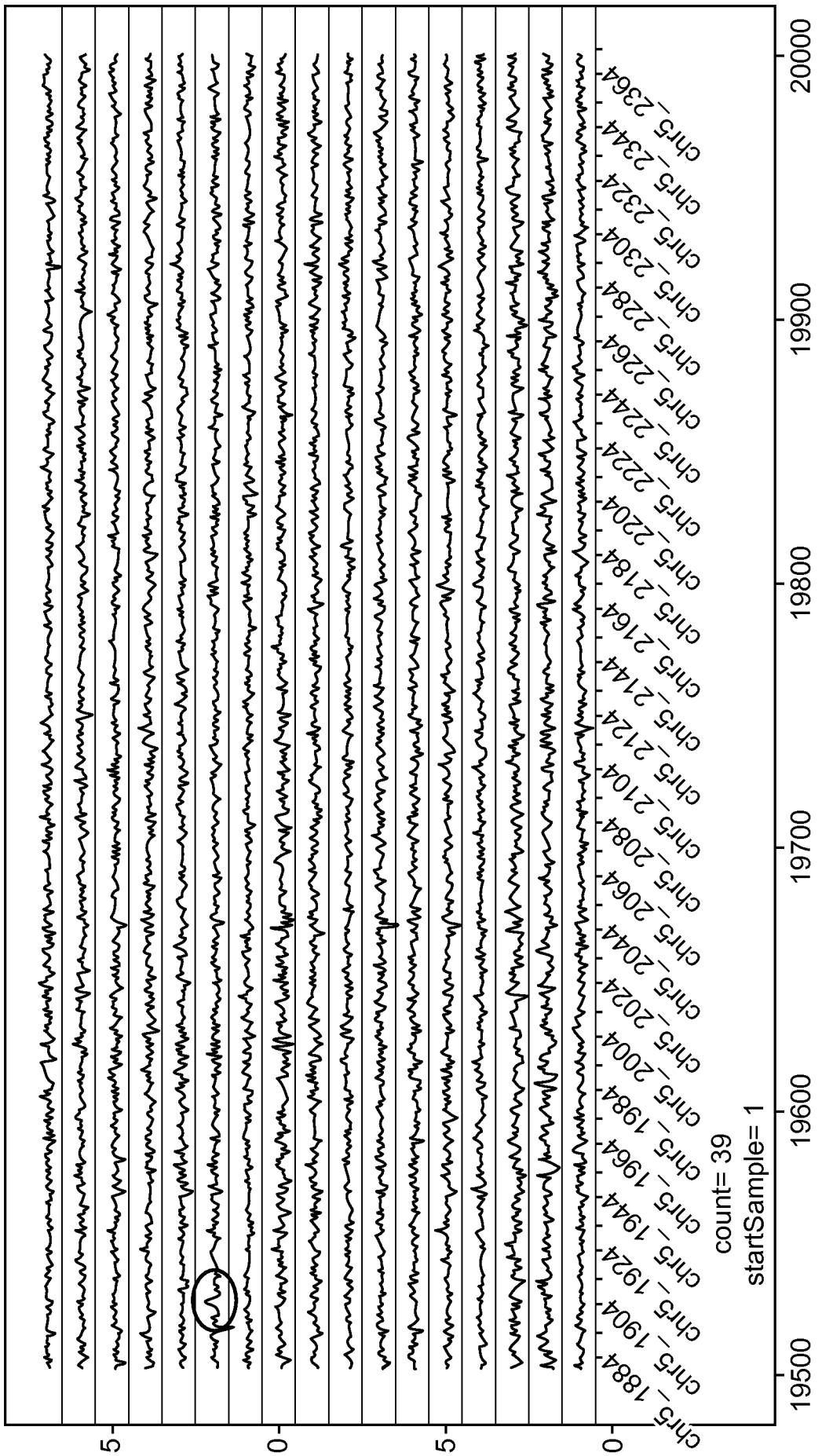

0
5
10
15
19500
19600
19700
19800
19900
20000
startSample= 1
count= 39
chr5_1884A
chr5_1904A
chr5_1924A
chr5_1944A
chr5_1964A
chr5_1984A
chr5_2004A
chr5_2024A
chr5_2044A
chr5_2064A
chr5_2084A
chr5_2104A
chr5_2124A
chr5_2144A
chr5_2164A
chr5_2184A
chr5_2204A
chr5_2224A
chr5_2244A
chr5_2264A
chr5_2284A
chr5_2304A
chr5_2324A
chr5_2344A
chr5_2364A

FIG. 61D

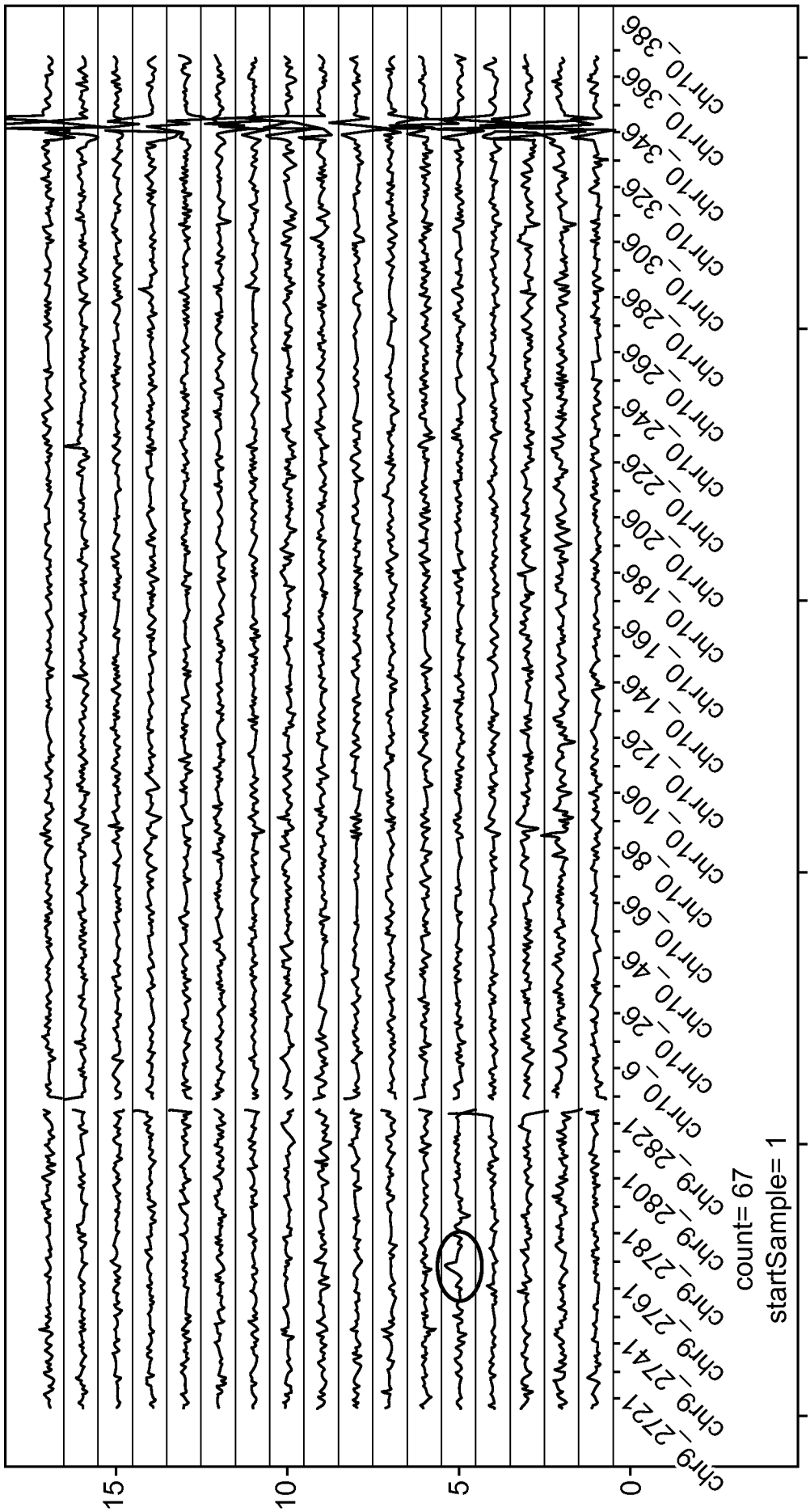

0
5
10
15
33500
33600
33700
33800
33900
34000
chr9_2721
chr9_2741
chr9_2761
chr9_2781
chr9_2801
chr9_2821
chr10_6
chr10_26
chr10_46
chr10_66
chr10_86
chr10_106
chr10_126
chr10_146
chr10_166
chr10_186
chr10_206
chr10_226
chr10_246
chr10_266
chr10_286
chr10_306
chr10_326
chr10_346
chr10_366
chr10_386
count= 67
startSample= 1

FIG. 61E

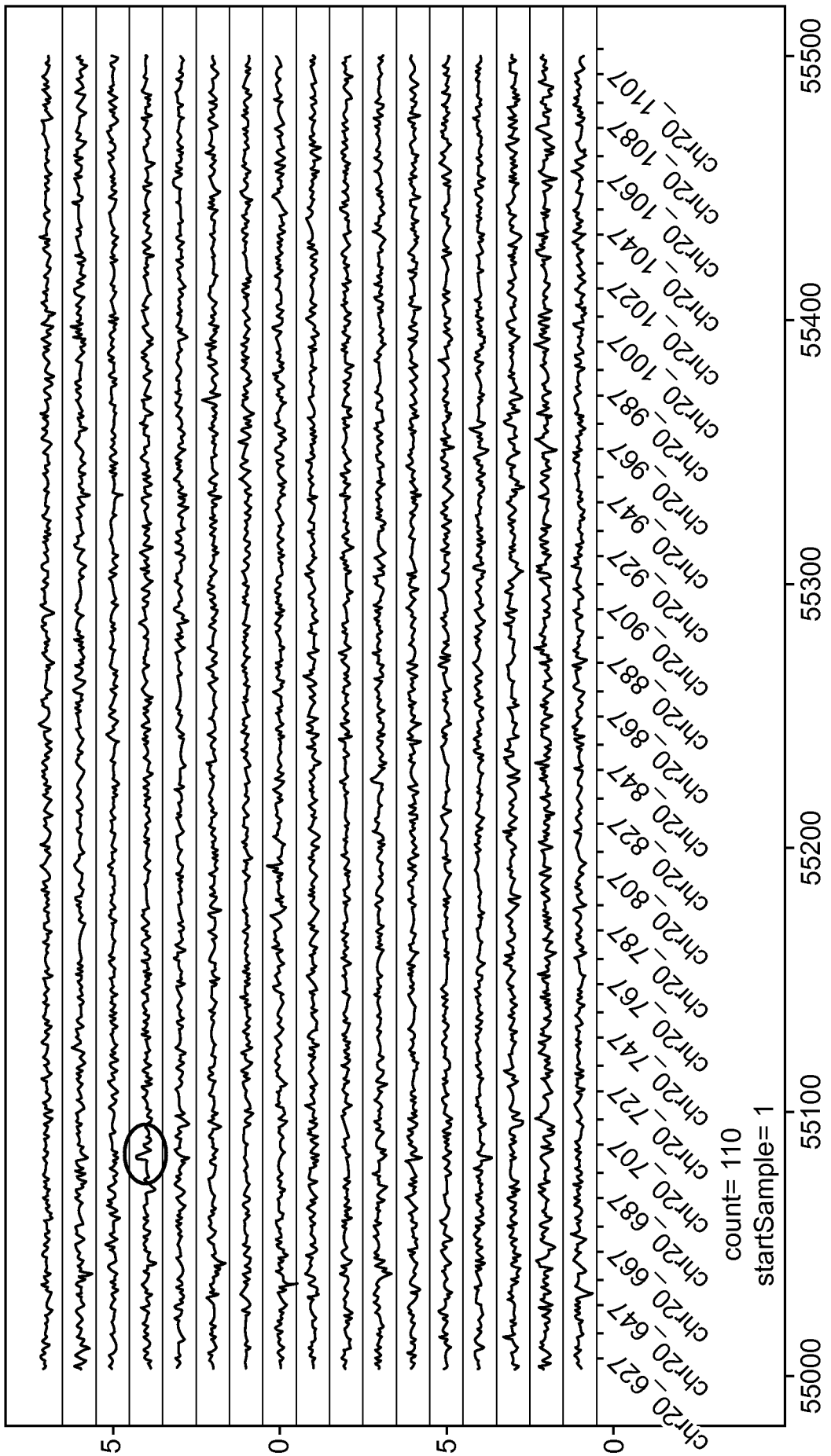

chr20_627
chr20_647
chr20_667
chr20_687
chr20_707
chr20_727
chr20_747
chr20_767
chr20_787
chr20_807
chr20_827
chr20_847
chr20_867
chr20_887
chr20_907
chr20_927
chr20_947
chr20_967
chr20_987
chr20_1007
chr20_1027
chr20_1047
chr20_1067
chr20_1087
chr20_1107
count= 110
startSample= 1
0
5
10
15
55000
55100
55200
55300
55400
55500

FIG. 61F

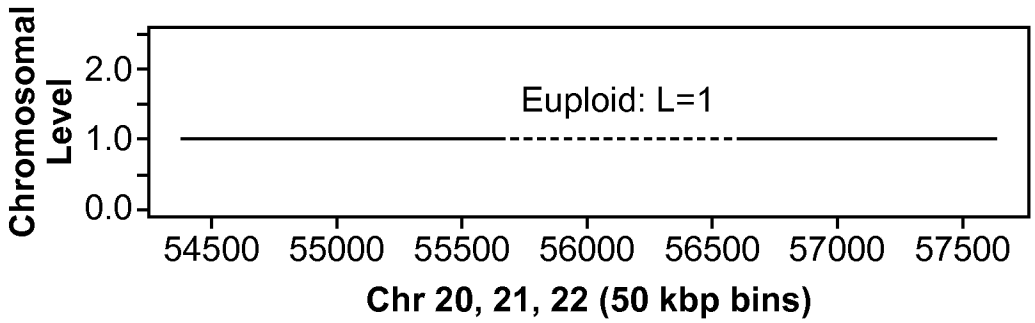

Chromosomal Level
2.0
1.0
0.0
Euploid: L=1
54500 55000 55500 56000 56500 57000 57500
Chr 20, 21, 22 (50 kbp bins)

FIG. 62

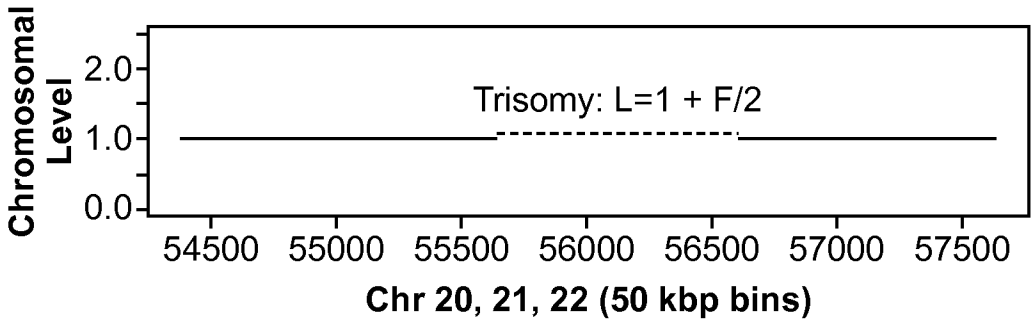

Chromosomal Level
2.0
1.0
0.0
Trisomy: L=1 + F/2
54500 55000 55500 56000 56500 57000 57500
Chr 20, 21, 22 (50 kbp bins)

FIG. 63

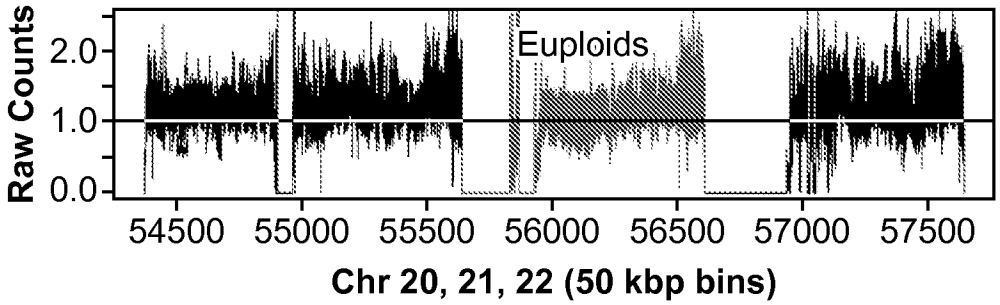

Raw Counts
2.0
1.0
0.0
Euploids
54500
55000
55500
56000
56500
57000
57500
Chr 20, 21, 22 (50 kbp bins)

FIG. 64

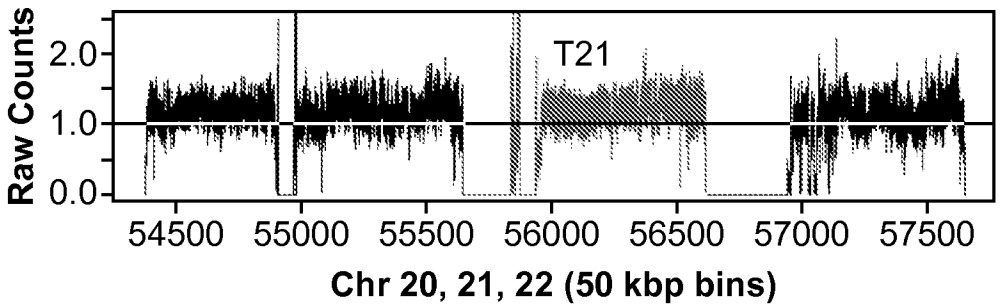

Raw Counts
2.0
1.0
0.0
T21
54500
55000
55500
56000
56500
57000
57500
Chr 20, 21, 22 (50 kbp bins)

FIG. 65

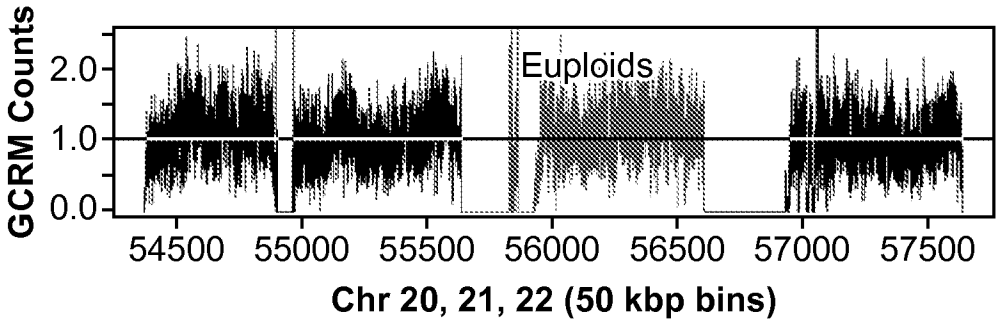

GCRM Counts
2.0
1.0
0.0
Euploids
54500
55000
55500
56000
56500
57000
57500
Chr 20, 21, 22 (50 kbp bins)

FIG. 66

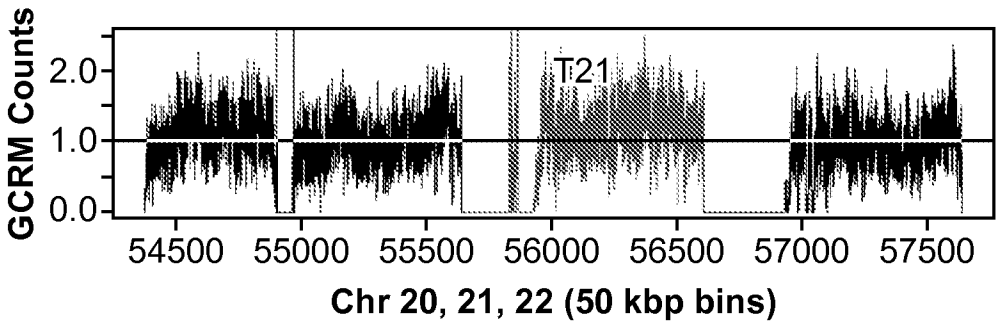

GCRM Counts
2.0
1.0
0.0
T21
54500
55000
55500
56000
56500
57000
57500
Chr 20, 21, 22 (50 kbp bins)

FIG. 67

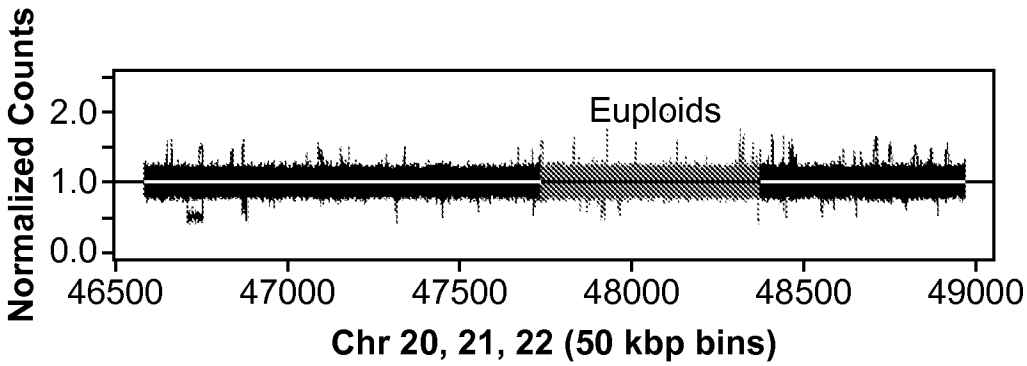

Normalized Counts
2.0
1.0
0.0
Euploids
46500
47000
47500
48000
48500
49000
Chr 20, 21, 22 (50 kbp bins)

FIG. 68

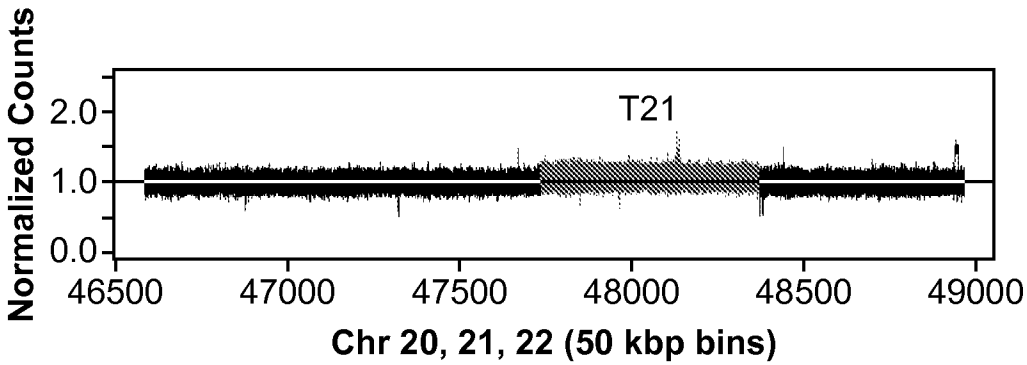
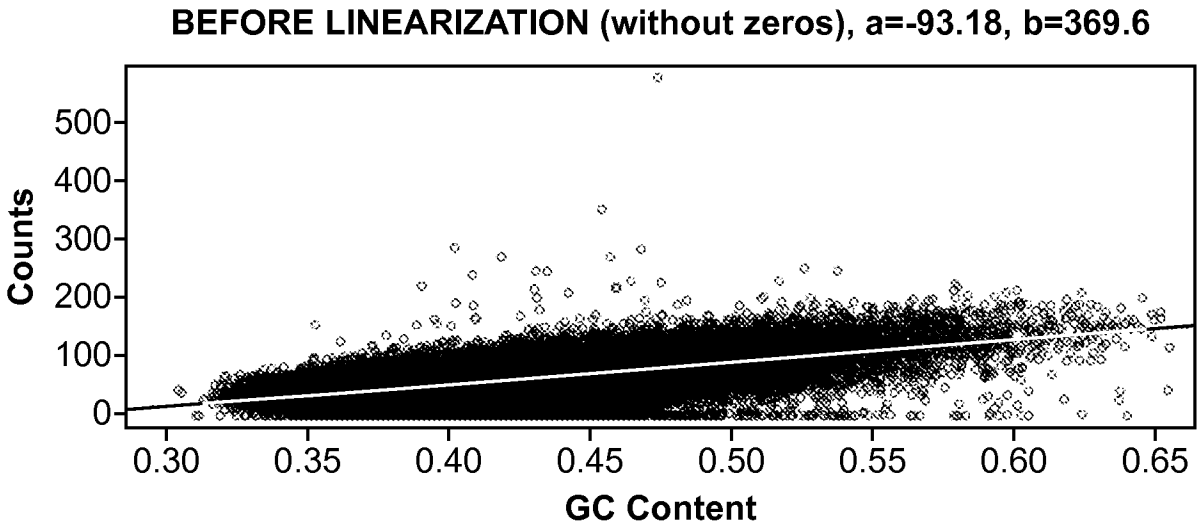

Normalized Counts
2.0
1.0
0.0
T21
46500
47000
47500
48000
48500
49000
Chr 20, 21, 22 (50 kbp bins)

FIG. 69


BEFORE LINEARIZATION (without zeros), a=-93.18, b=369.6
Counts
500
400
300
200
100
0
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
GC Content

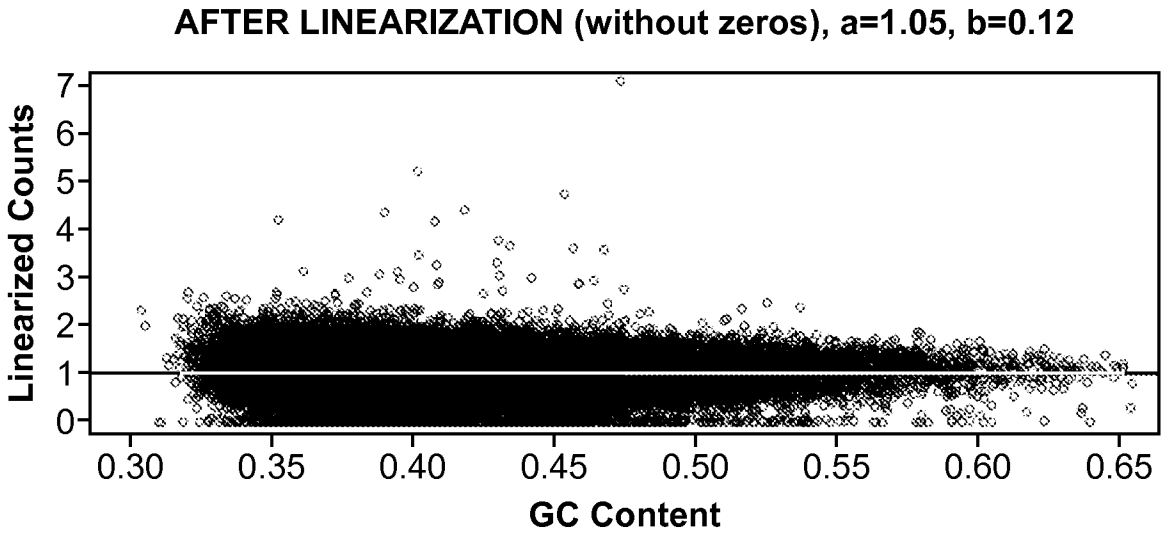

AFTER LINEARIZATION (without zeros), a=1.05, b=0.12
Linearized Counts
7
6
5
4
3
2
1
0
0.30
0.35
0.40
0.45
0.50
0.55
0.60
0.65
GC Content

FIG. 70

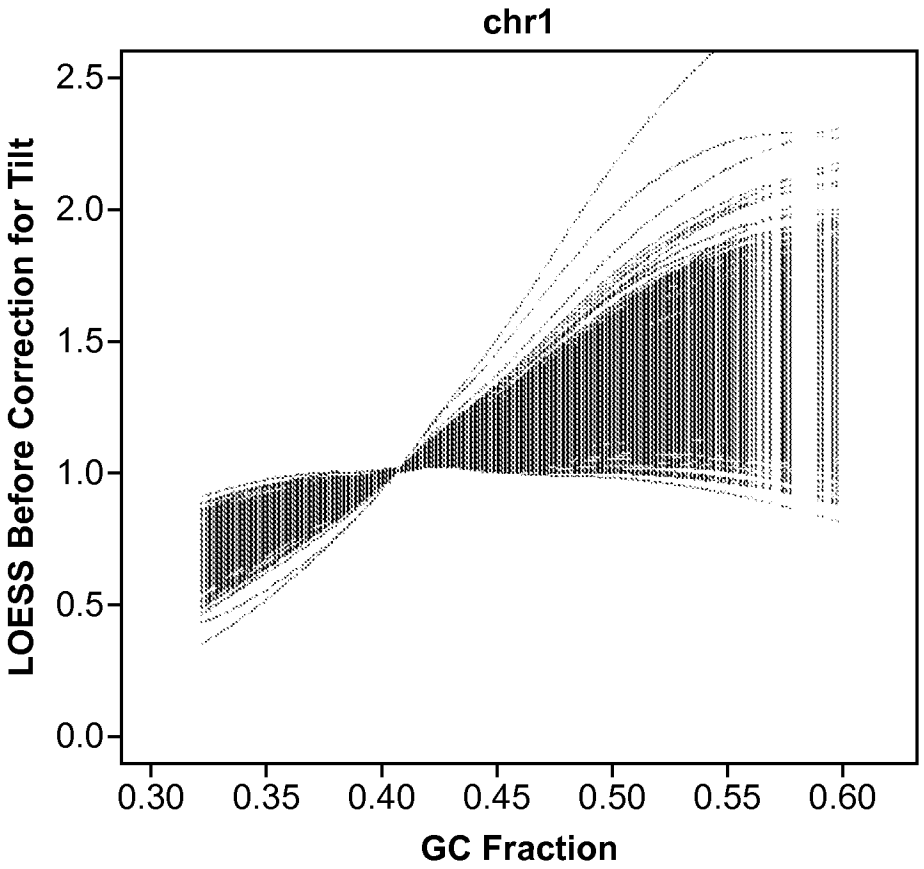

chr1
LOESS Before Correction for Tilt
2.5
2.0
1.5
1.0
0.5
0.0
0.30
0.35
0.40
0.45
0.50
0.55
0.60
GC Fraction

FIG. 71

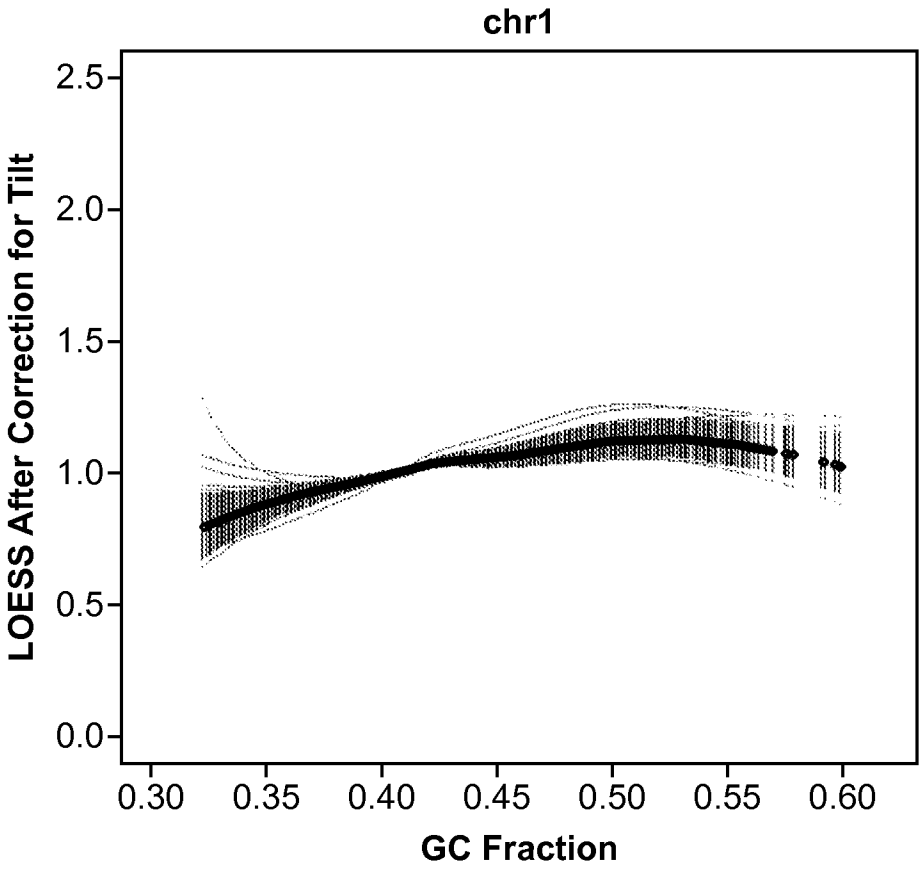

chr1
LOESS After Correction for Tilt
2.5
2.0
1.5
1.0
0.5
0.0
0.30
0.35
0.40
0.45
0.50
0.55
0.60
GC Fraction

FIG. 72

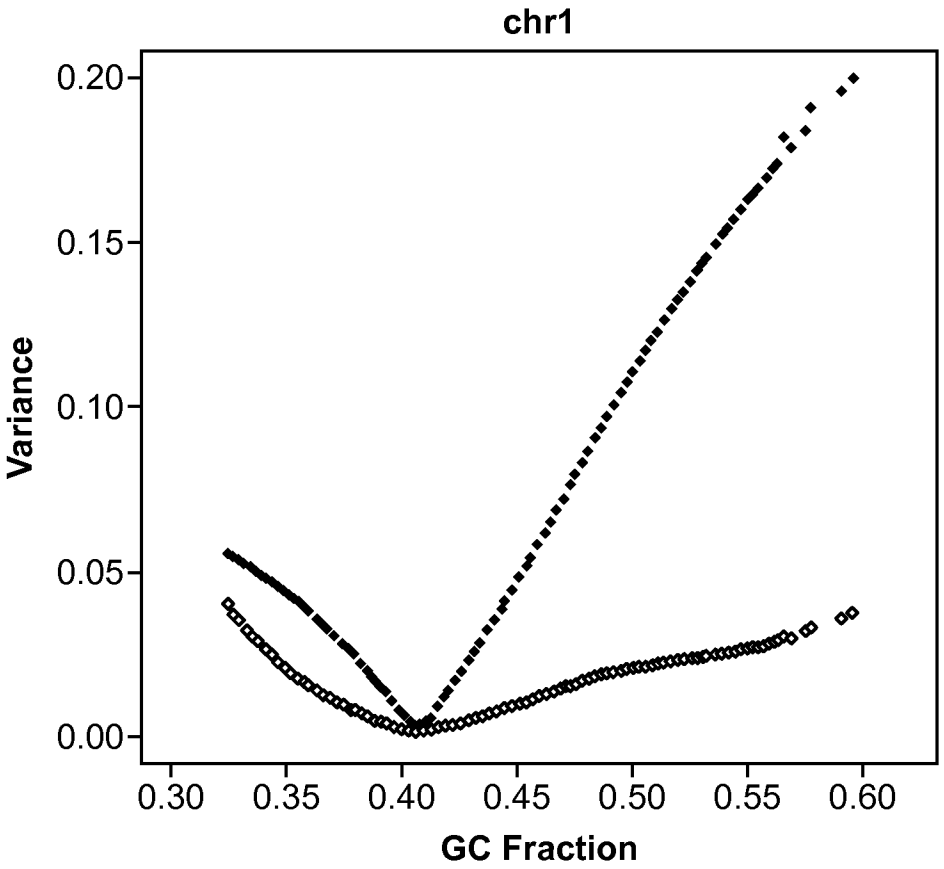

chr1
Variance
0.20
0.15
0.10
0.05
0.00
0.30
0.35
0.40
0.45
0.50
0.55
0.60
GC Fraction

FIG. 73

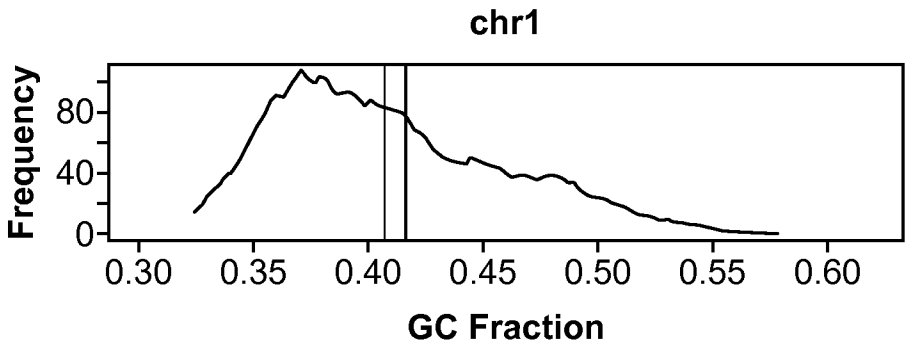


FIG. 74

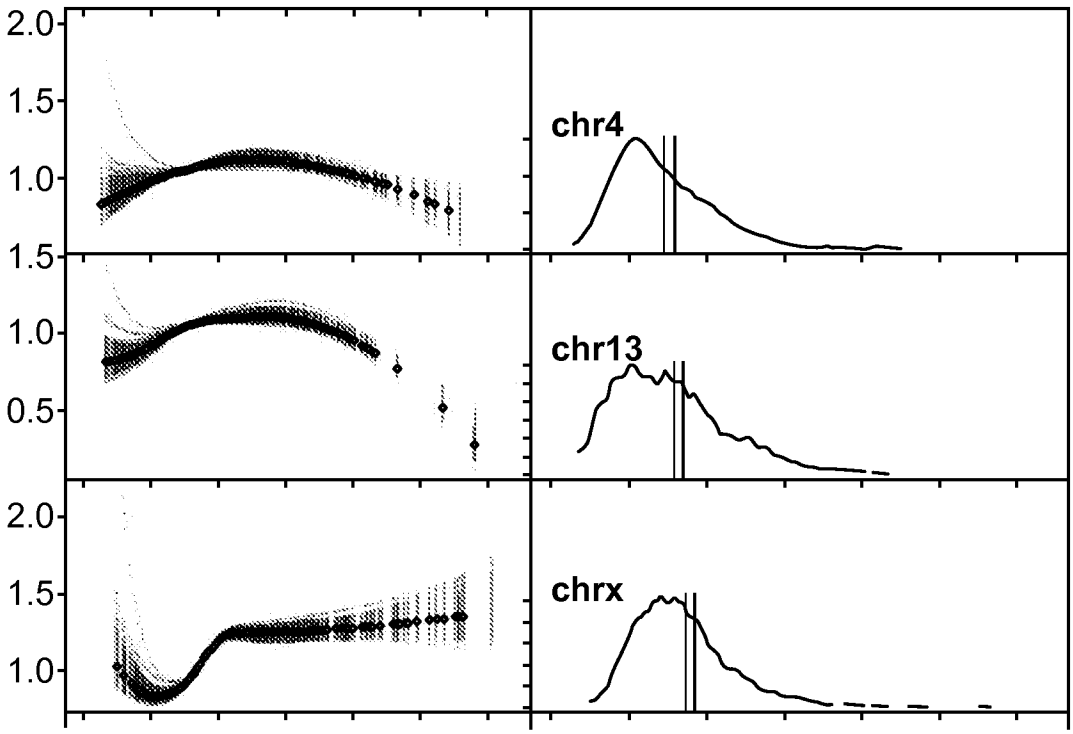


FIG. 75A

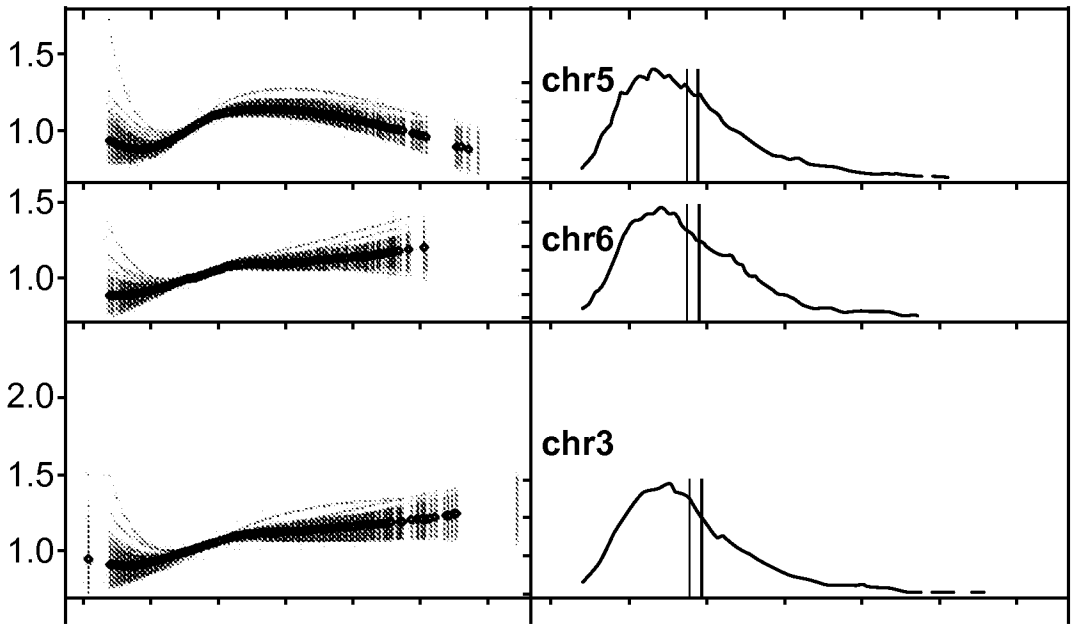


FIG. 75B

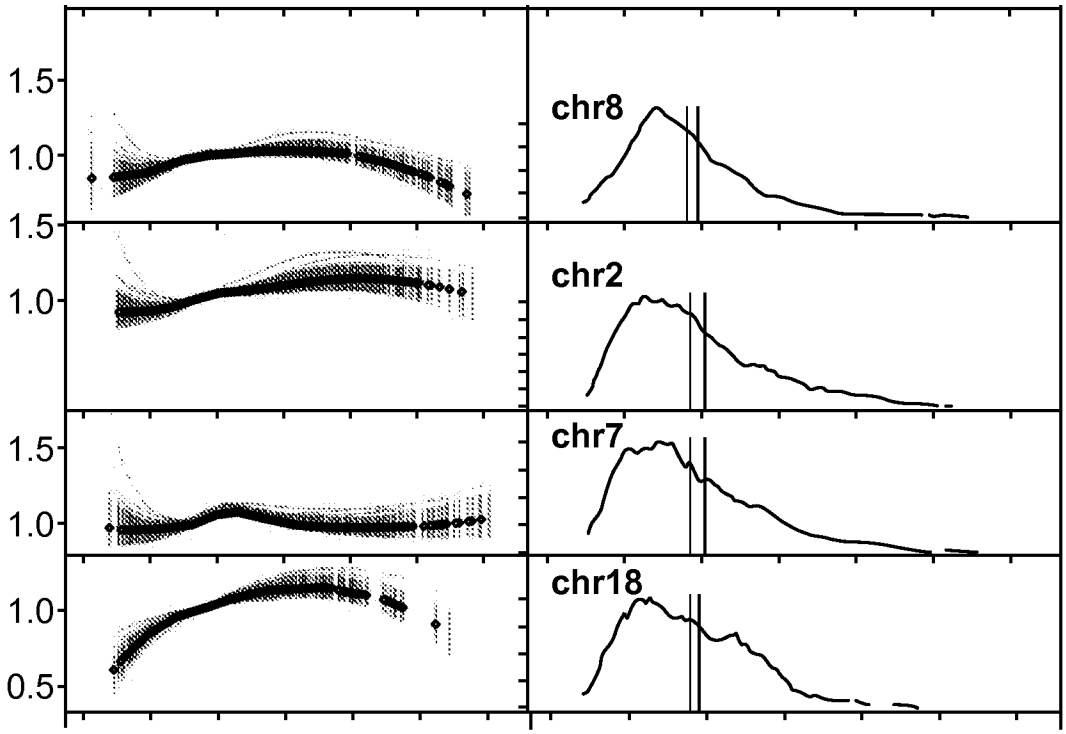


FIG. 75C

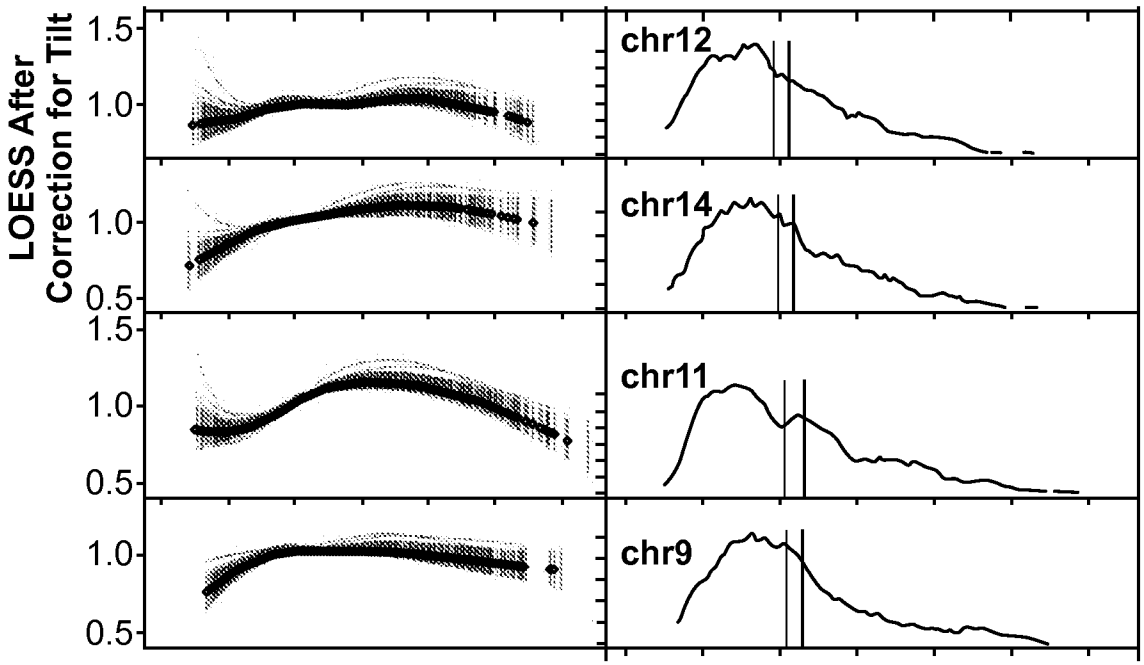


FIG. 75D

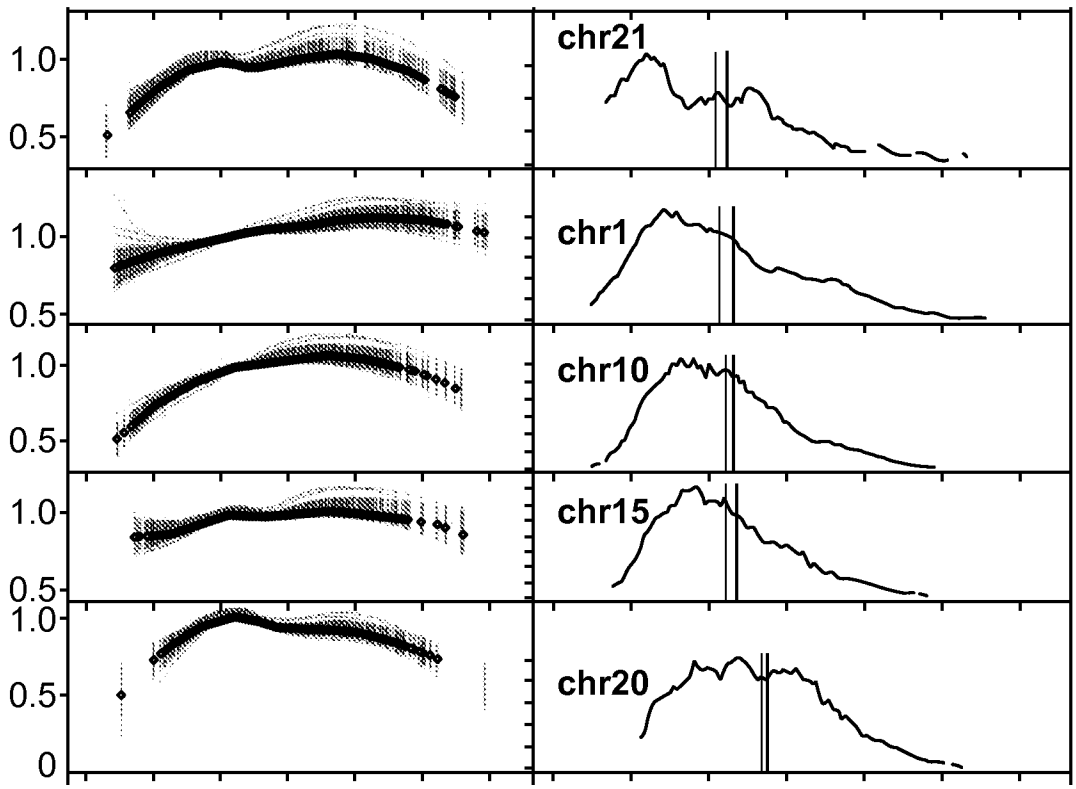


FIG. 75E

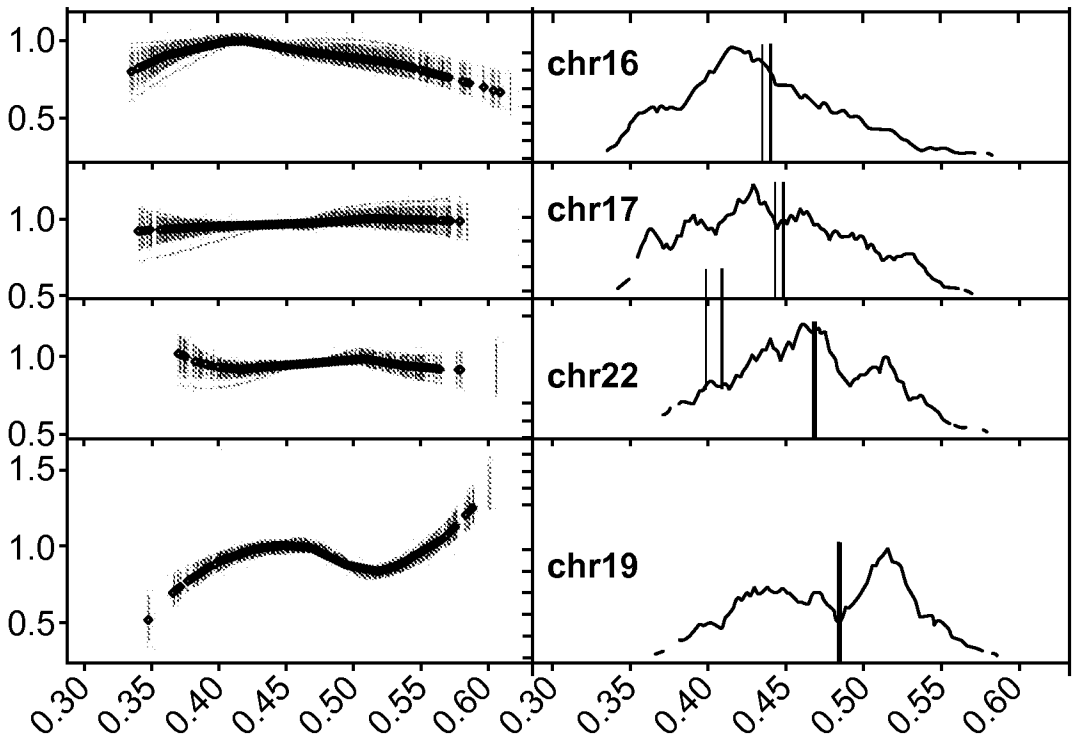


FIG. 75F

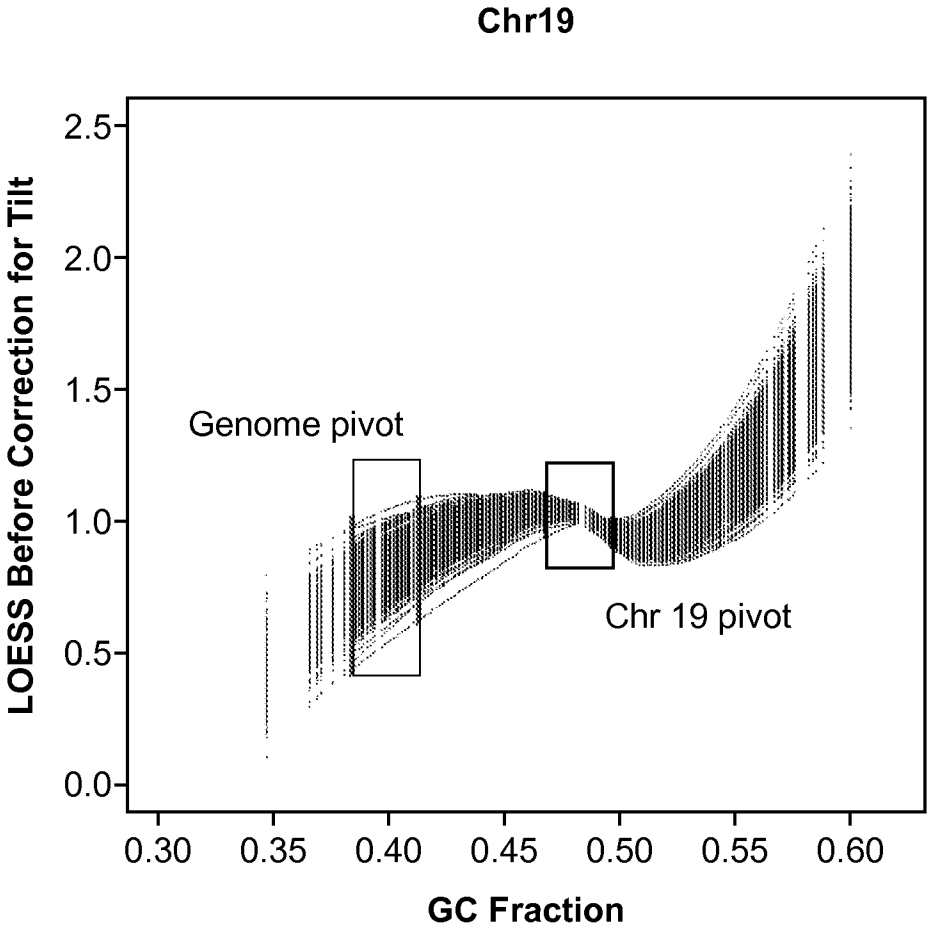

Chr19
LOESS Before Correction for Tilt
2.5
2.0
1.5
1.0
0.5
0.0
Genome pivot
Chr 19 pivot
0.30
0.35
0.40
0.45
0.50
0.55
0.60
GC Fraction

FIG. 76

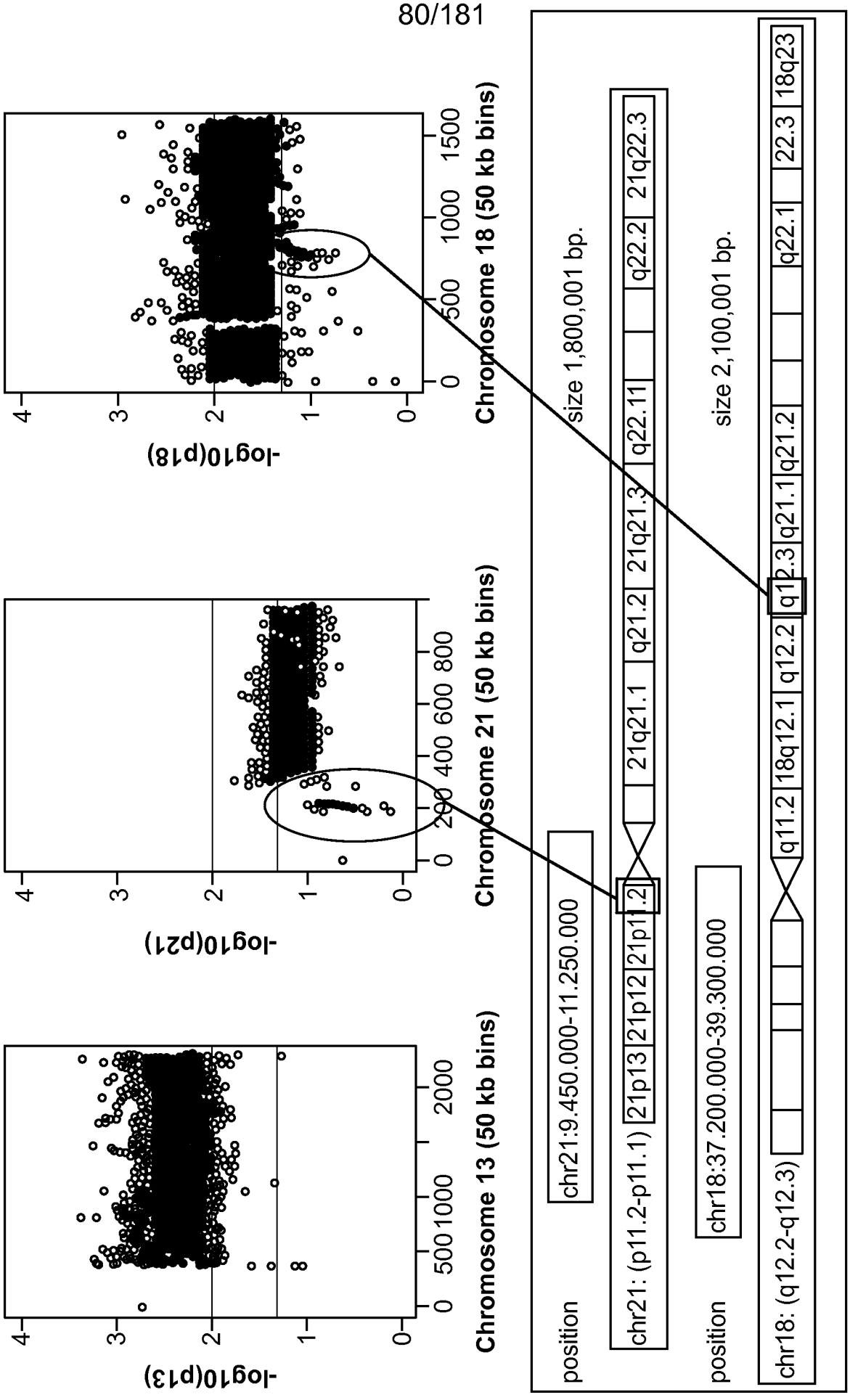

-log10(p13)
Chromosome 13 (50 kb bins)
-log10(p21)
Chromosome 21 (50 kb bins)
-log10(p18)
Chromosome 18 (50 kb bins)
position
chr21:9.450.000-11.250.000
size 1,800,001 bp.
chr21: (p11.2-p11.1)
21p13
21p12
21p11.2
21q11.1
21q11.2
21q21.1
21q21.3
q22.11
q22.2
21q22.3
position
chr18:37.200.000-39.300.000
size 2,100,001 bp.
chr18: (q12.2-q12.3)
q11.2
18q12.1
q12.2
q12.3
q21.1
q21.2
q22.1
22.3
18q23

FIG. 77

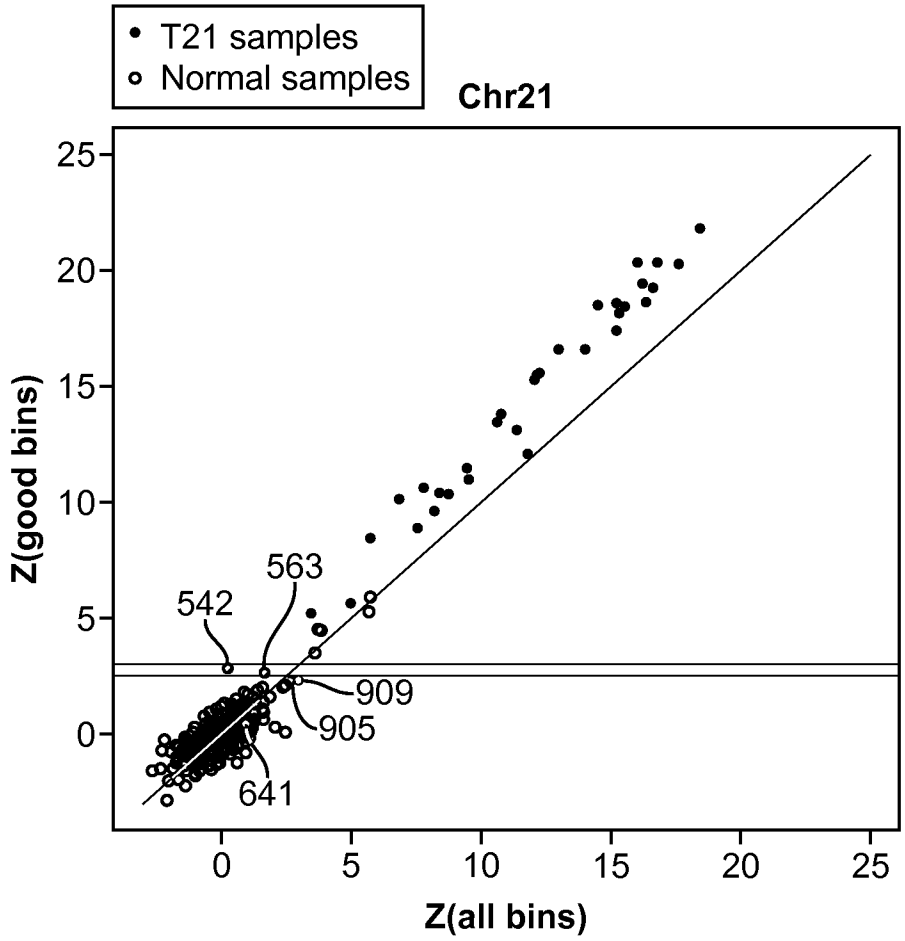

T21 samples
Normal samples
Chr21
Z(good bins)
Z(all bins)
25
20
15
10
5
0
0
5
10
15
20
25
563
542
909
905
641

FIG. 78

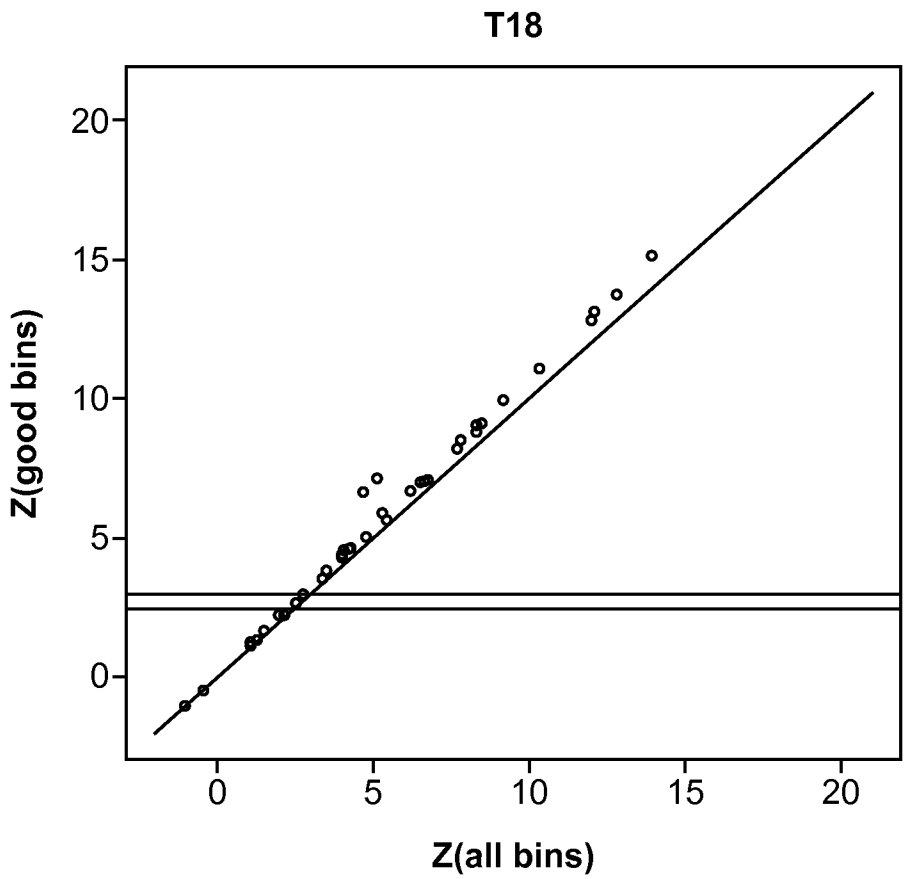


FIG. 79

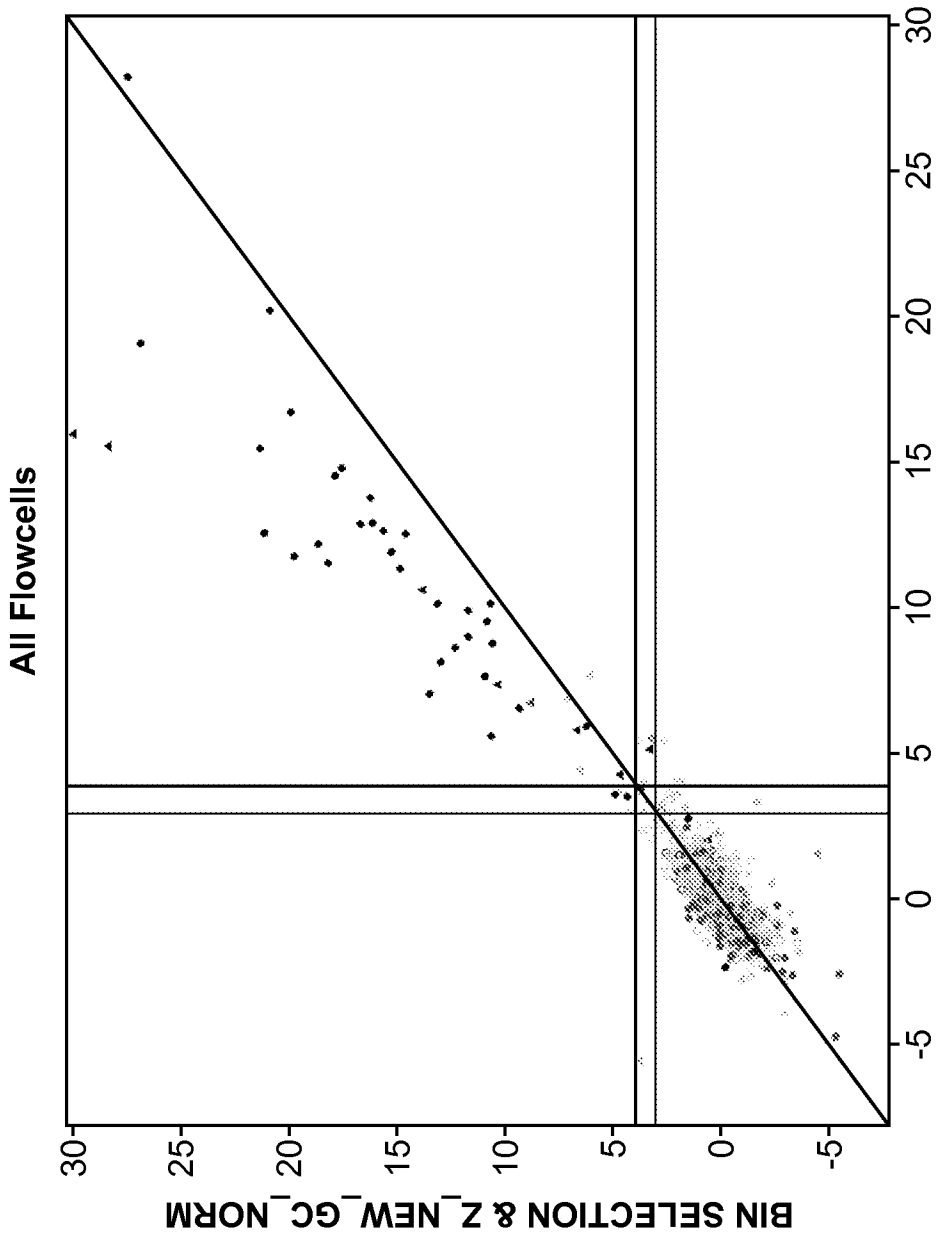

All Flowcells
BIN SELECTION & Z_NEW_GC_NORM
Z_C_18
30
25
20
15
10
5
0
-5

FIG. 80

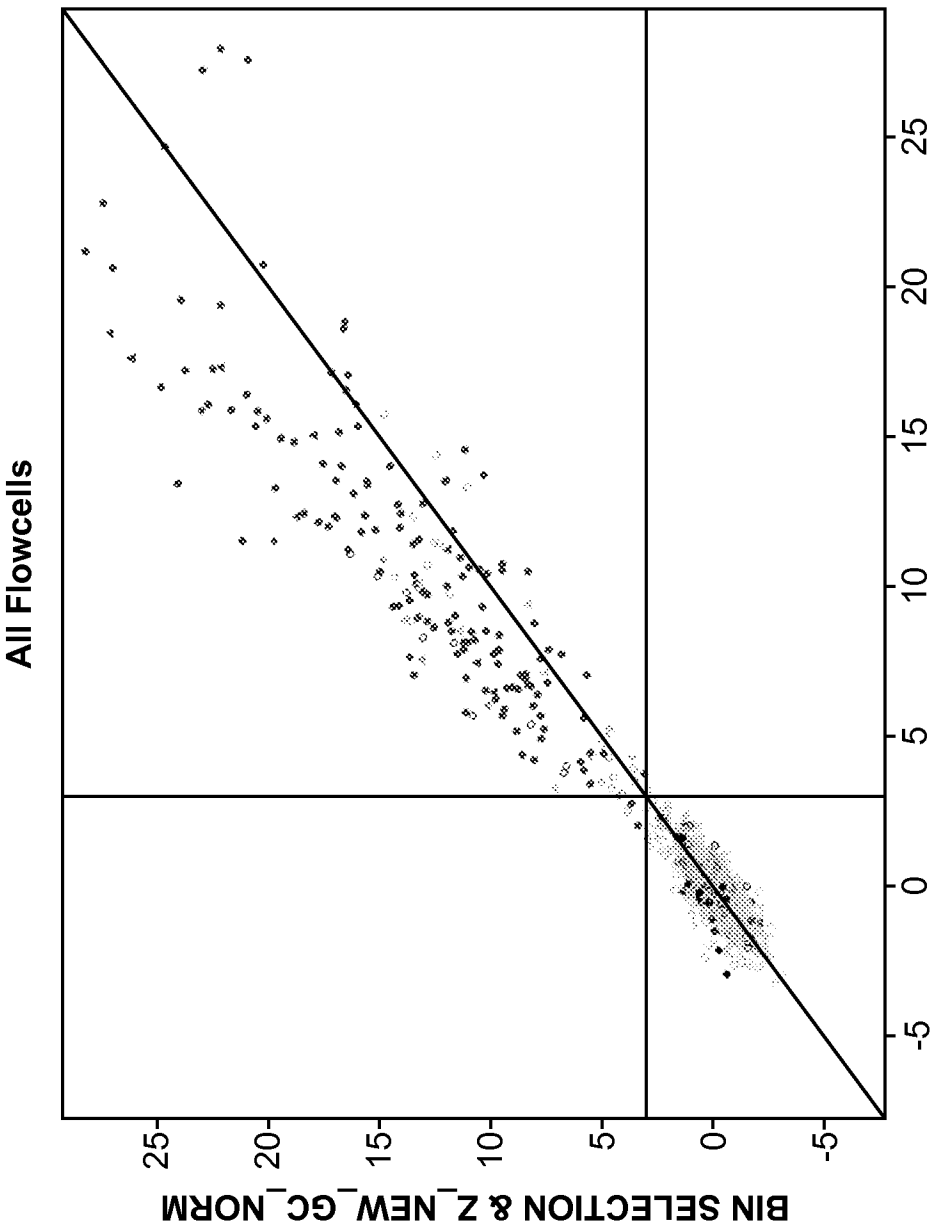

All Flowcells
BIN SELECTION & Z_NEW_GC_NORM
Z_C_21
25
20
15
10
5
0
-5
-5
0
5
10
15
20
25

FIG. 81

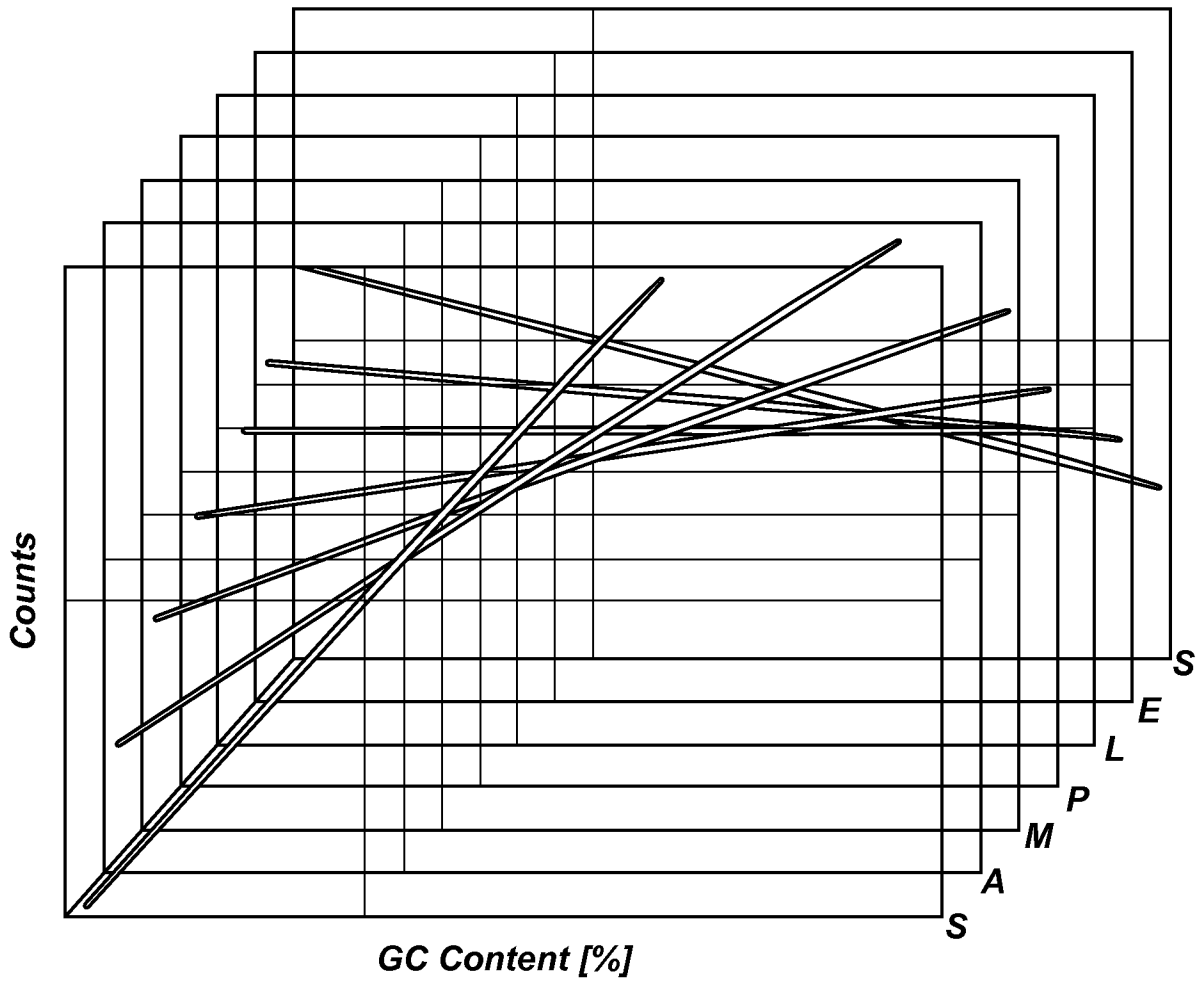


FIG. 82

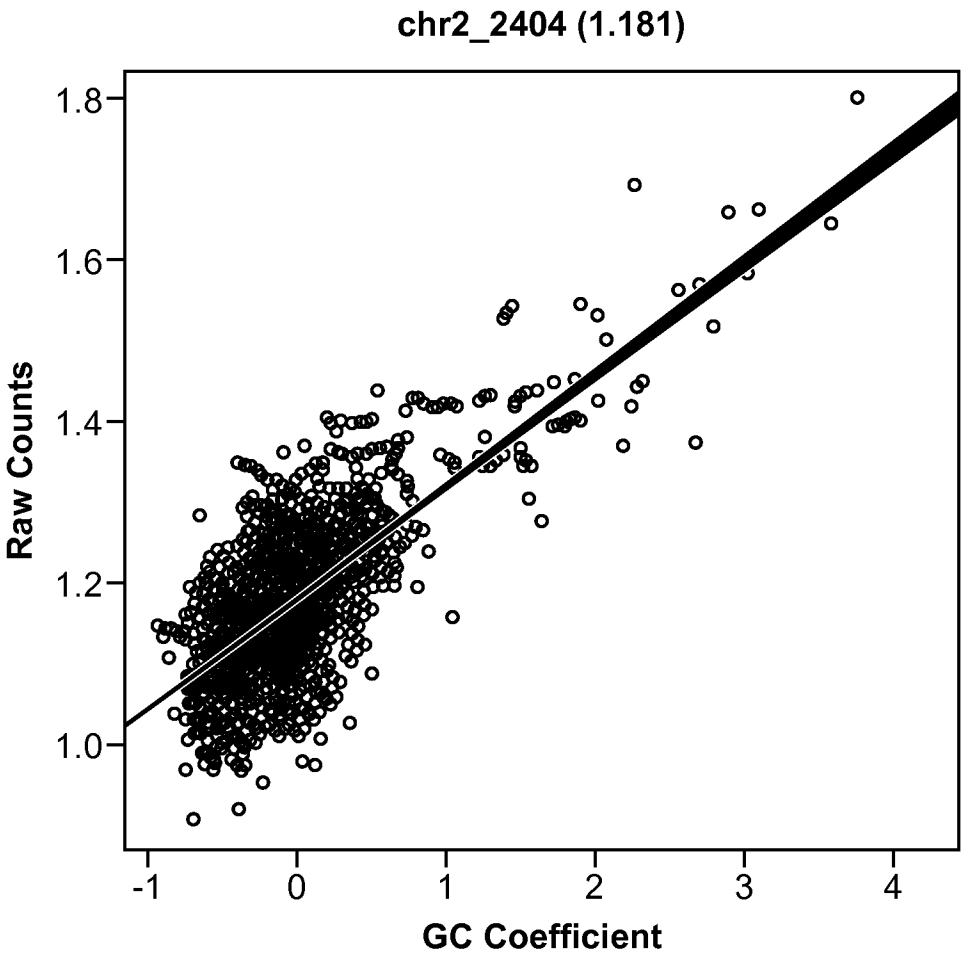

chr2_2404 (1.181)
Raw Counts
1.8
1.6
1.4
1.2
1.0
-1
0
1
2
3
4
GC Coefficient

FIG. 83

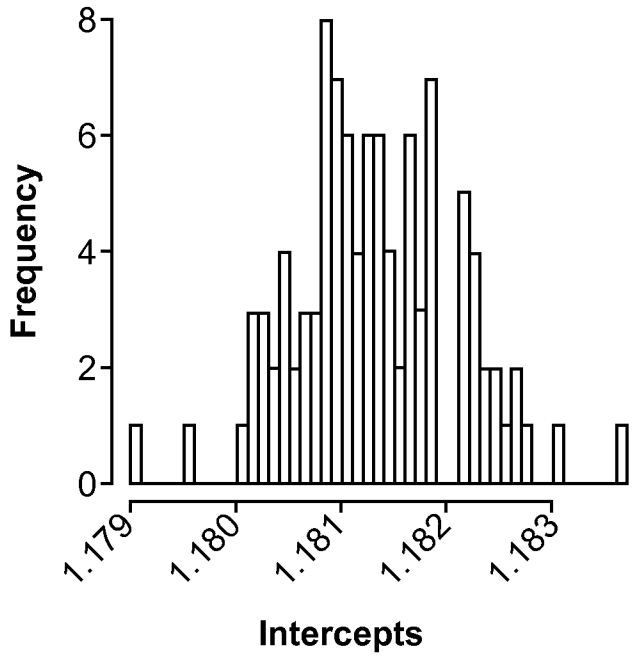

Frequency
8
6
4
2
0
1.179
1.180
1.181
1.182
1.183
Intercepts

FIG. 84

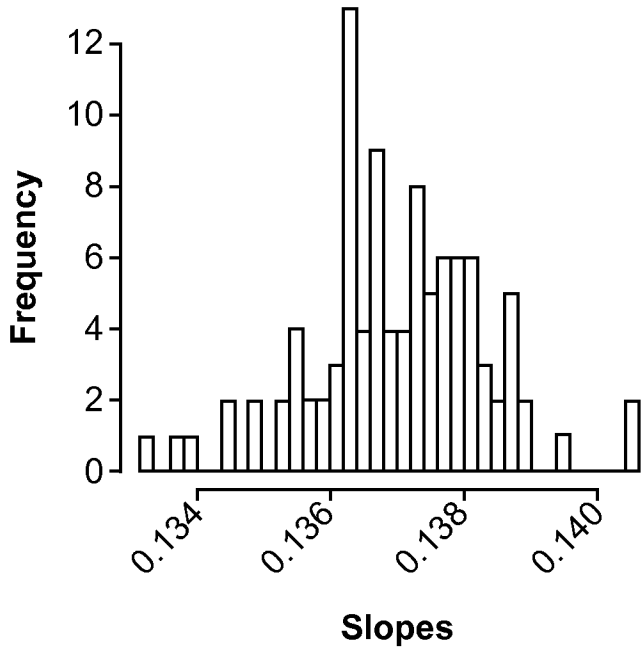

Frequency
12
10
8
6
4
2
0
0.134
0.136
0.138
0.140
Slopes

FIG. 85

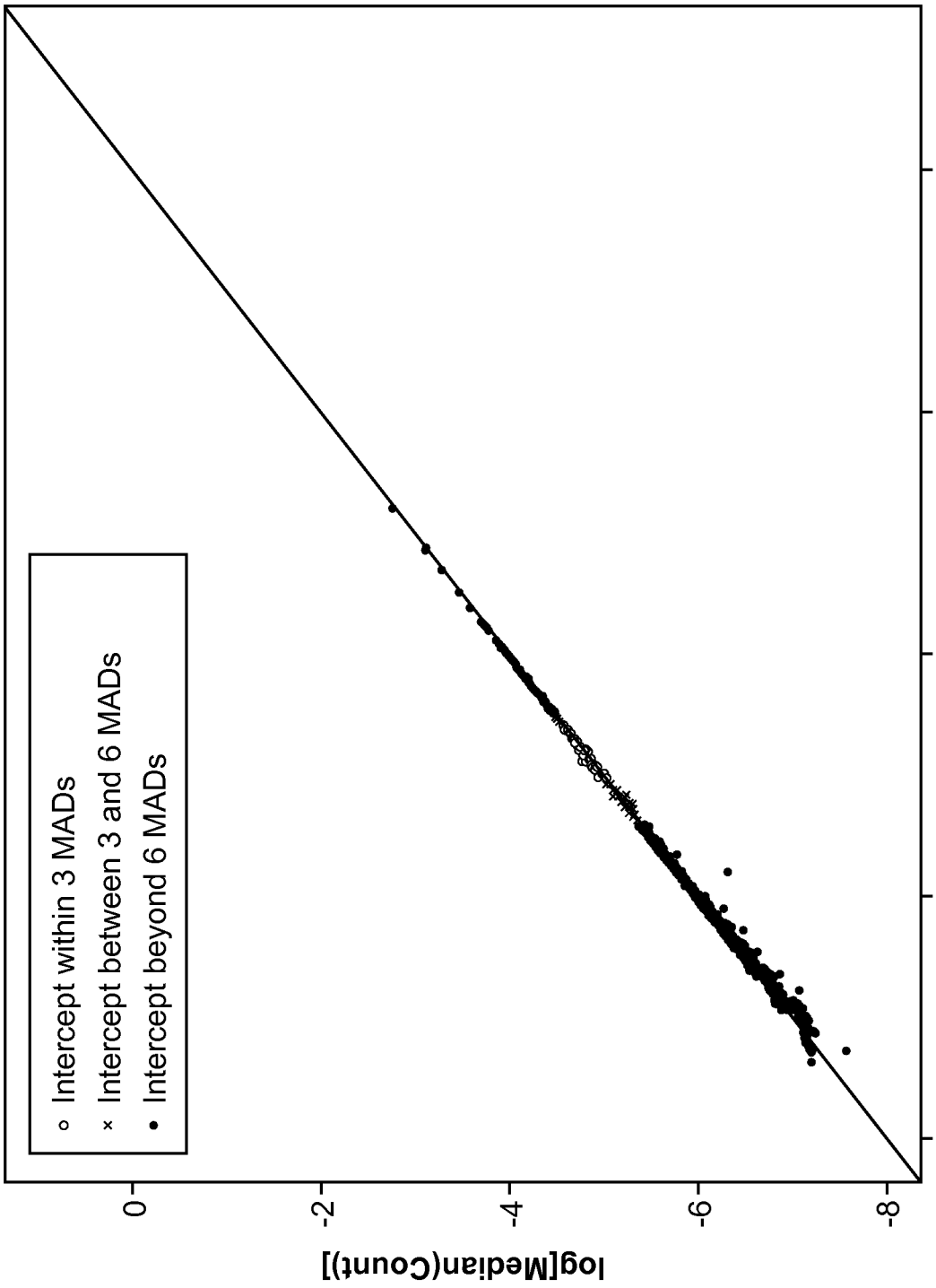

log[Median(Count)]
0
-2
-4
-6
-8
log(intercept)
0
-2
-4
-6
-8
∘ Intercept within 3 MADs
× Intercept between 3 and 6 MADs
• Intercept beyond 6 MADs

FIG. 86

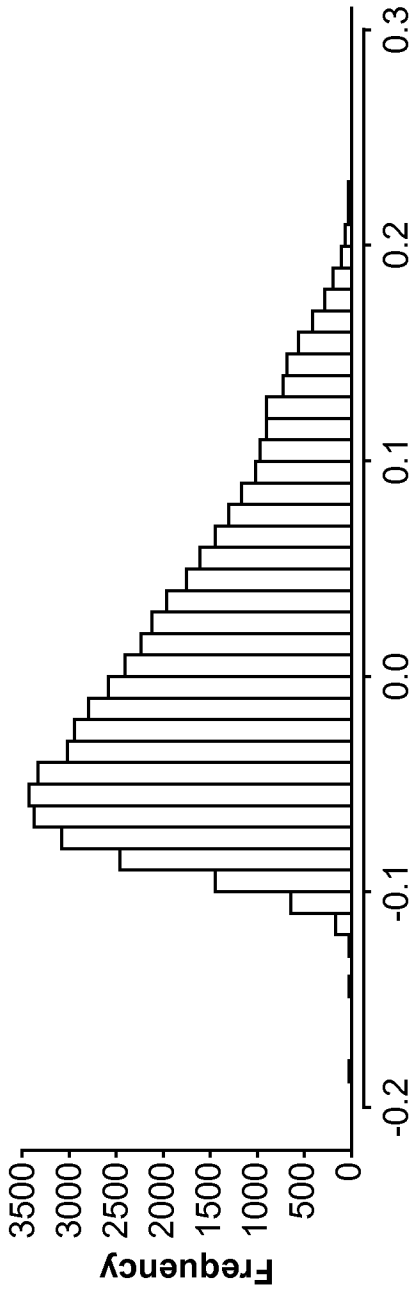

Frequency
3500
3000
2500
2000
1500
1000
500
0
-0.2
-0.1
0.0
0.1
0.2
0.3
Slope

FIG. 87

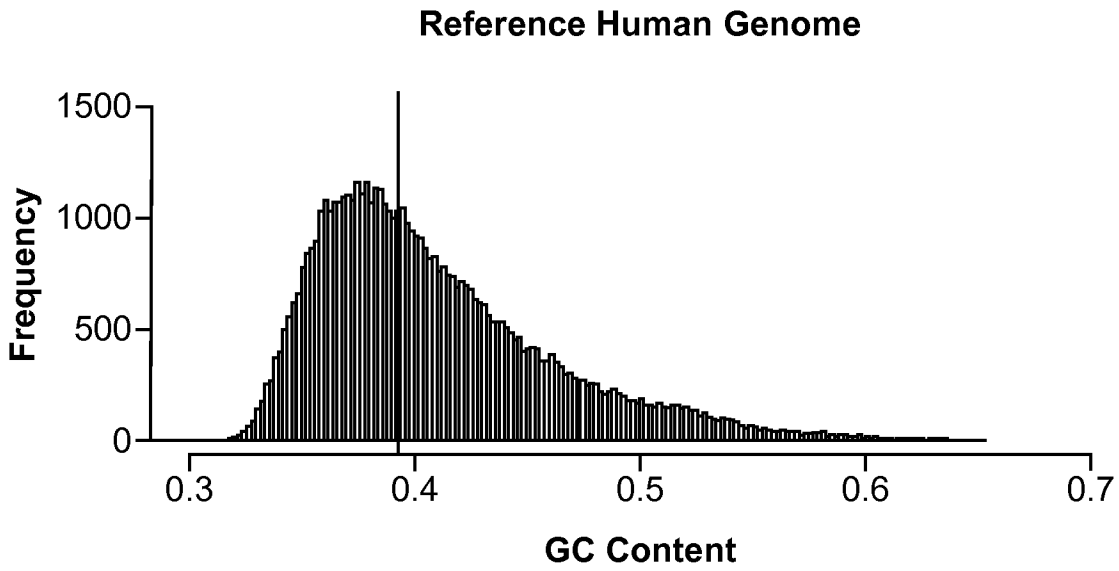

Reference Human Genome
1500
1000
500
0
Frequency
0.3
0.4
0.5
0.6
0.7
GC Content

FIG. 88

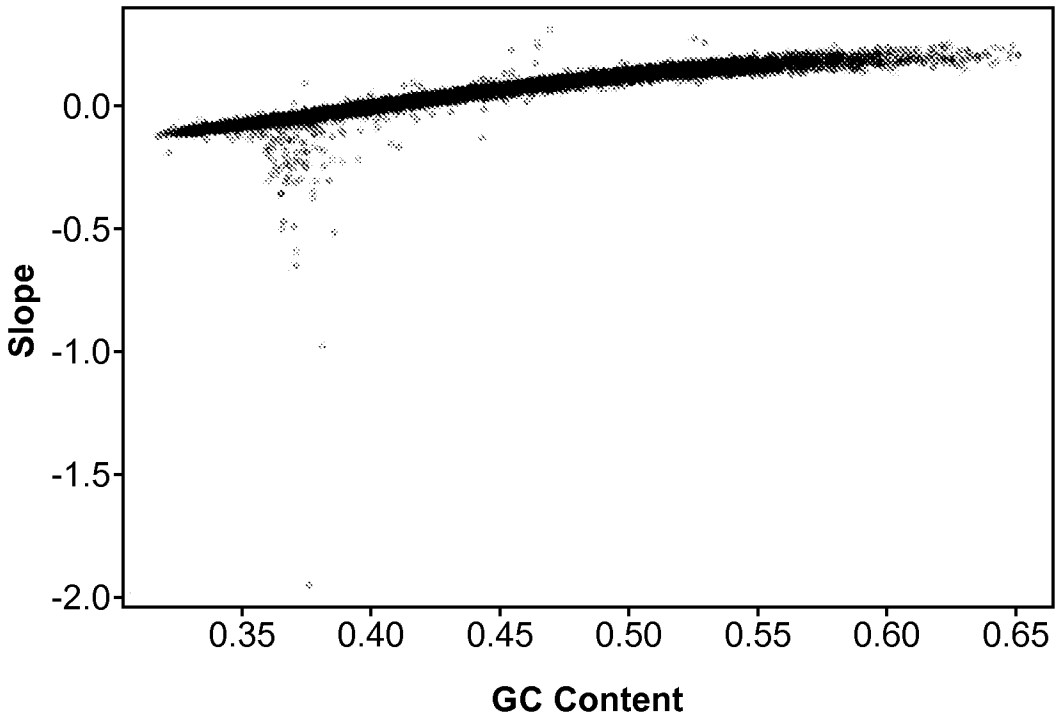


FIG. 89

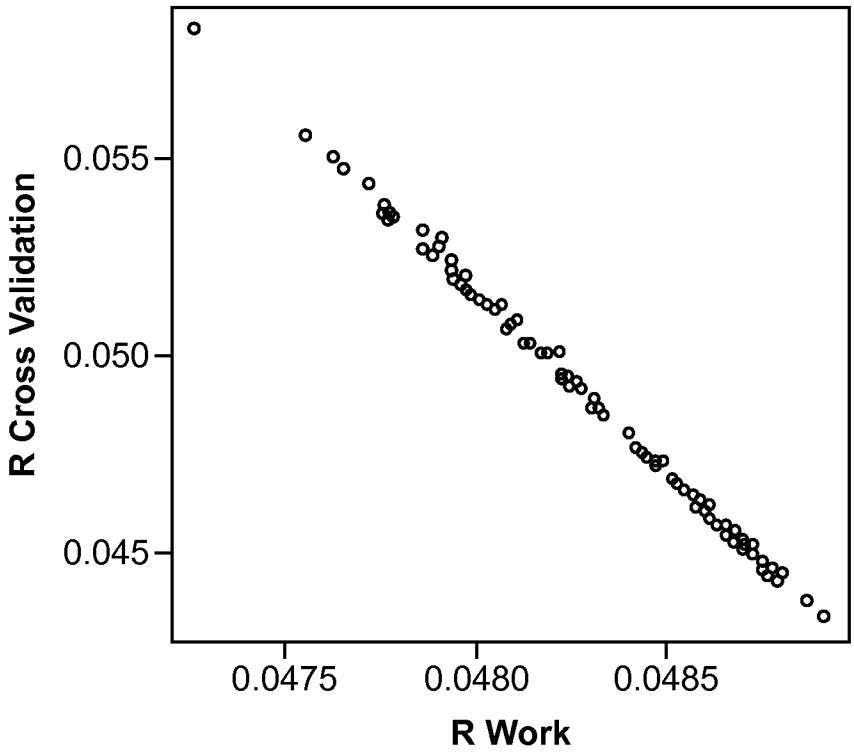


FIG. 90

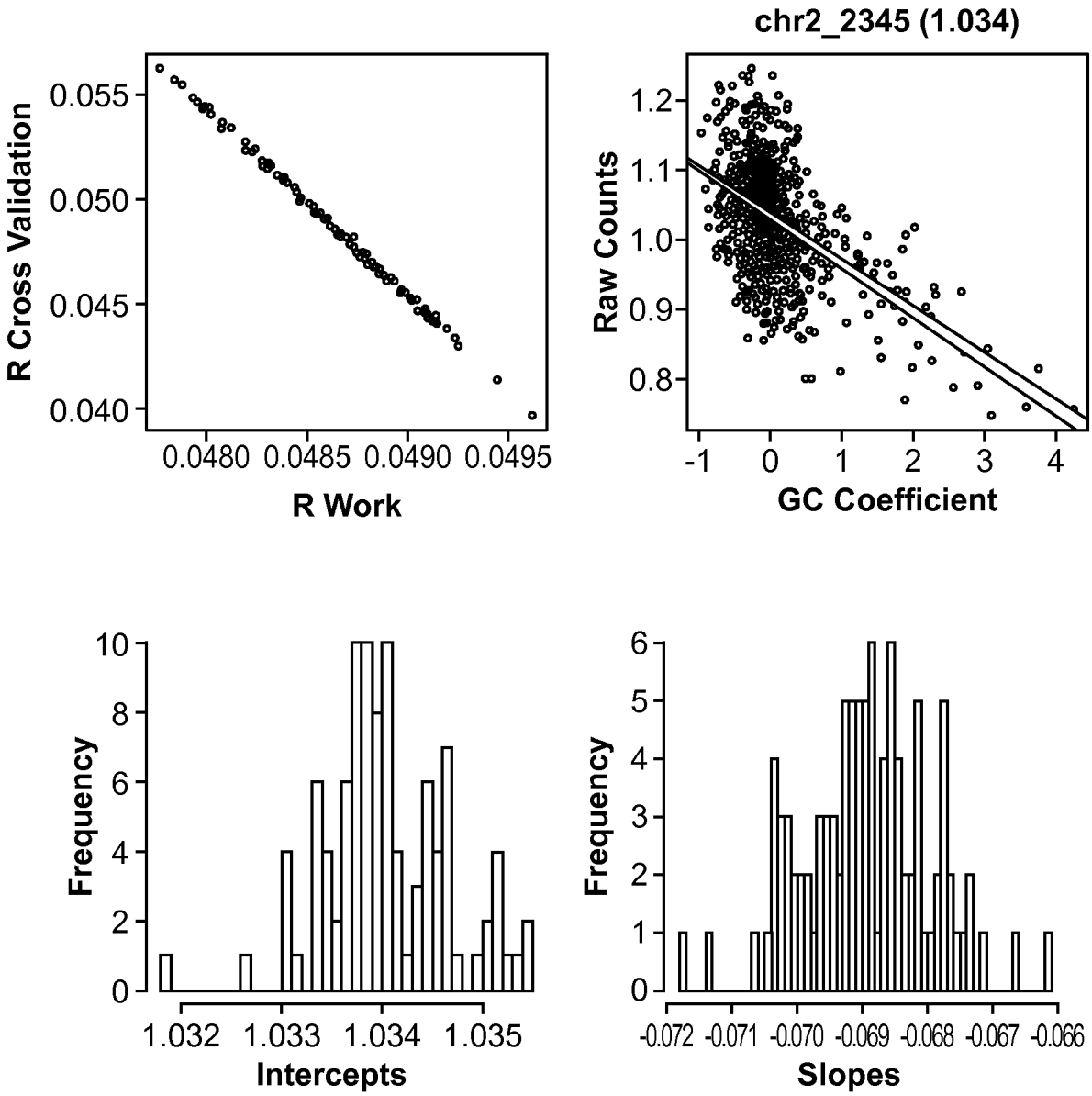

chr2_2345 (1.034)
R Cross Validation
0.055
0.050
0.045
0.040
0.0480 0.0485 0.0490 0.0495
R Work
Raw Counts
1.2
1.1
1.0
0.9
0.8
-1 0 1 2 3 4
GC Coefficient
Frequency
10
8
6
4
2
0
1.032 1.033 1.034 1.035
Intercepts
Frequency
6
5
4
3
2
1
0
-0.072 -0.071 -0.070 -0.069 -0.068 -0.067 -0.066
Slopes

FIG. 91

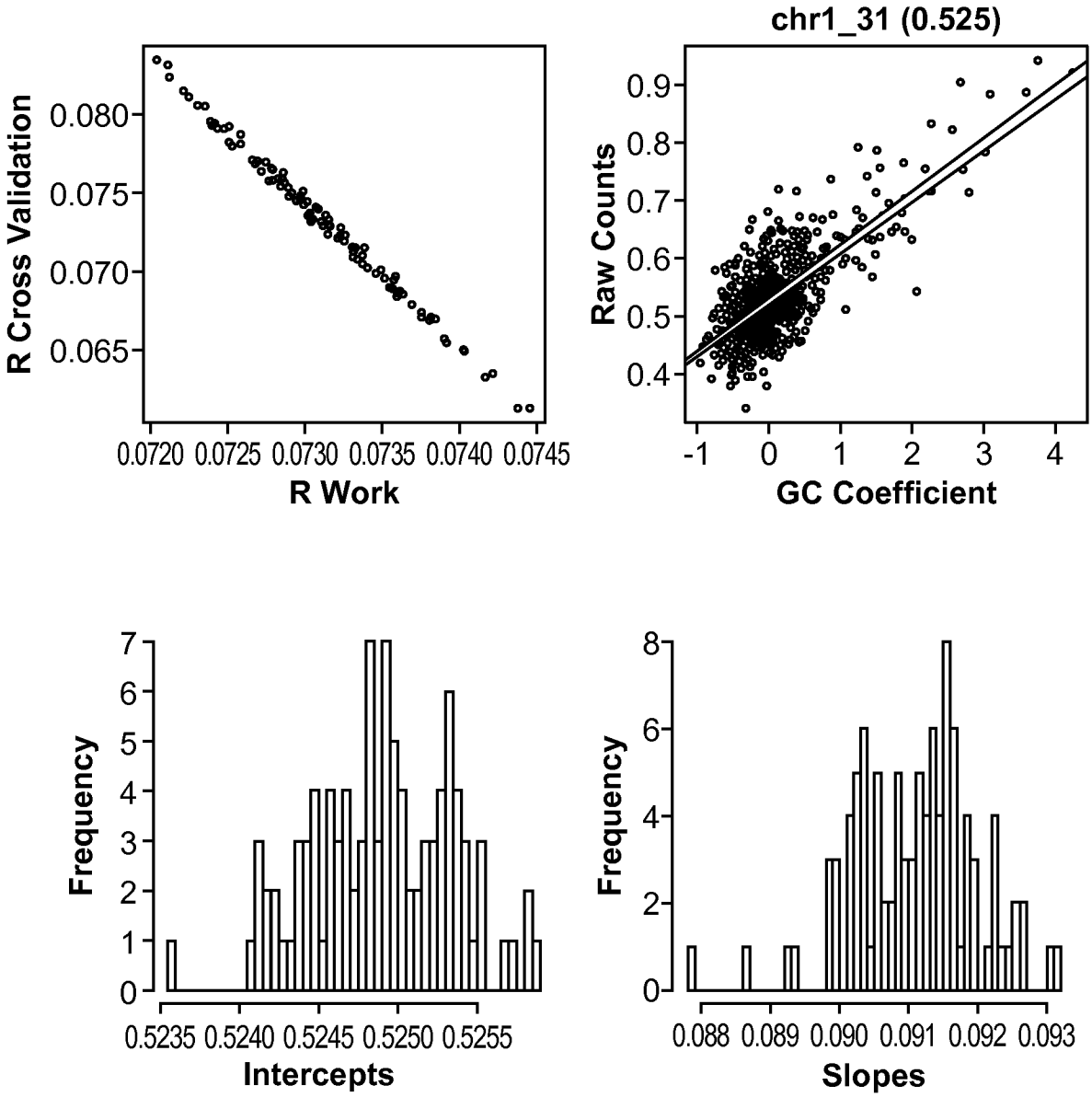

R Cross Validation
0.080
0.075
0.070
0.065
0.0720 0.0725 0.0730 0.0735 0.0740 0.0745
R Work
chr1_31 (0.525)
Raw Counts
0.9
0.8
0.7
0.6
0.5
0.4
-1 0 1 2 3 4
GC Coefficient
Frequency
7
6
5
4
3
2
1
0
0.5235 0.5240 0.5245 0.5250 0.5255
Intercepts
Frequency
8
6
4
2
0
0.088 0.089 0.090 0.091 0.092 0.093
Slopes

FIG. 92

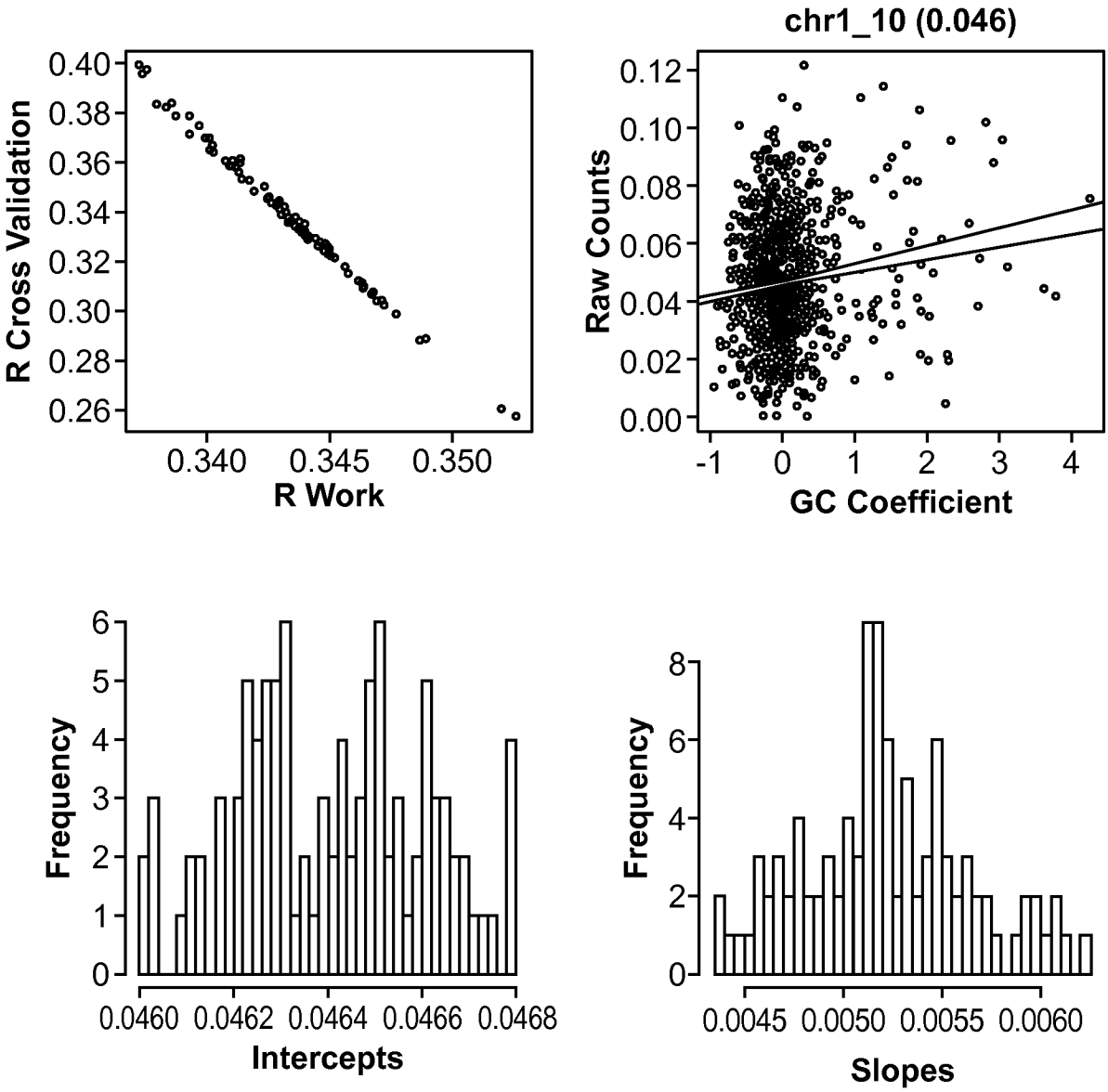

R Cross Validation
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.340
0.345
0.350
R Work
chr1_10 (0.046)
Raw Counts
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-1
0
1
2
3
4
GC Coefficient
Frequency
6
5
4
3
2
1
0
0.0460
0.0462
0.0464
0.0466
0.0468
Intercepts
Frequency
8
6
4
2
0
0.0045
0.0050
0.0055
0.0060
Slopes

FIG. 93

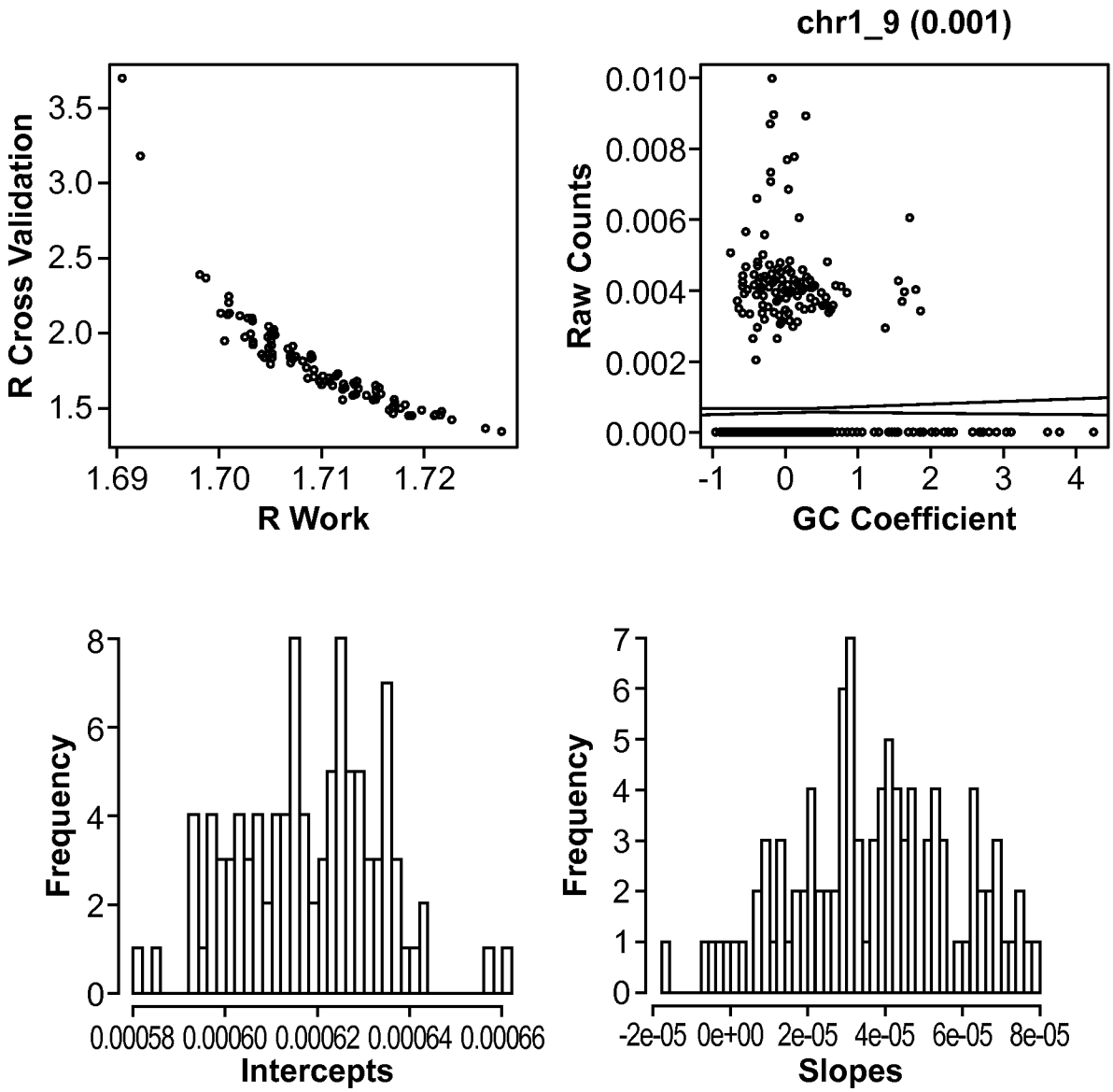

chr1_9 (0.001)
R Cross Validation
3.5
3.0
2.5
2.0
1.5
1.69
1.70
1.71
1.72
R Work
Raw Counts
0.010
0.008
0.006
0.004
0.002
0.000
-1
0
1
2
3
4
GC Coefficient
Frequency
8
6
4
2
0
0.00058
0.00060
0.00062
0.00064
0.00066
Intercepts
Frequency
7
6
5
4
3
2
1
0
-2e-05
0e+00
2e-05
4e-05
6e-05
8e-05
Slopes

FIG. 94

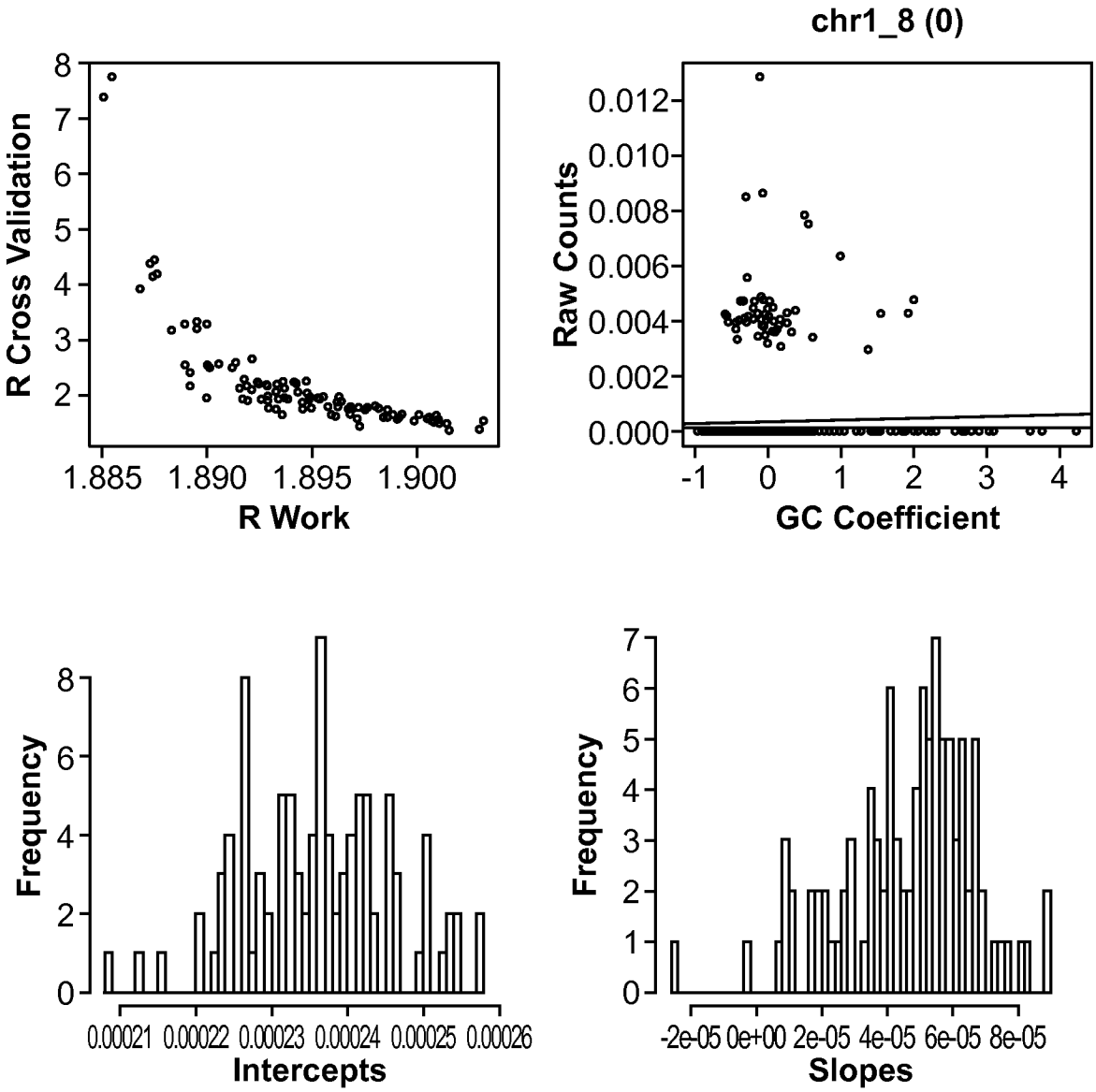

chr1_8 (0)
R Cross Validation
R Work
Raw Counts
GC Coefficient
Frequency
Intercepts
Frequency
Slopes

FIG. 95

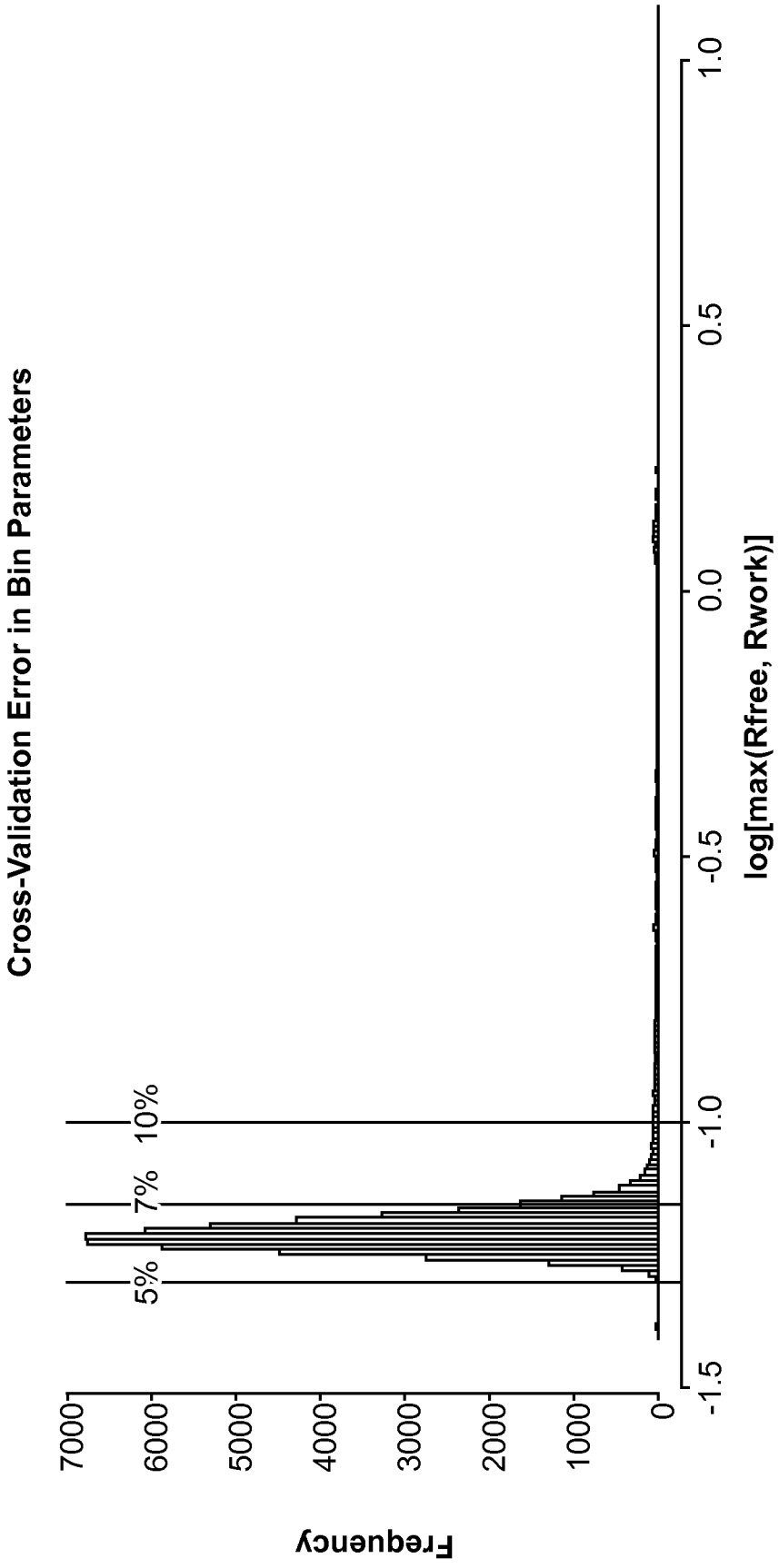

Cross-Validation Error in Bin Parameters
Frequency
7000
6000
5000
4000
3000
2000
1000
0
-1.5
-1.0
-0.5
0.0
0.5
1.0
log[max(Rfree, Rwork)]
5%
7%
10%

FIG. 96

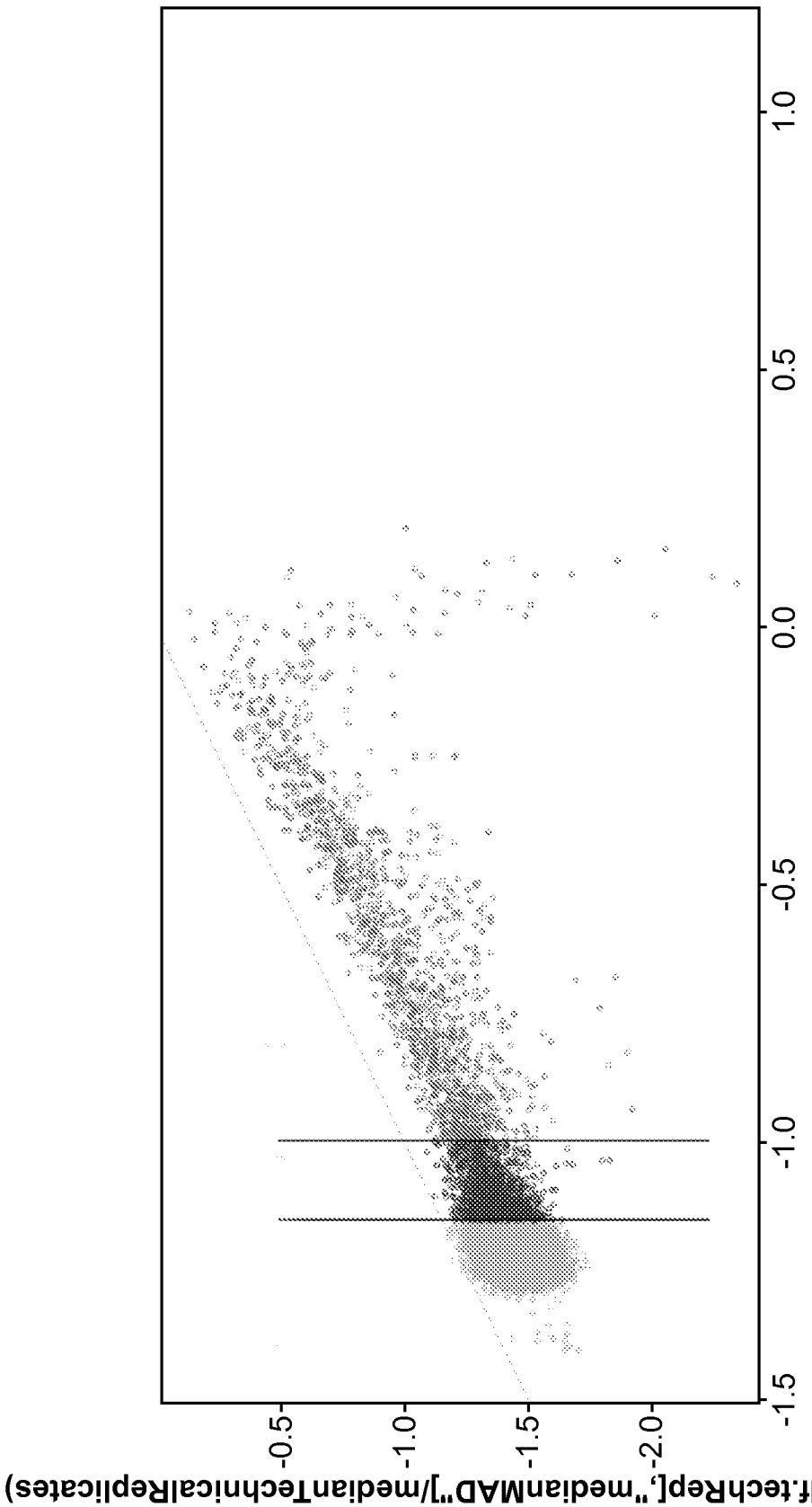

log10(df.techRep[,"medianMAD"]/medianTechnicalReplicates)
-0.5
-1.0
-1.5
-2.0
log10(max Cross Validation Errors)
-1.5
-1.0
-0.5
0.0
0.5
1.0

FIG. 97

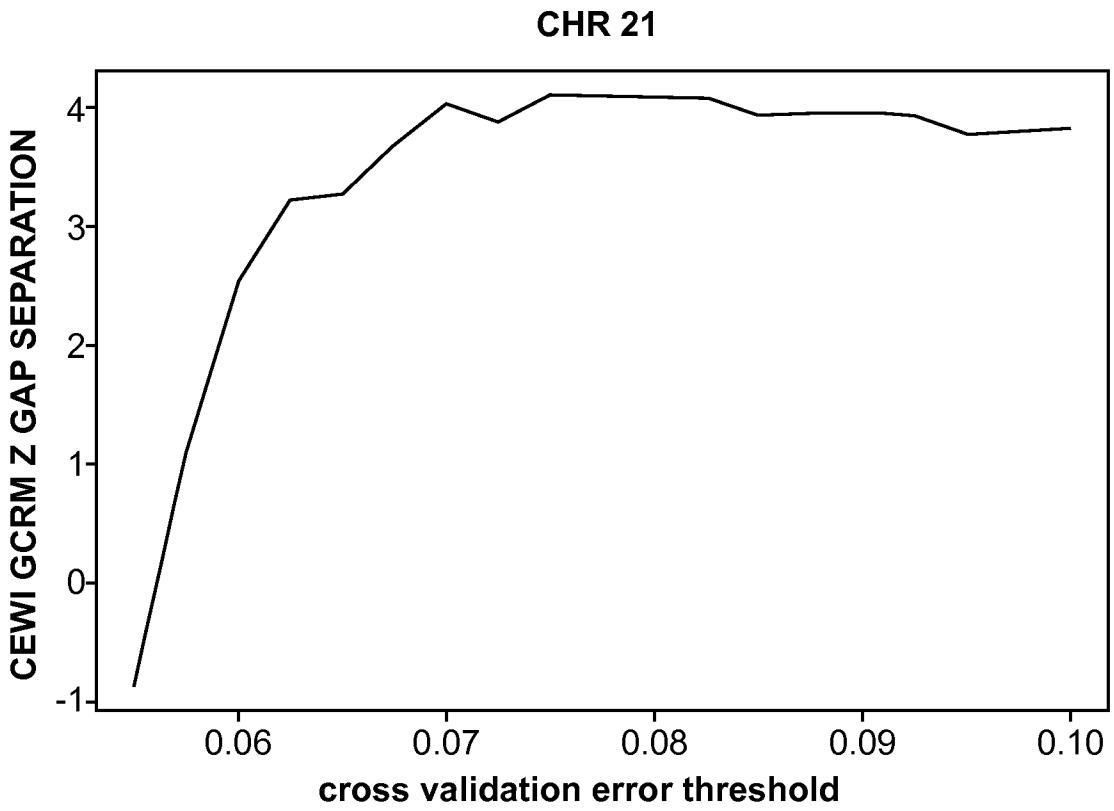

CHR 21
CEWI GCRM Z GAP SEPARATION
4
3
2
1
0
-1
0.06
0.07
0.08
0.09
0.10
cross validation error threshold

FIG. 98

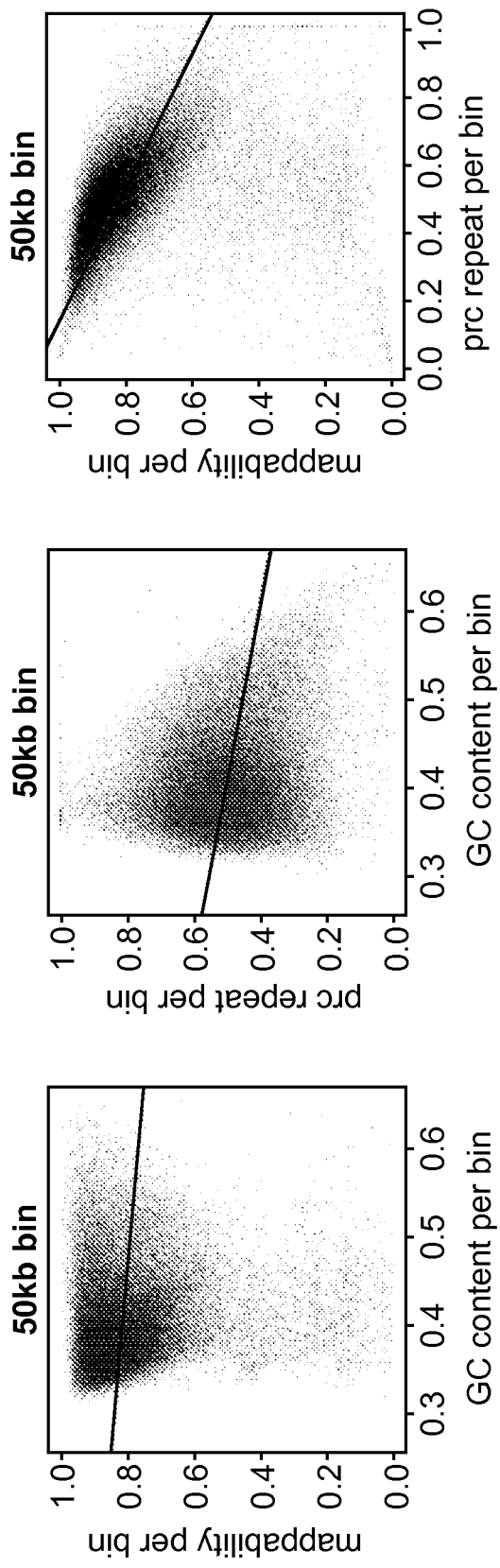


FIG. 99A

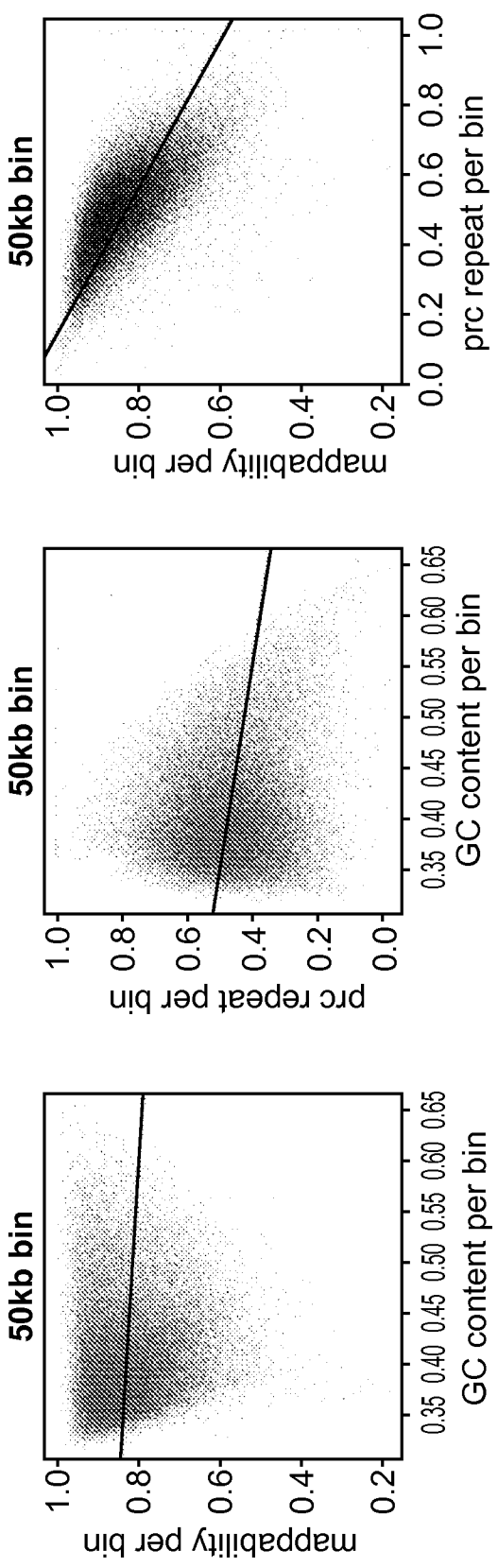


FIG. 99B

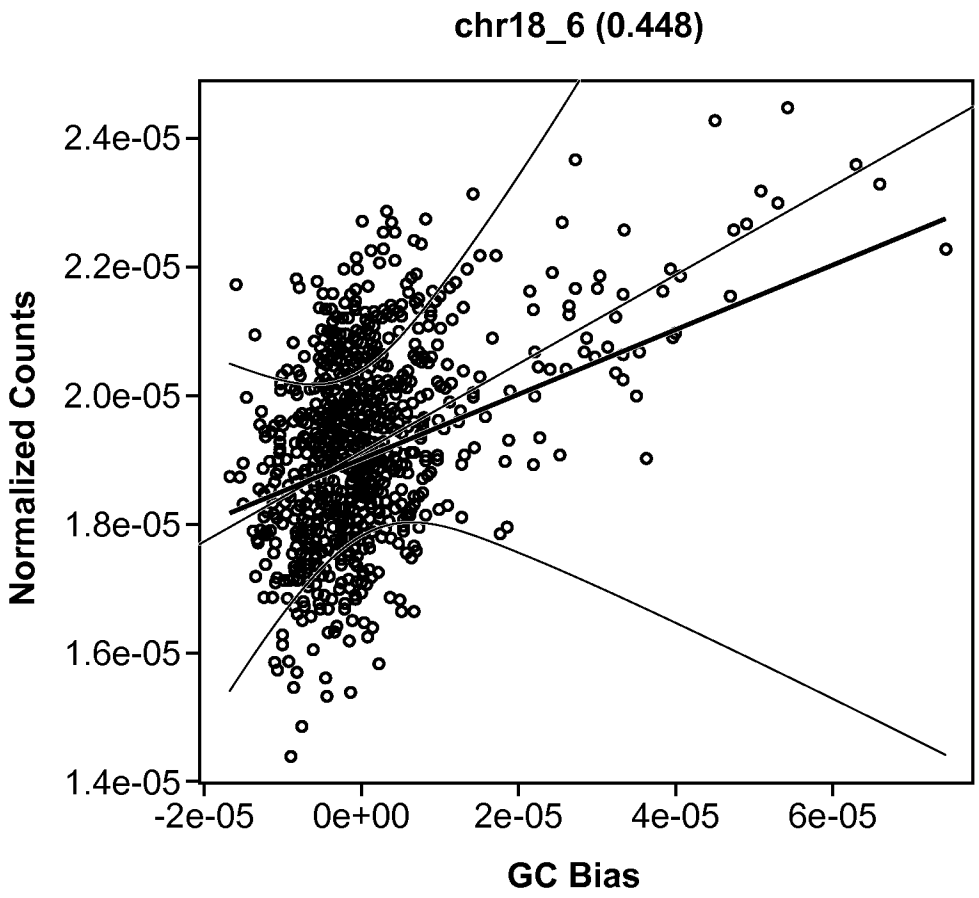

chr18_6 (0.448)
Normalized Counts
2.4e-05
2.2e-05
2.0e-05
1.8e-05
1.6e-05
1.4e-05
-2e-05
0e+00
2e-05
4e-05
6e-05
GC Bias

FIG. 100

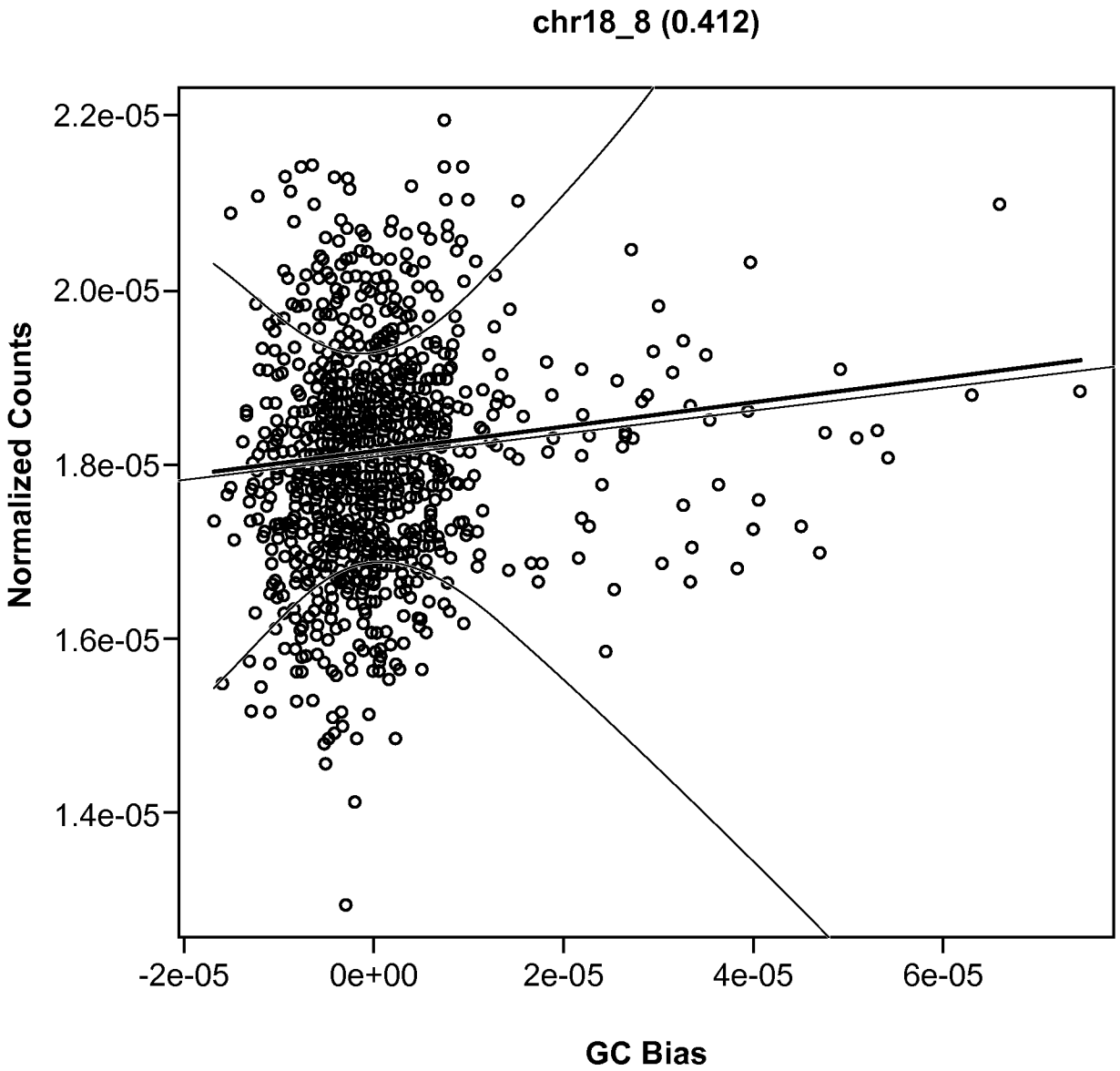

chr18_8 (0.412)
2.2e-05
2.0e-05
1.8e-05
1.6e-05
1.4e-05
Normalized Counts
-2e-05
0e+00
2e-05
4e-05
6e-05
GC Bias

FIG. 101

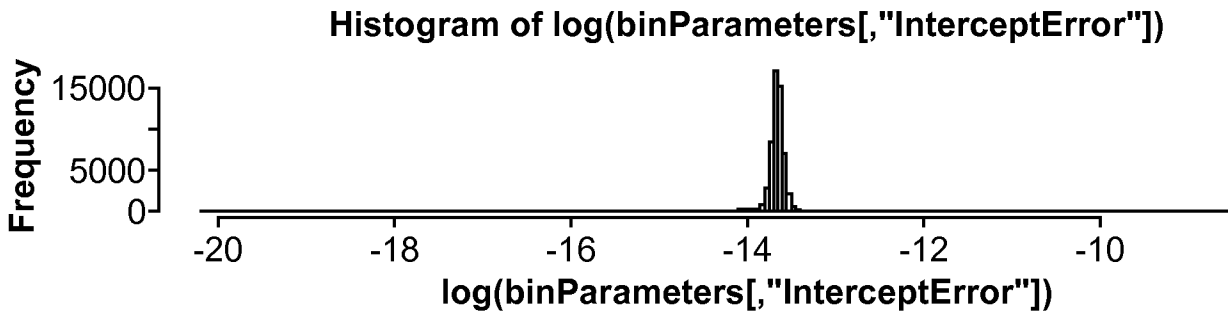

Histogram of log(binParameters[,"InterceptError"])
Frequency
15000
5000
0
-20
-18
-16
-14
-12
-10
log(binParameters[,"InterceptError"])

FIG. 102

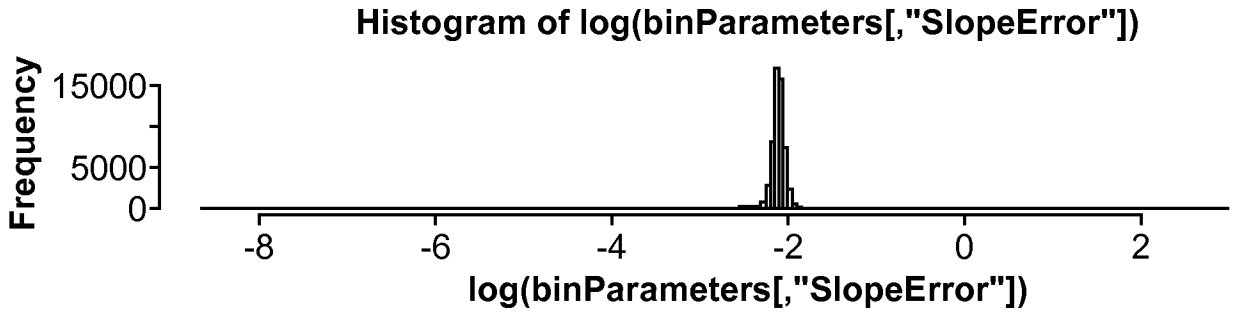

Histogram of log(binParameters[,"SlopeError"])
Frequency
15000
5000
0
-8
-6
-4
-2
0
2
log(binParameters[,"SlopeError"])

FIG. 103

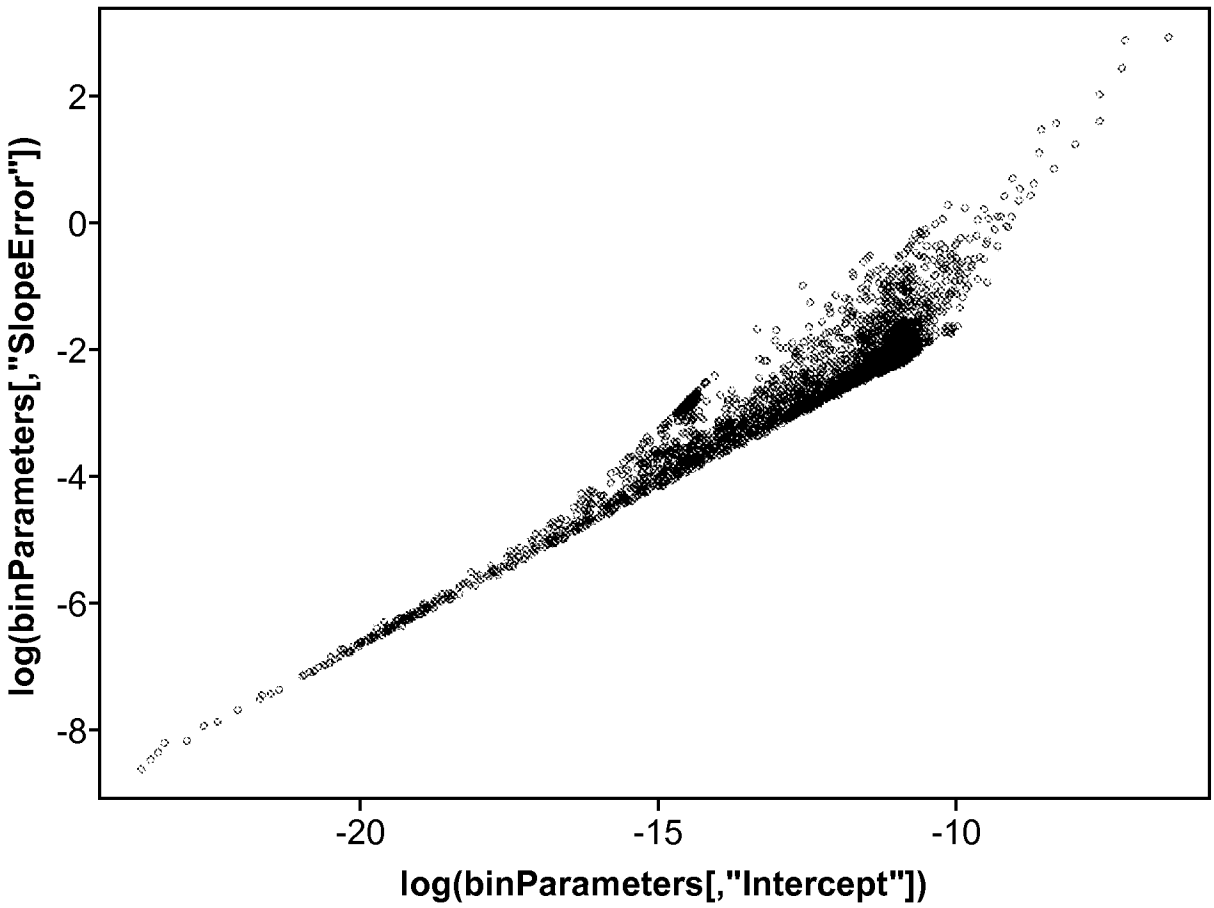

log(binParameters[,"SlopeError"])
2
0
-2
-4
-6
-8
-20
-15
-10
log(binParameters[,"Intercept"])

FIG. 104

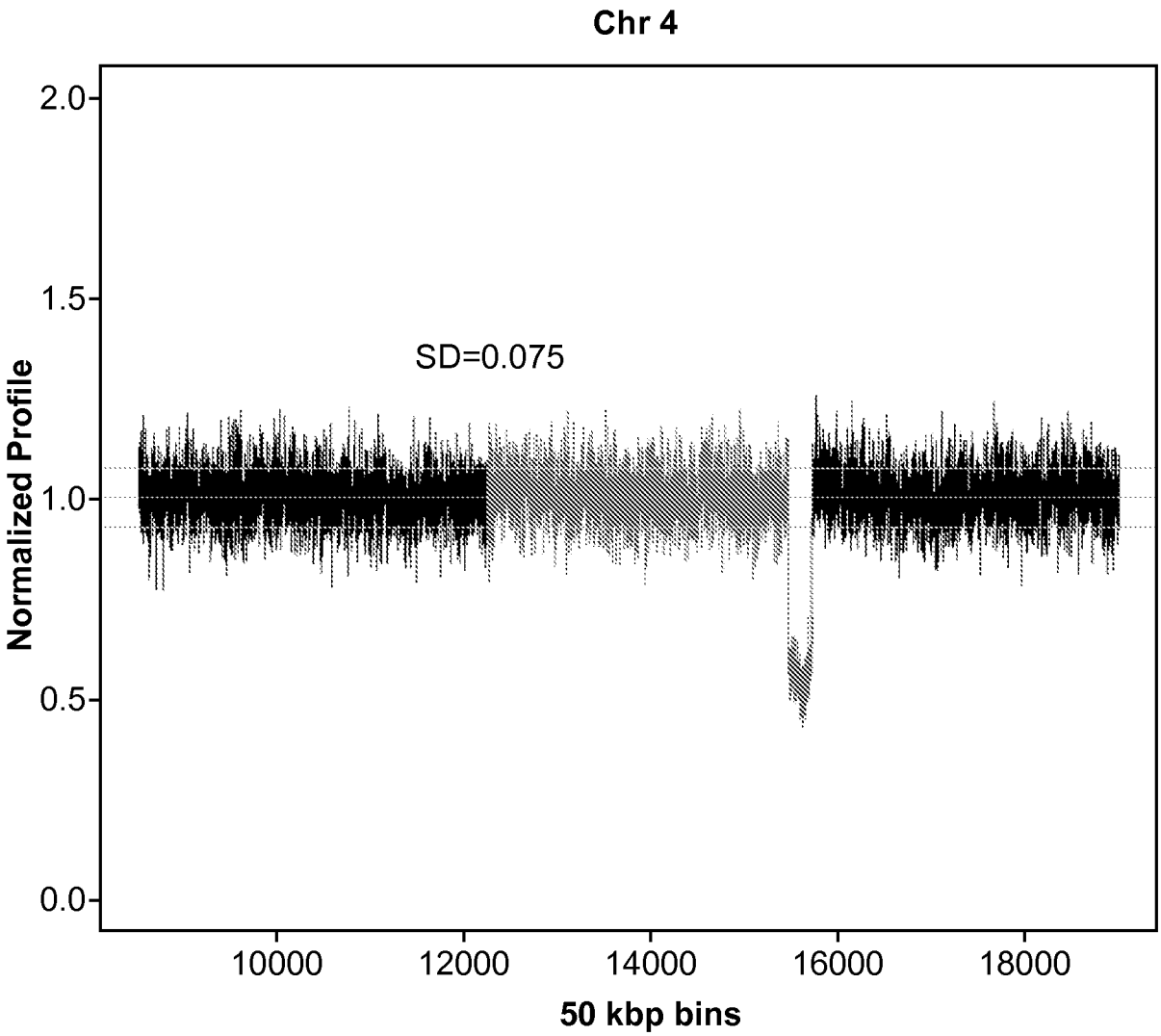

Chr 4
2.0
1.5
1.0
0.5
0.0
SD=0.075
Normalized Profile
10000
12000
14000
16000
18000
50 kbp bins

FIG. 105

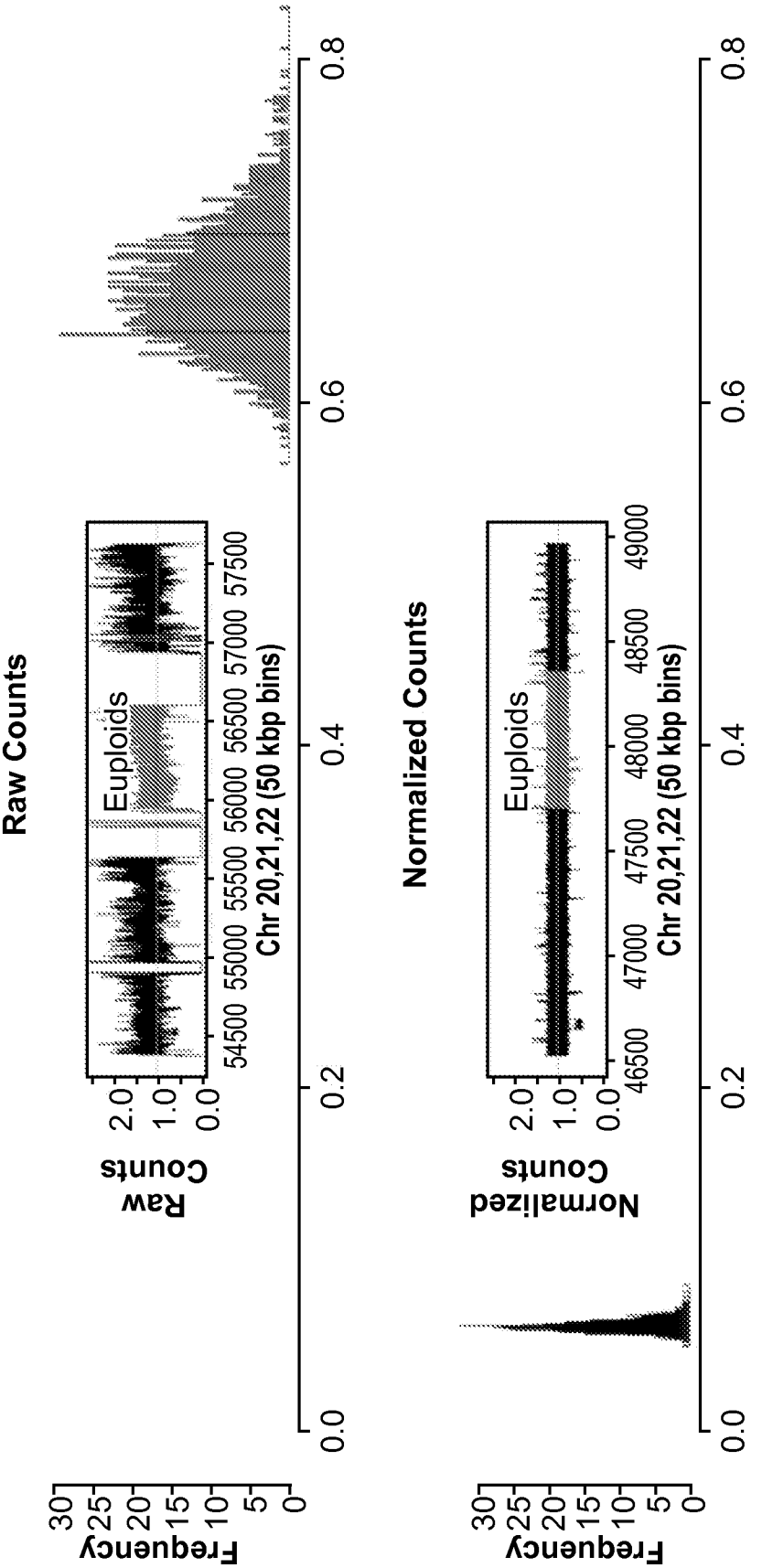

Raw Counts
Euploids
Raw Counts
Chr 20,21,22 (50 kbp bins)
Frequency
SD
Normalized Counts
Euploids
Normalized Counts
Chr 20,21,22 (50 kbp bins)
Frequency
SD

FIG. 106

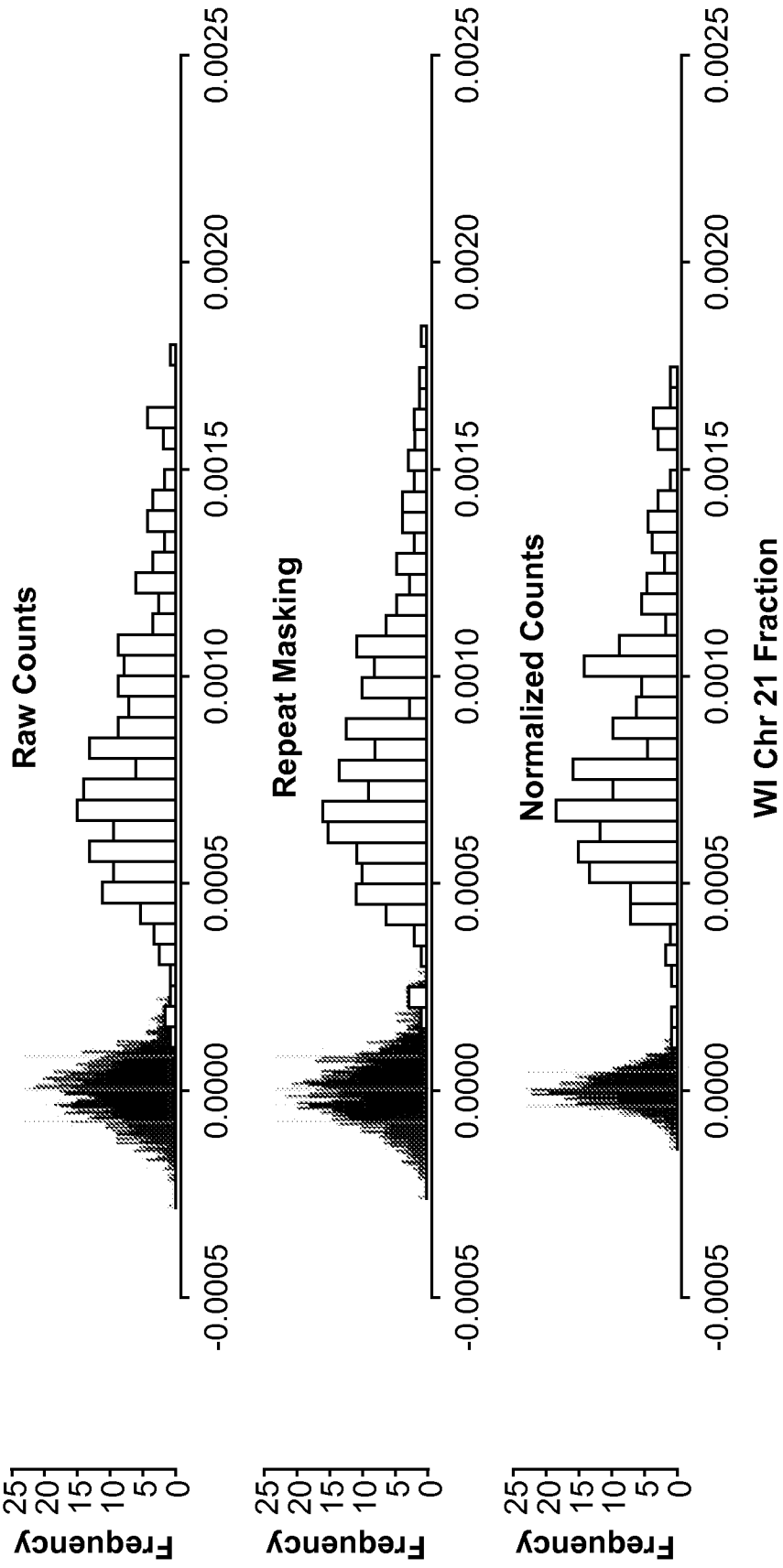

Raw Counts
Repeat Masking
Normalized Counts
Frequency
25
20
15
10
5
0
-0.0005
0.0000
0.0005
0.0010
0.0015
0.0020
0.0025
WI Chr 21 Fraction

FIG. 107

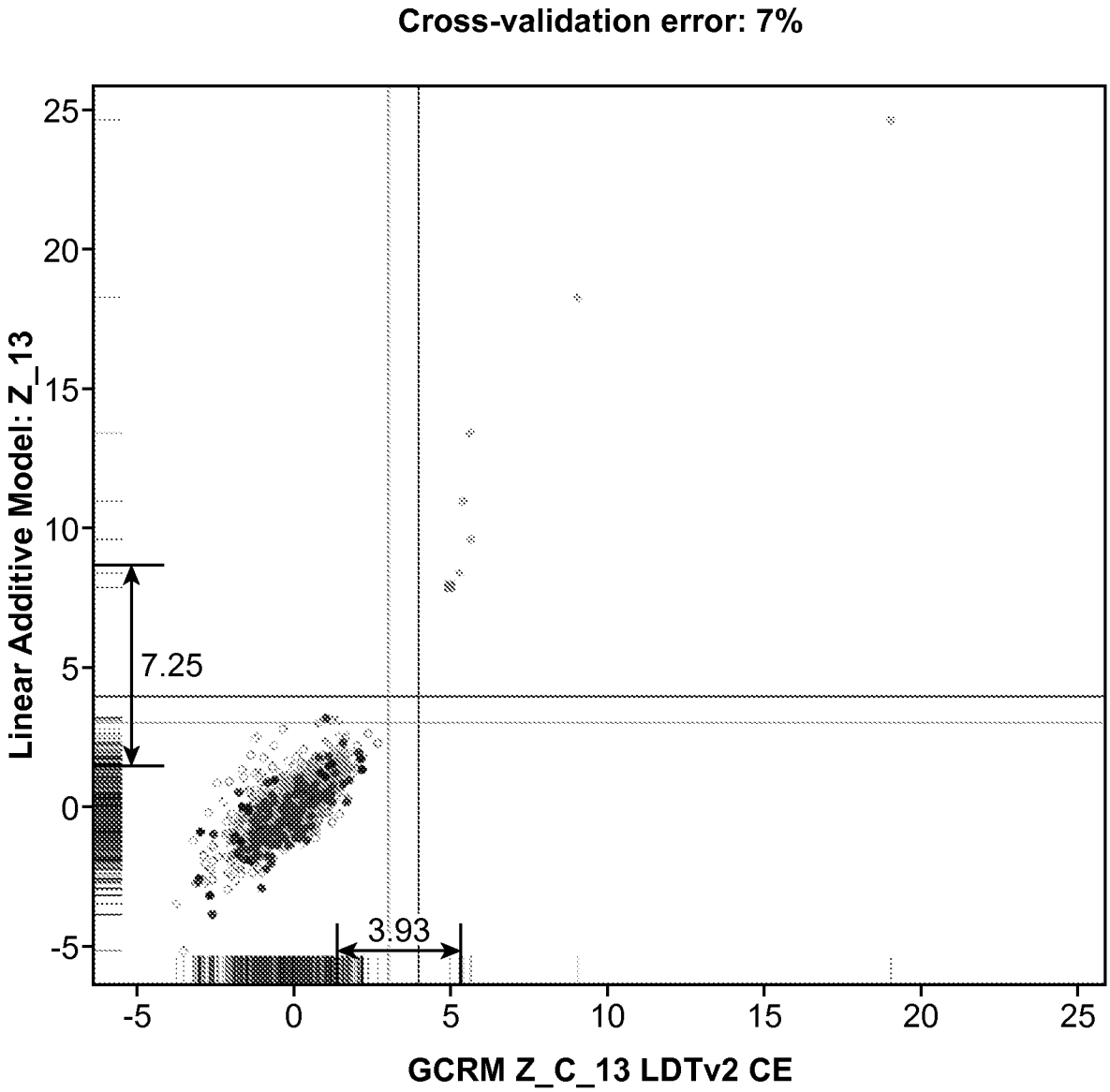

Cross-validation error: 7%
Linear Additive Model: Z_13
GCRM Z_C_13 LDTv2 CE
7.25
3.93
25
20
15
10
5
0
-5
-5
0
5
10
15
20
25

FIG. 108

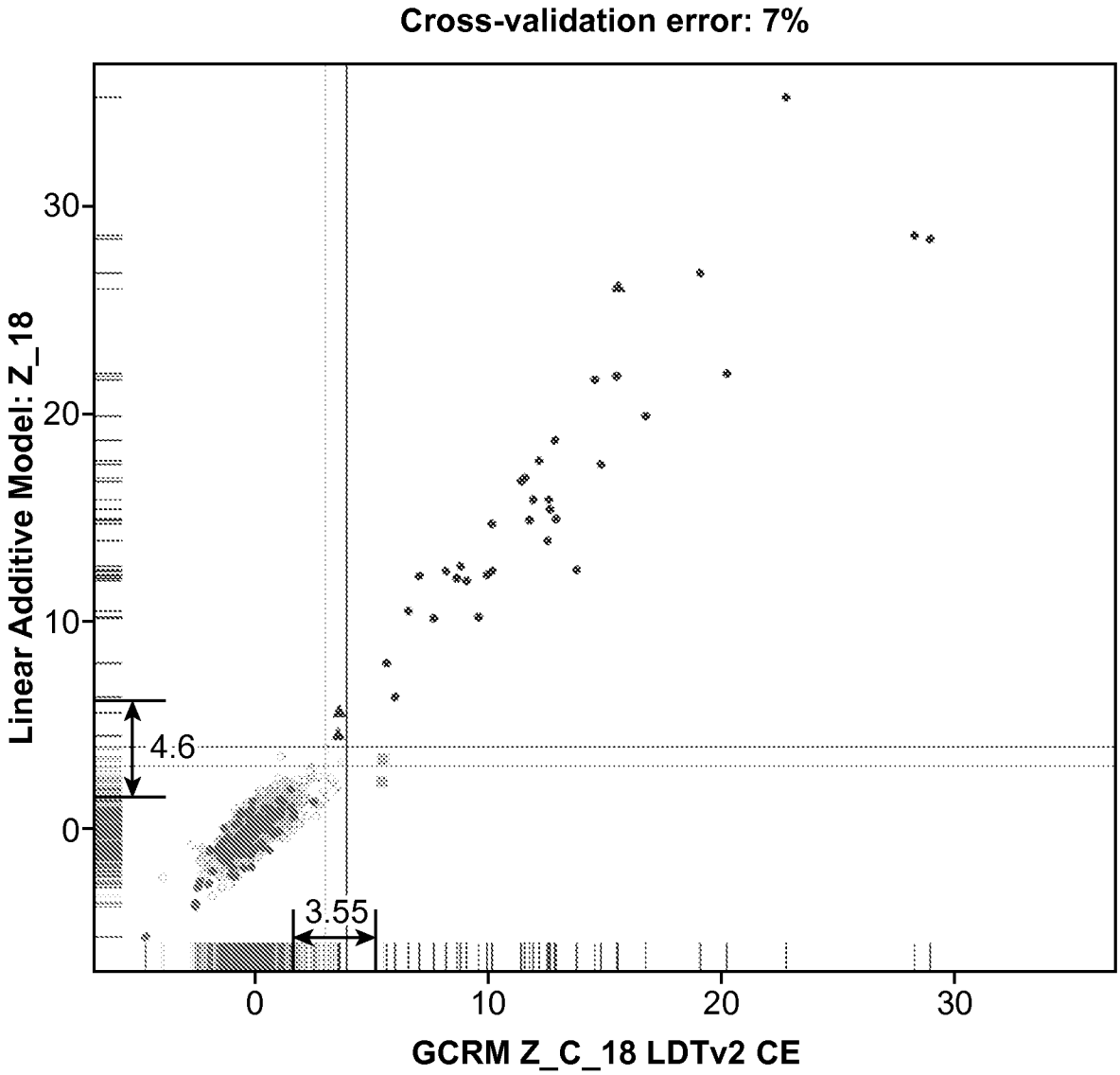

Cross-validation error: 7%
Linear Additive Model: Z_18
GCRM Z_C_18 LDTv2 CE
30
20
10
0
4.6
3.55
0
10
20
30

FIG. 109

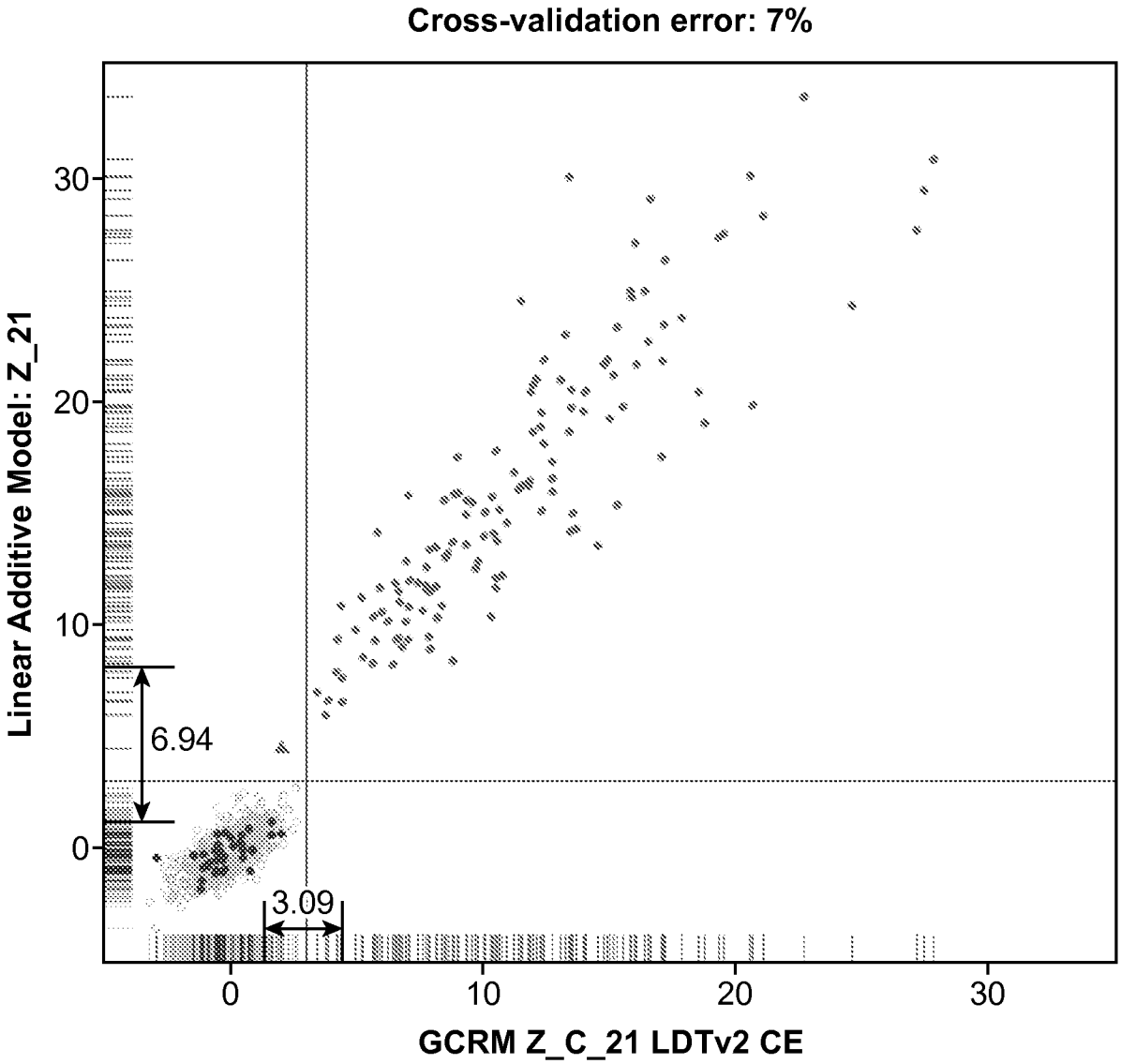

Cross-validation error: 7%
Linear Additive Model: Z_21
GCRM Z_C_21 LDTv2 CE
6.94
3.09
0
10
20
30

FIG. 110

high negative control >3, low fetal percentage with boderline Z-score

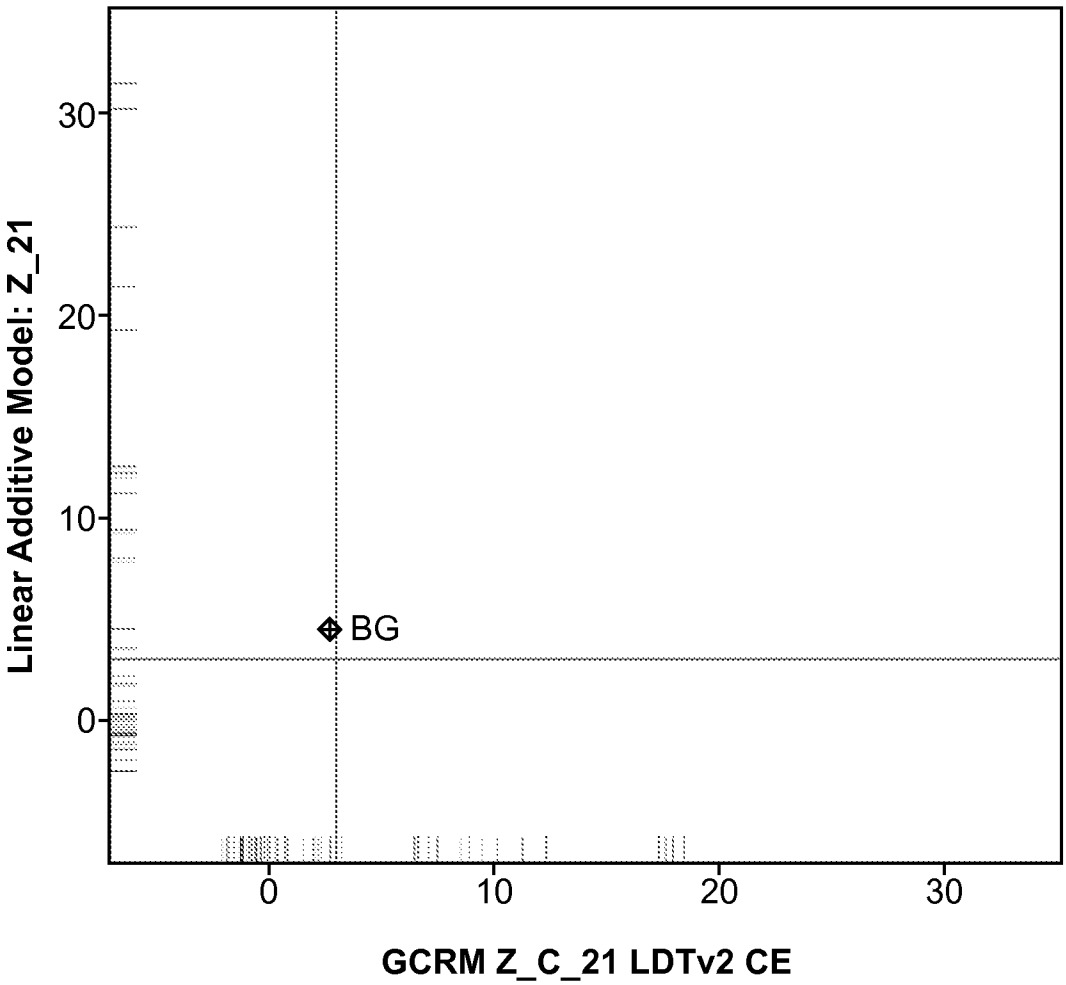


FIG. 111

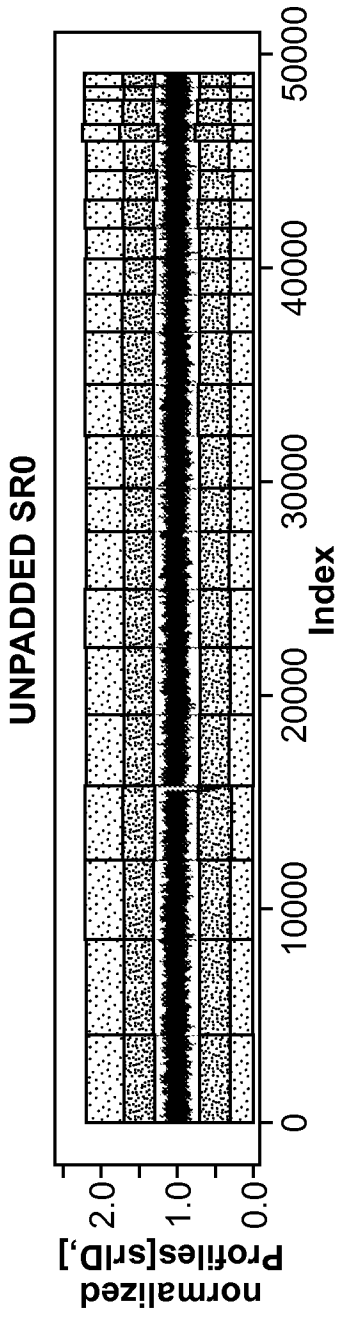


FIG. 112A

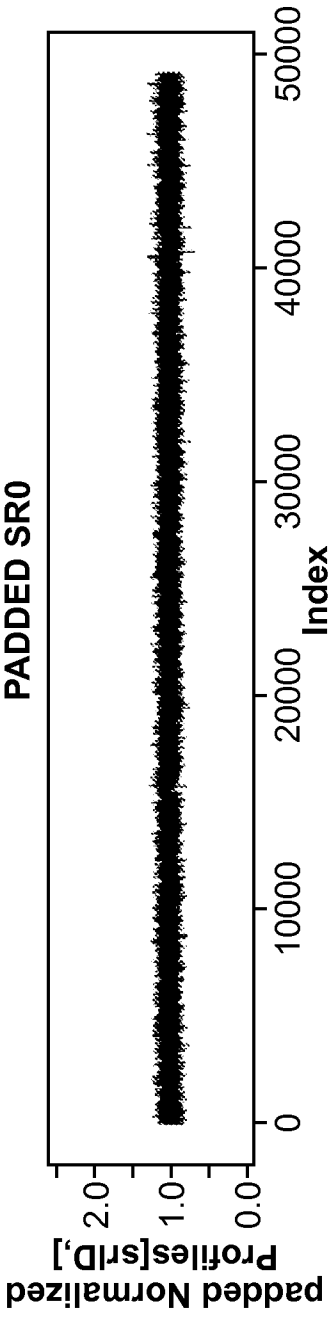


FIG. 112B

FIG. 112C

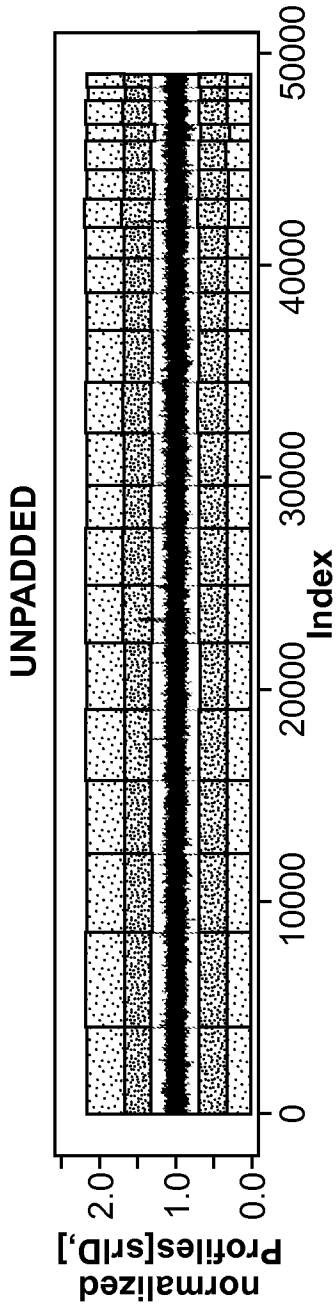


FIG. 113A

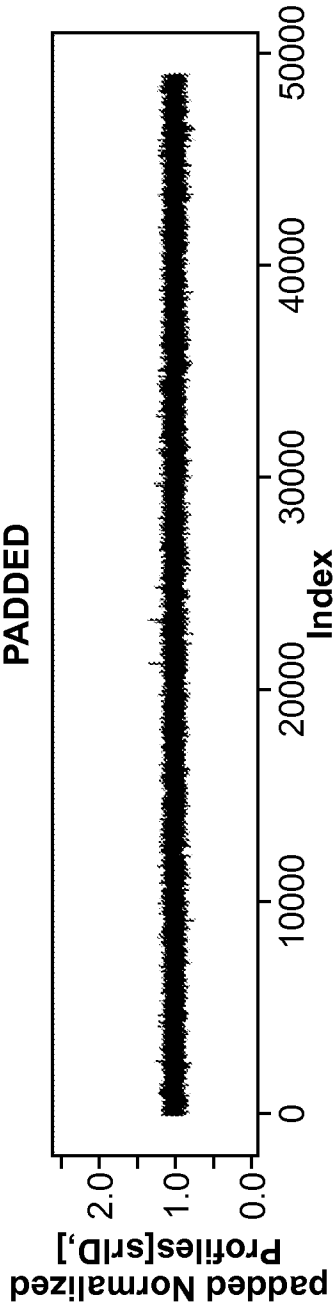


FIG. 113B

FIG. 113C

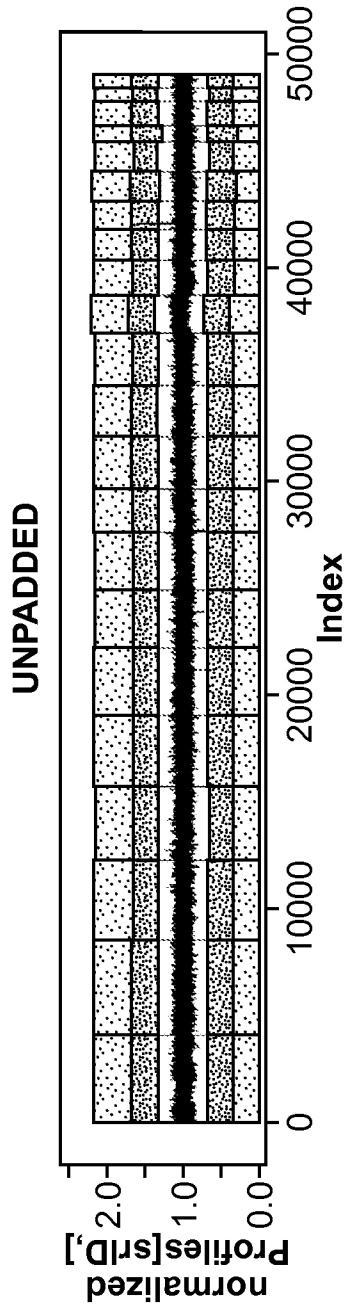


FIG. 114A

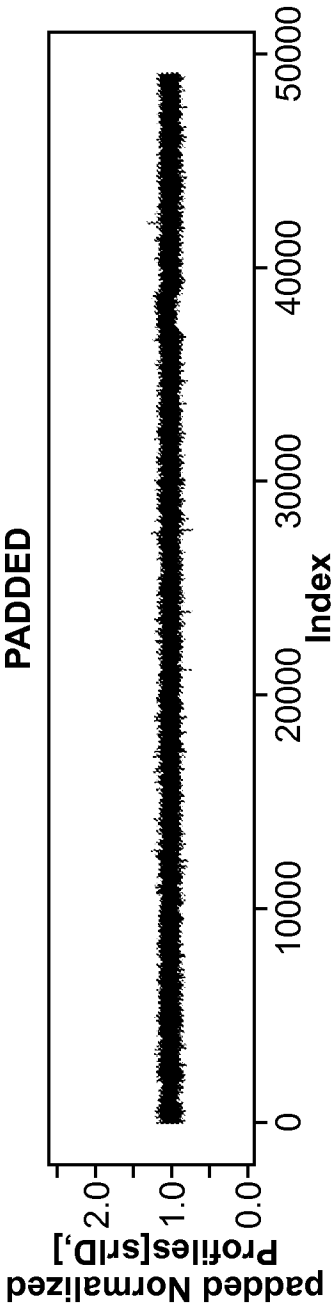


FIG. 114B

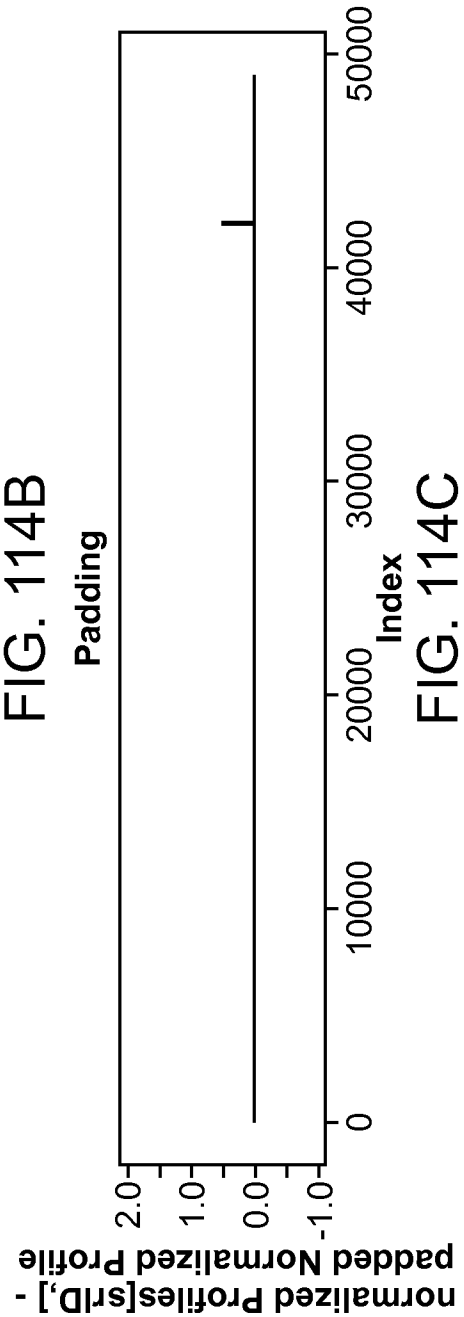


FIG. 114C

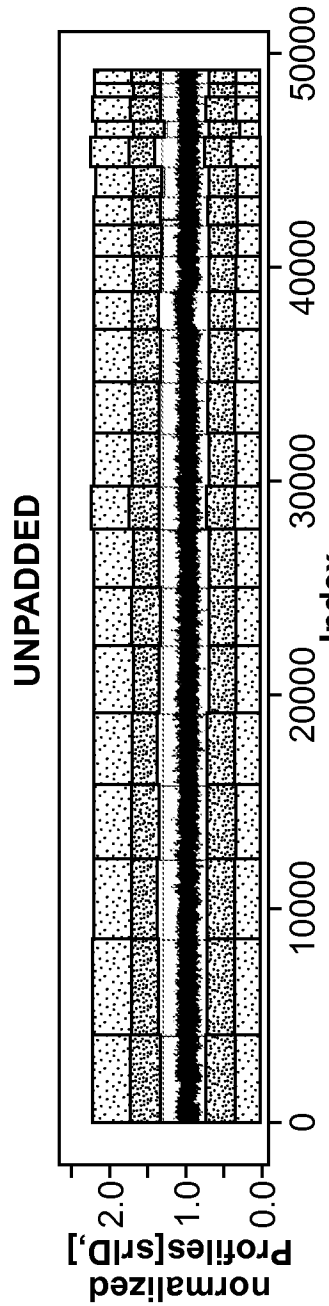


FIG. 115A

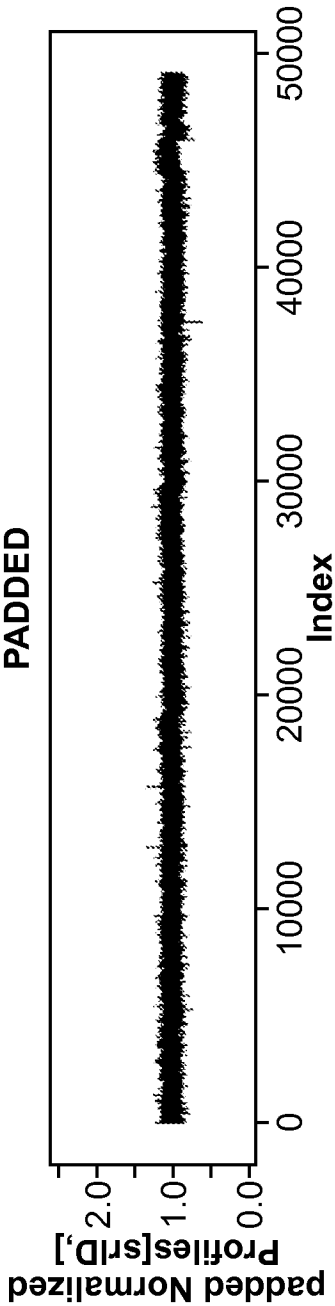


FIG. 115B

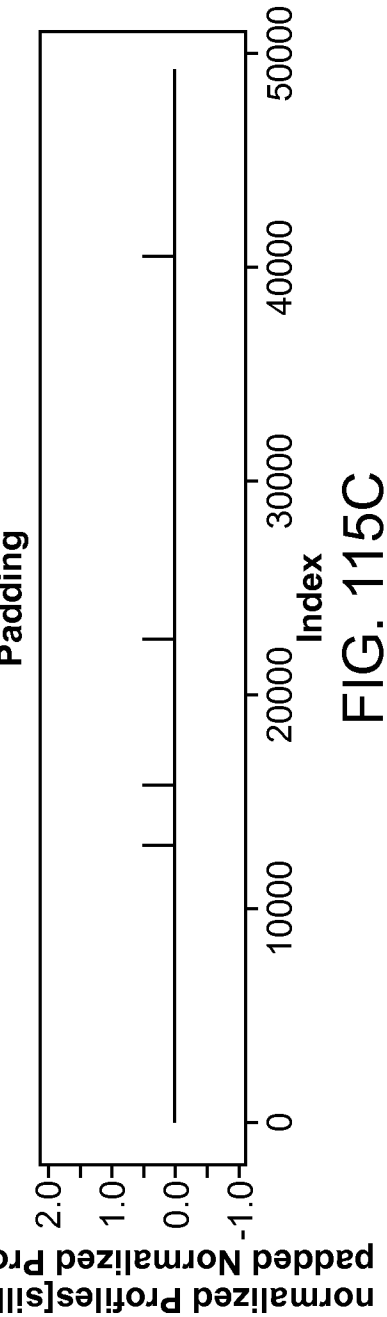


FIG. 115C

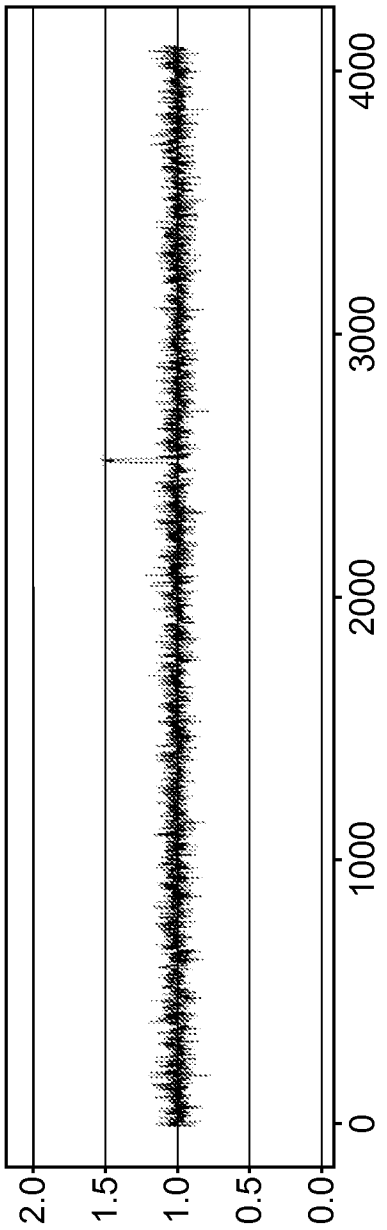


FIG. 116

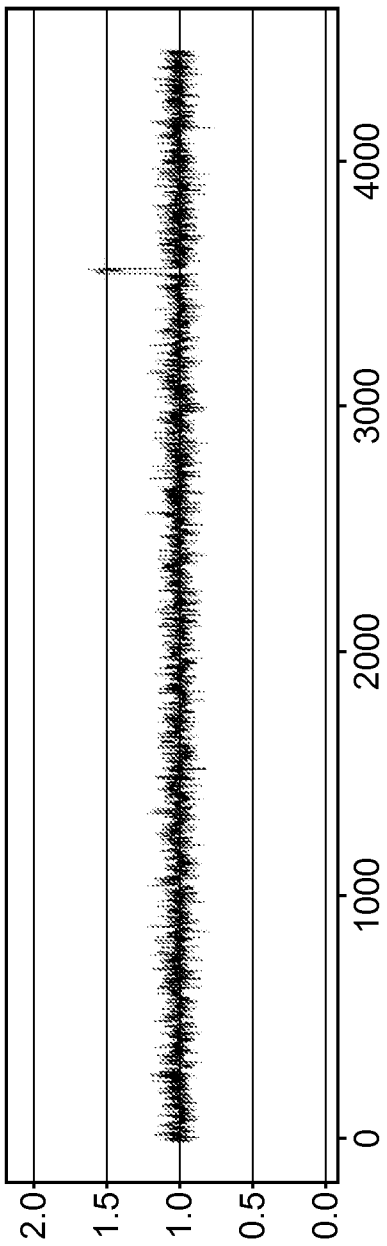


FIG. 117

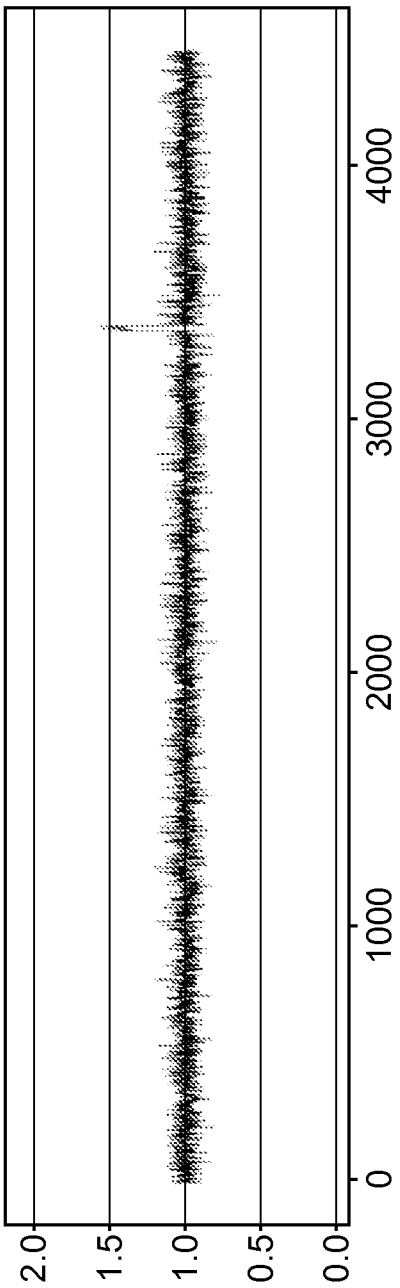


FIG. 118

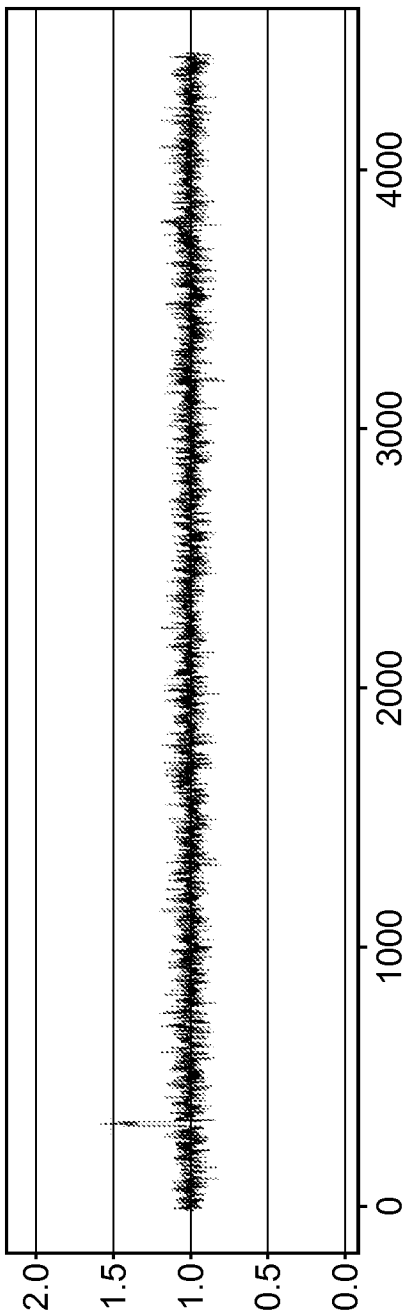


FIG. 119

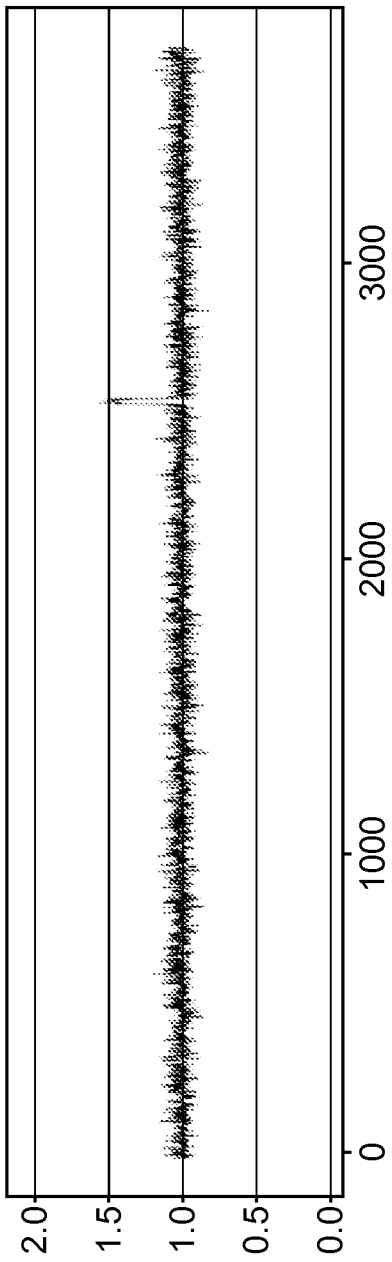


FIG. 120

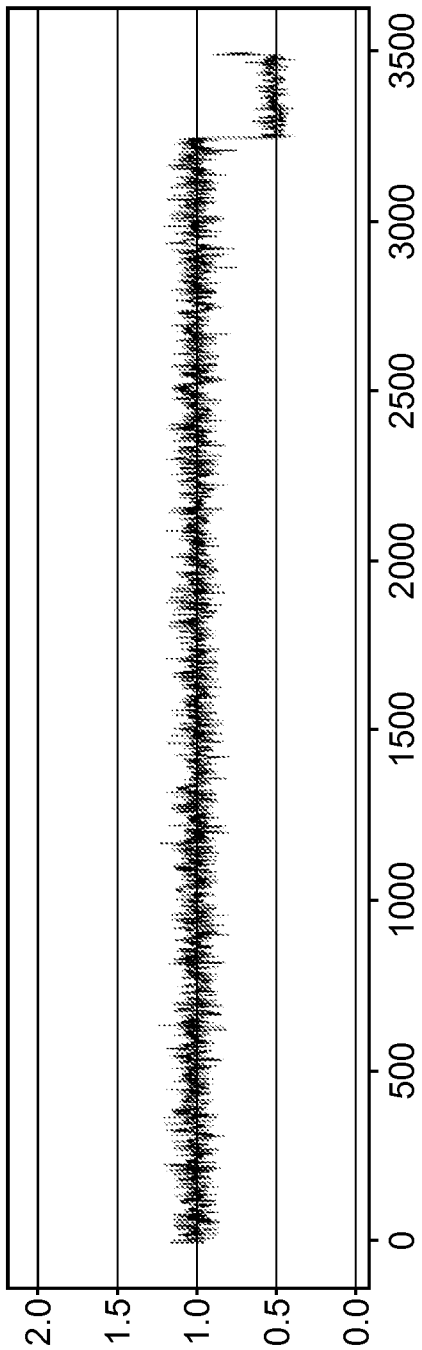


FIG. 121

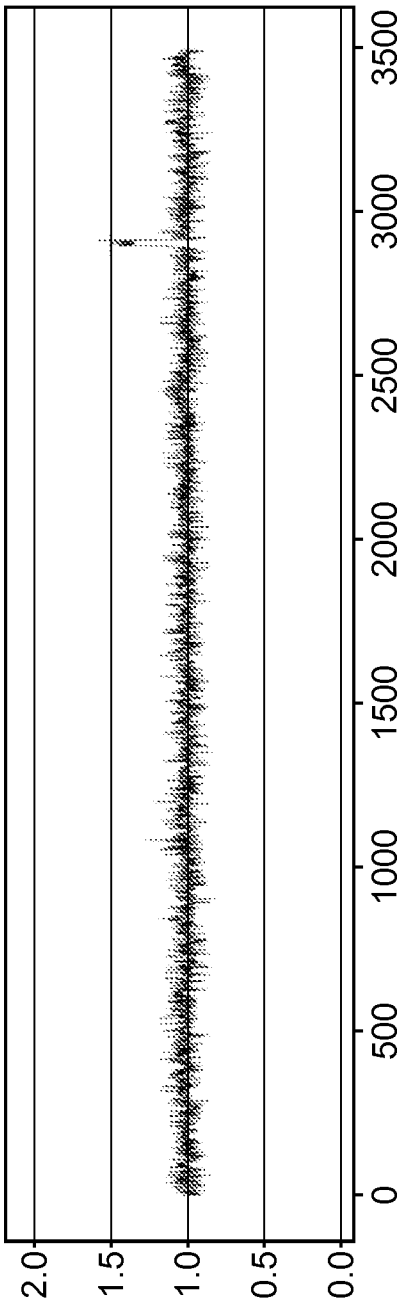


FIG. 122

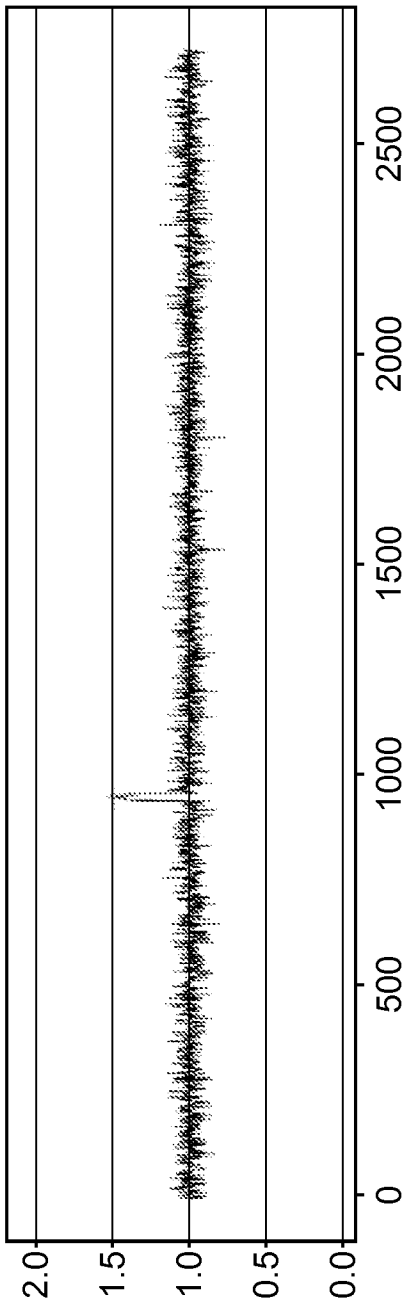


FIG. 123

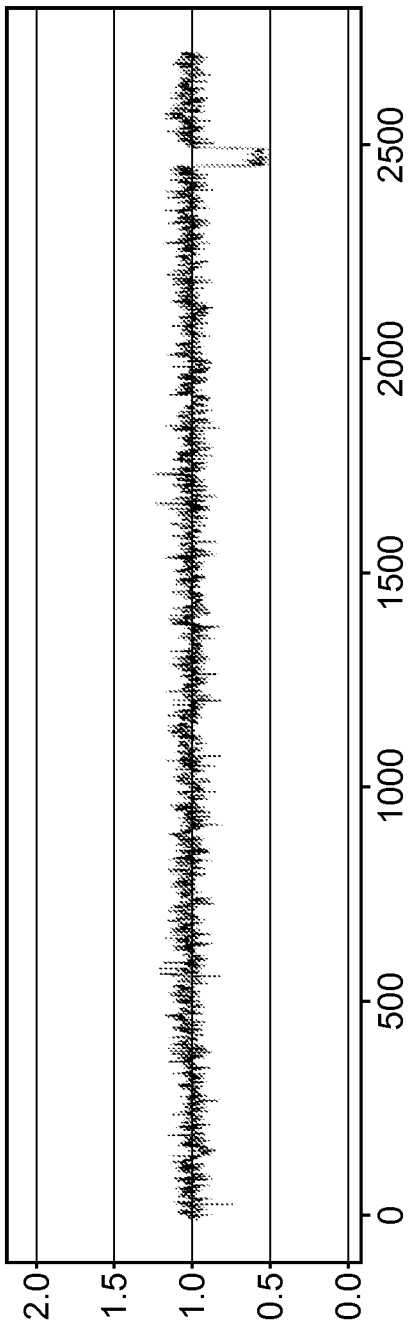


FIG. 124

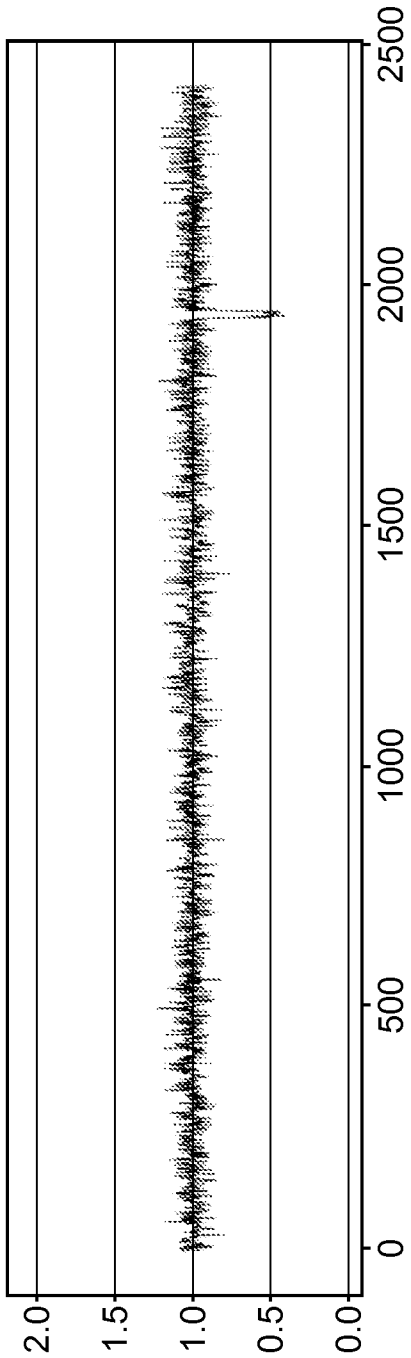


FIG. 125

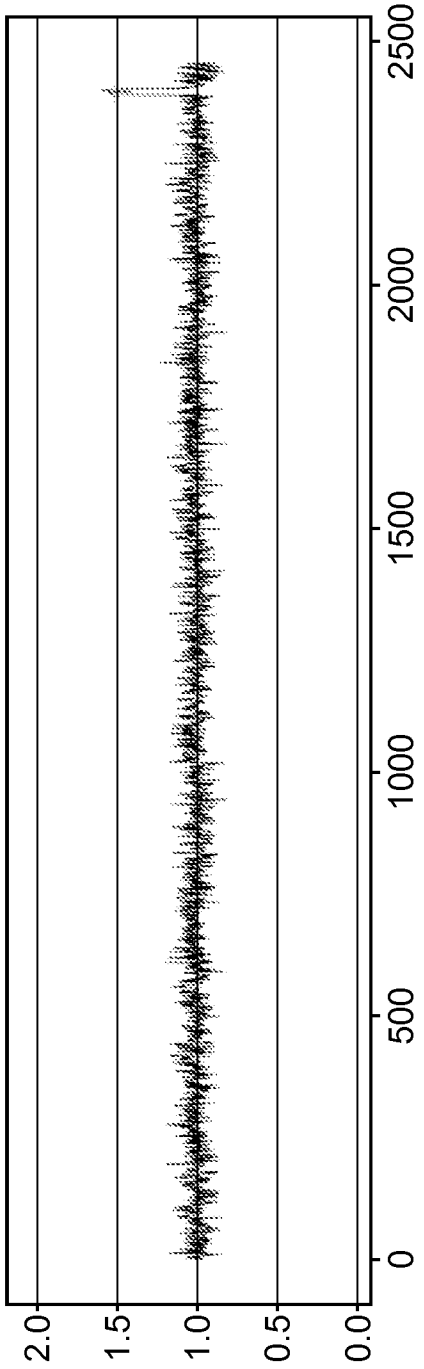


FIG. 126

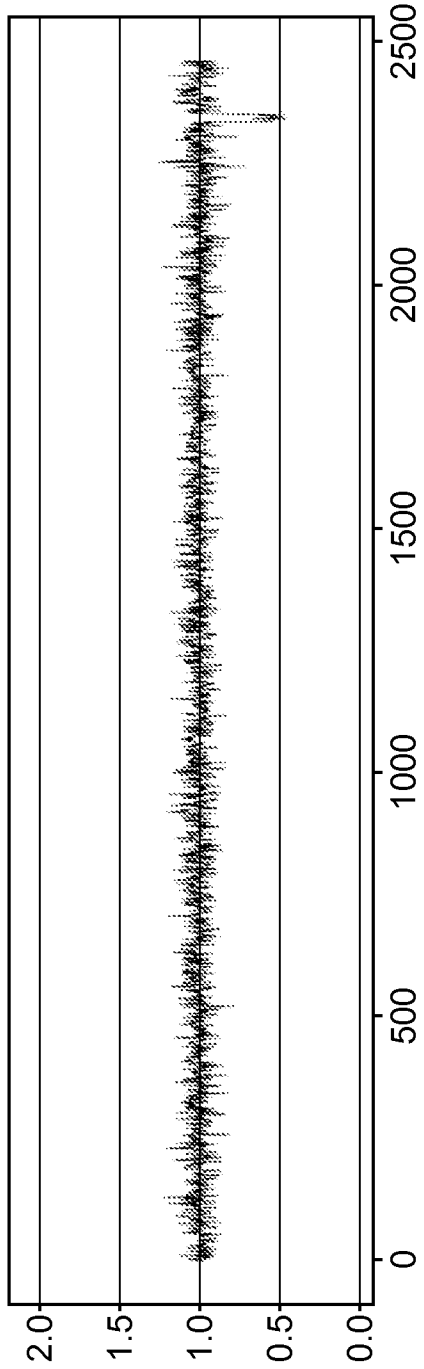


FIG. 127

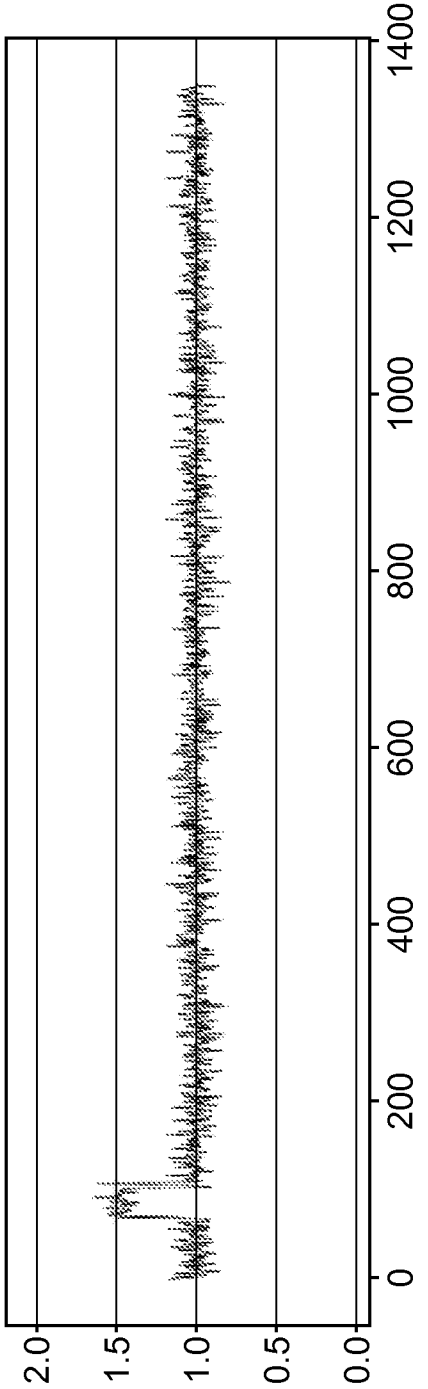


FIG. 128

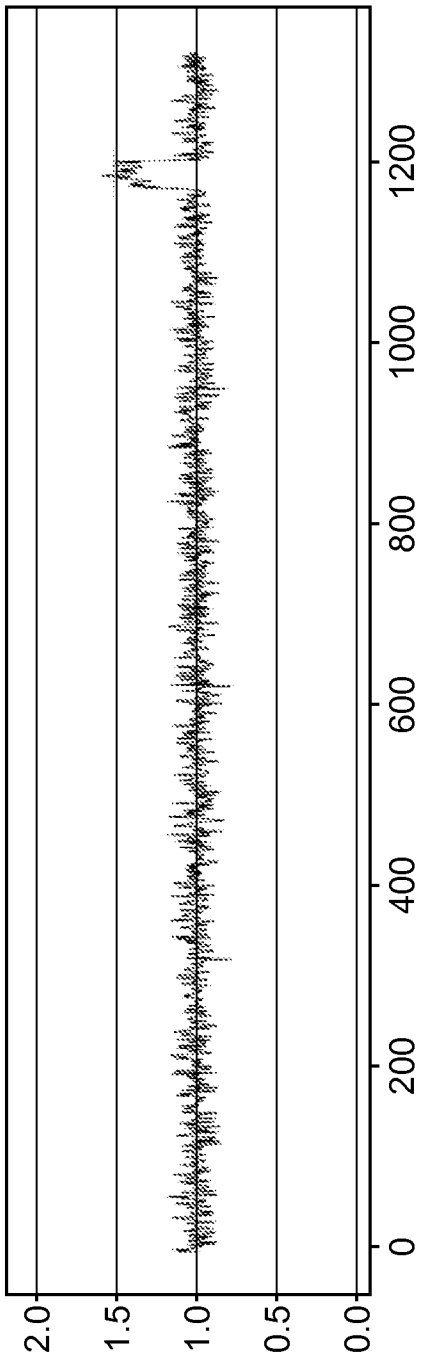


FIG. 129

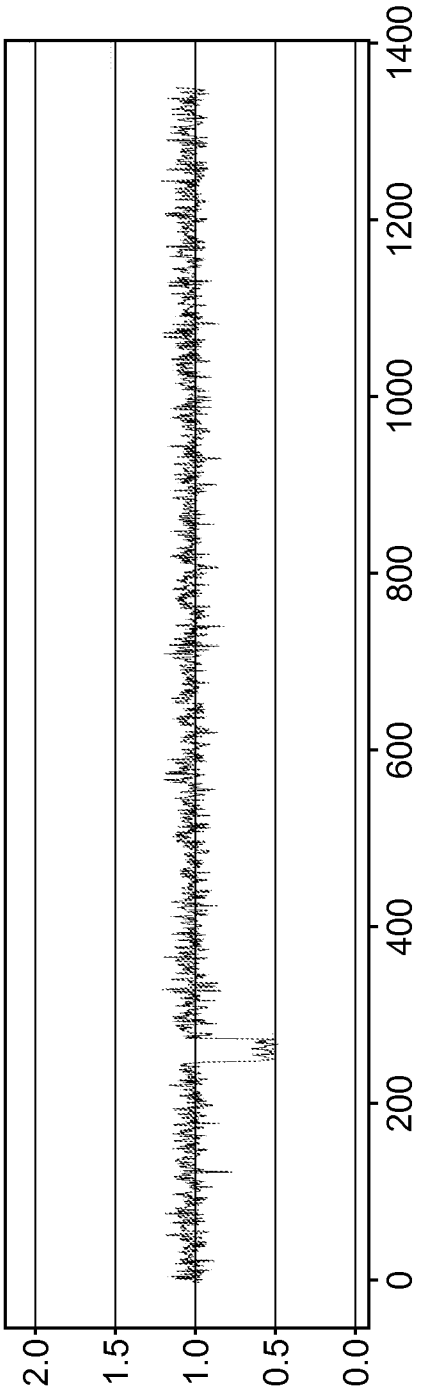


FIG. 130

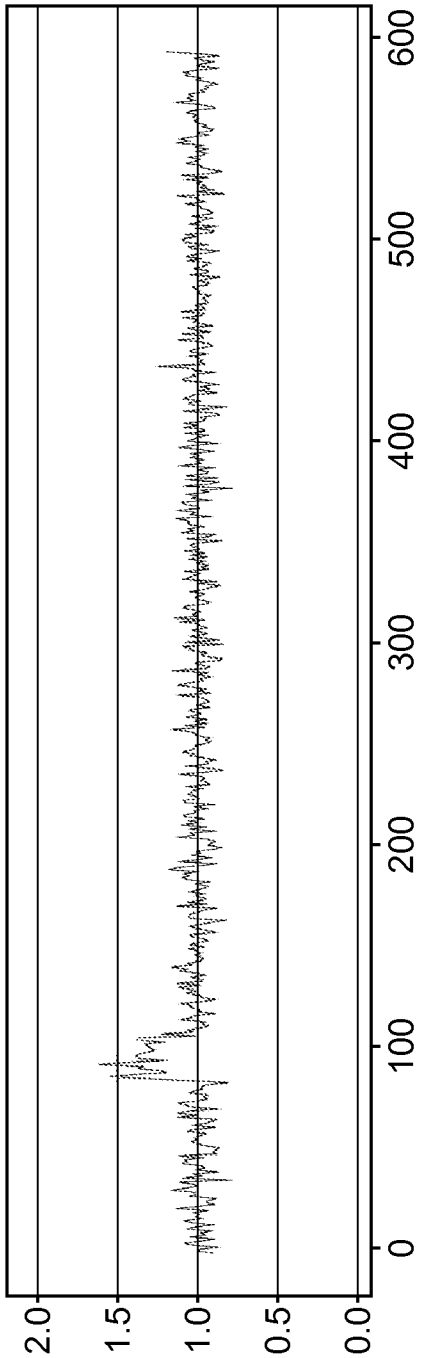


FIG. 131

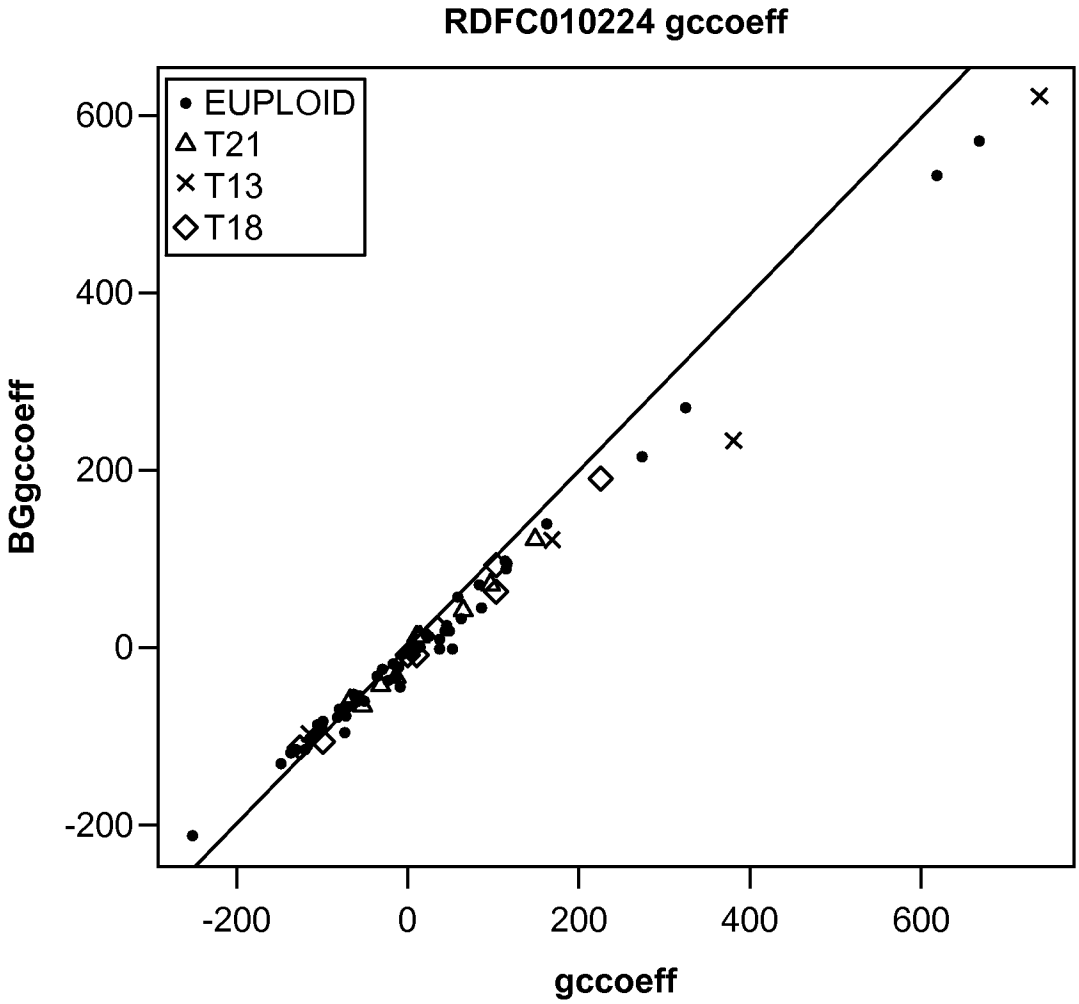

RDFC010224 gccoeff
EUPLOID
T21
T13
T18
600
400
200
0
-200
BGgccoeff
-200
0
200
400
600
gccoeff

FIG. 132

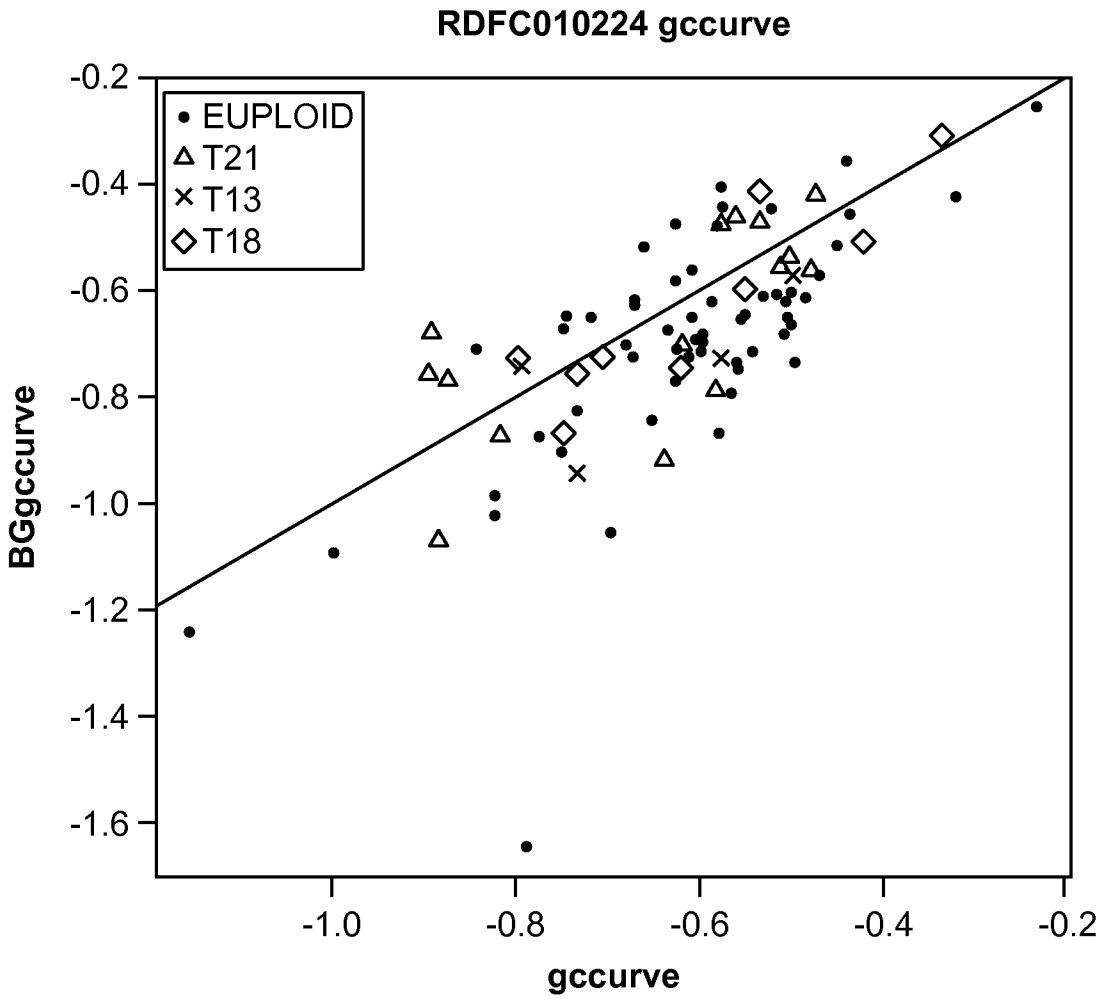

RDFC010224 gccurve
EUPLOID
T21
T13
T18
BGgccurve
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.0
-0.8
-0.6
-0.4
-0.2
gccurve

FIG. 133

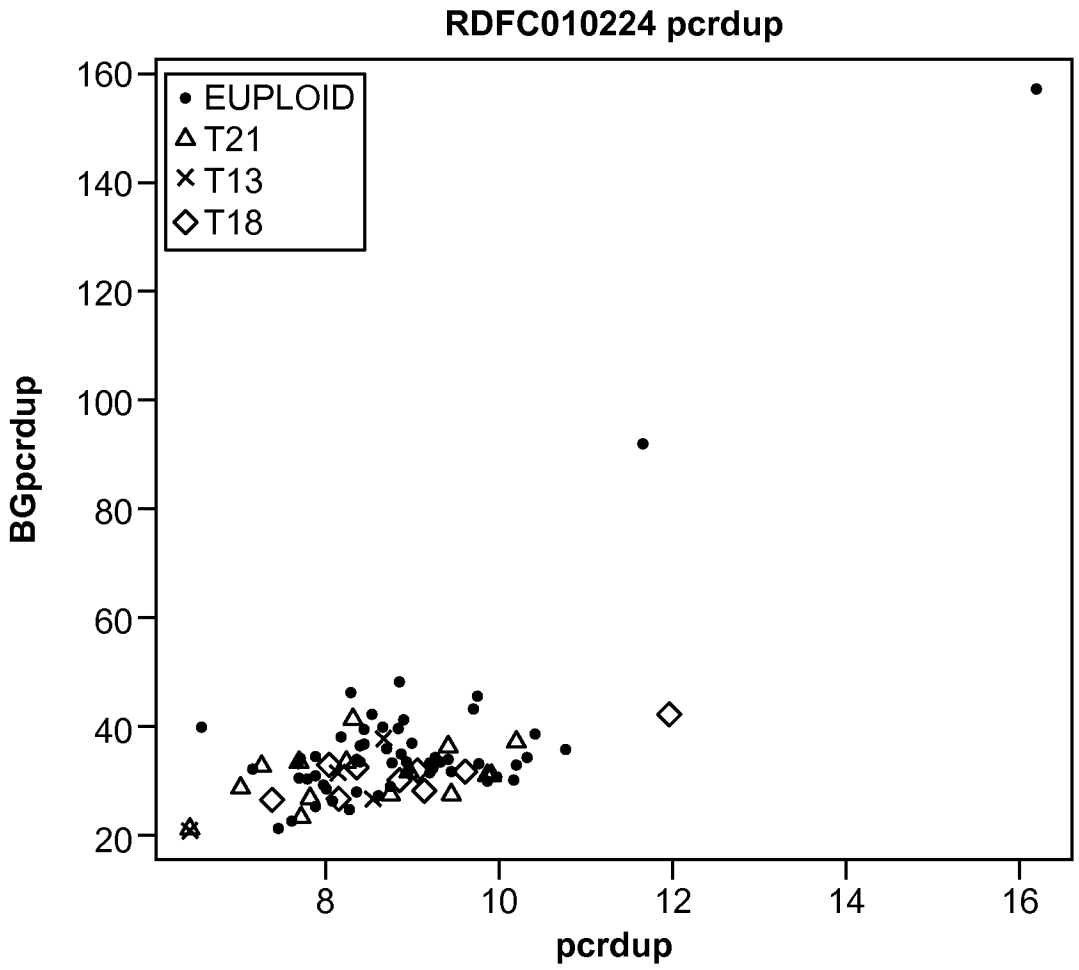

RDFC010224 pcrdup
EUPLOID
T21
T13
T18
BGpcrdup
160
140
120
100
80
60
40
20
8
10
12
14
16
pcrdup

FIG. 134

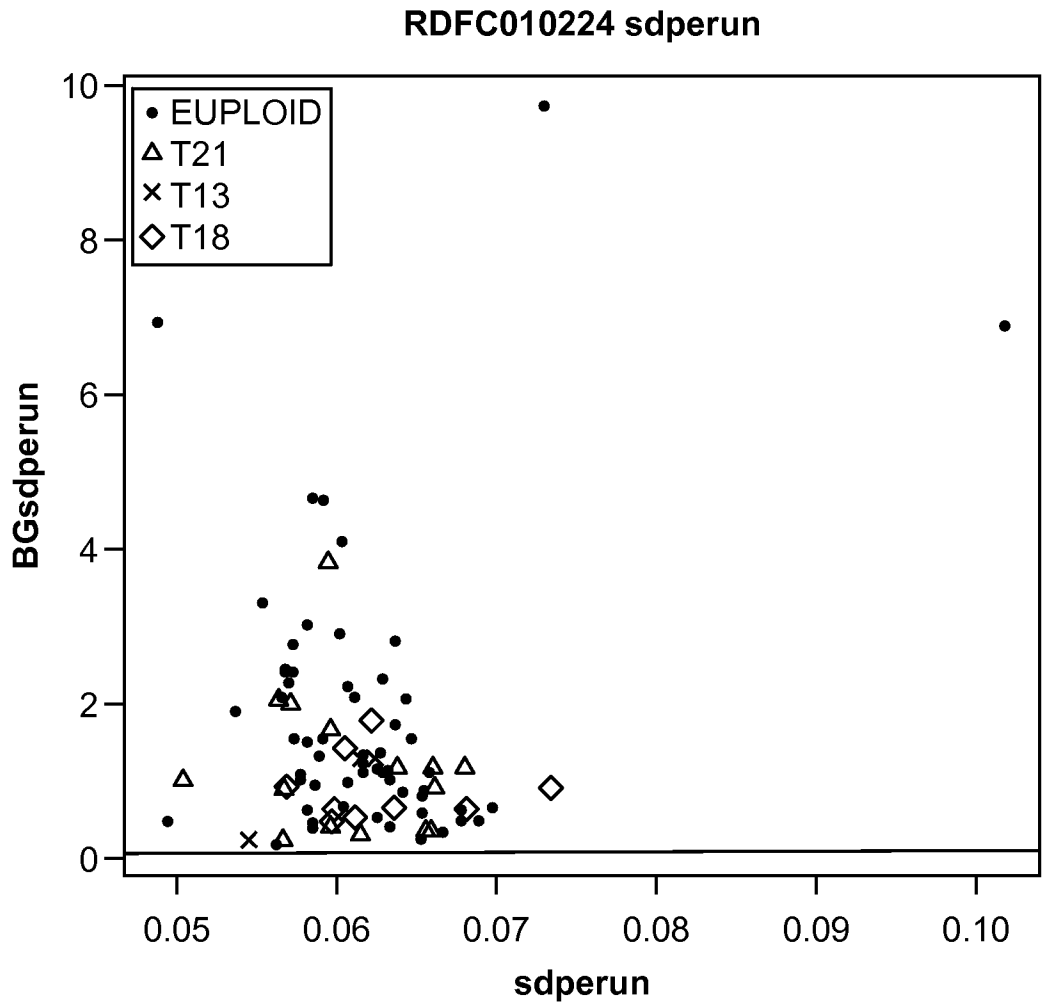

RDFC010224 sdperun
EUPLOID
T21
T13
T18
BGsdperun
sdperun
0
2
4
6
8
10
0.05
0.06
0.07
0.08
0.09
0.10

FIG. 135

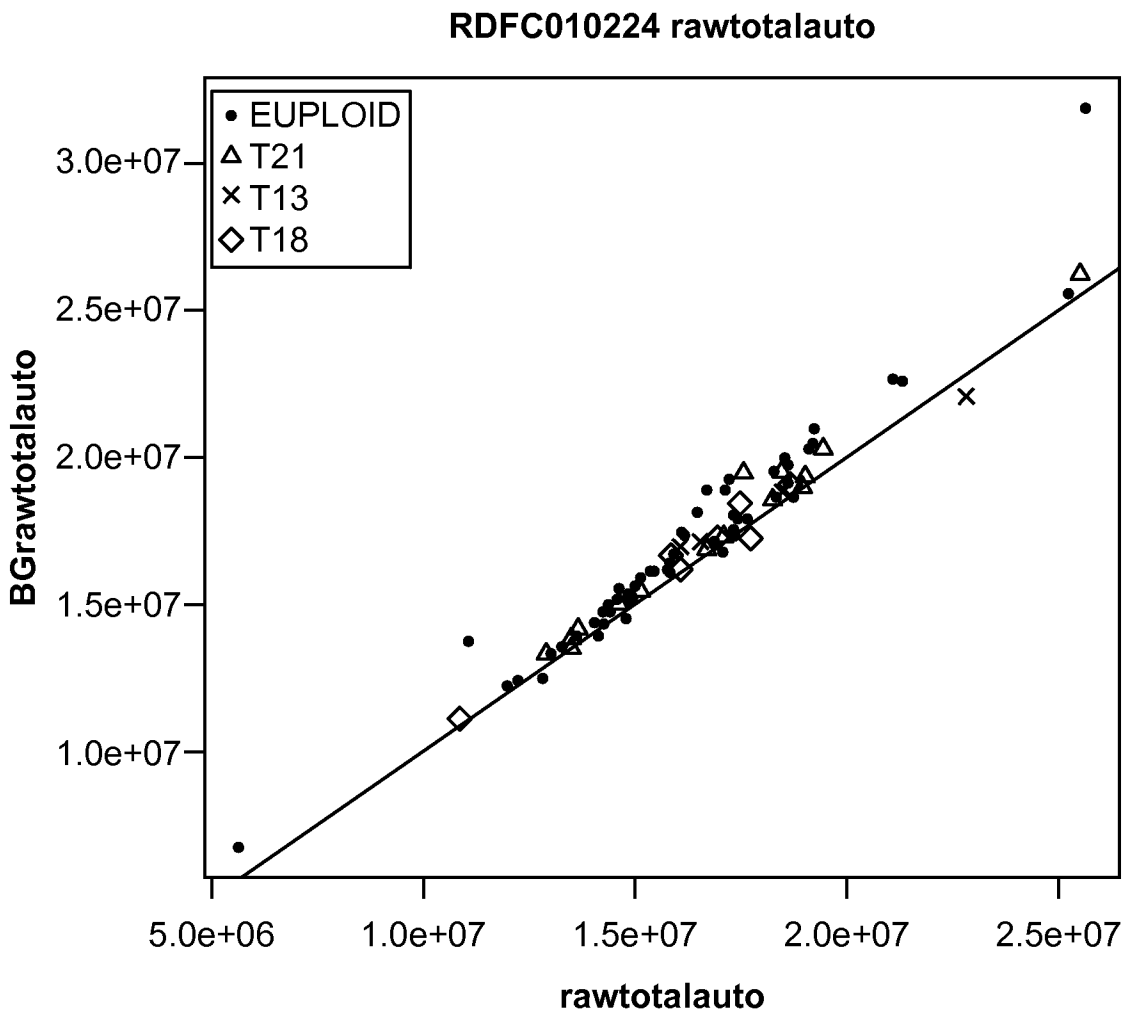

RDFC010224 rawtotalauto
EUPLOID
T21
T13
T18
3.0e+07
2.5e+07
2.0e+07
1.5e+07
1.0e+07
BGrawtotalauto
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
rawtotalauto

FIG. 136

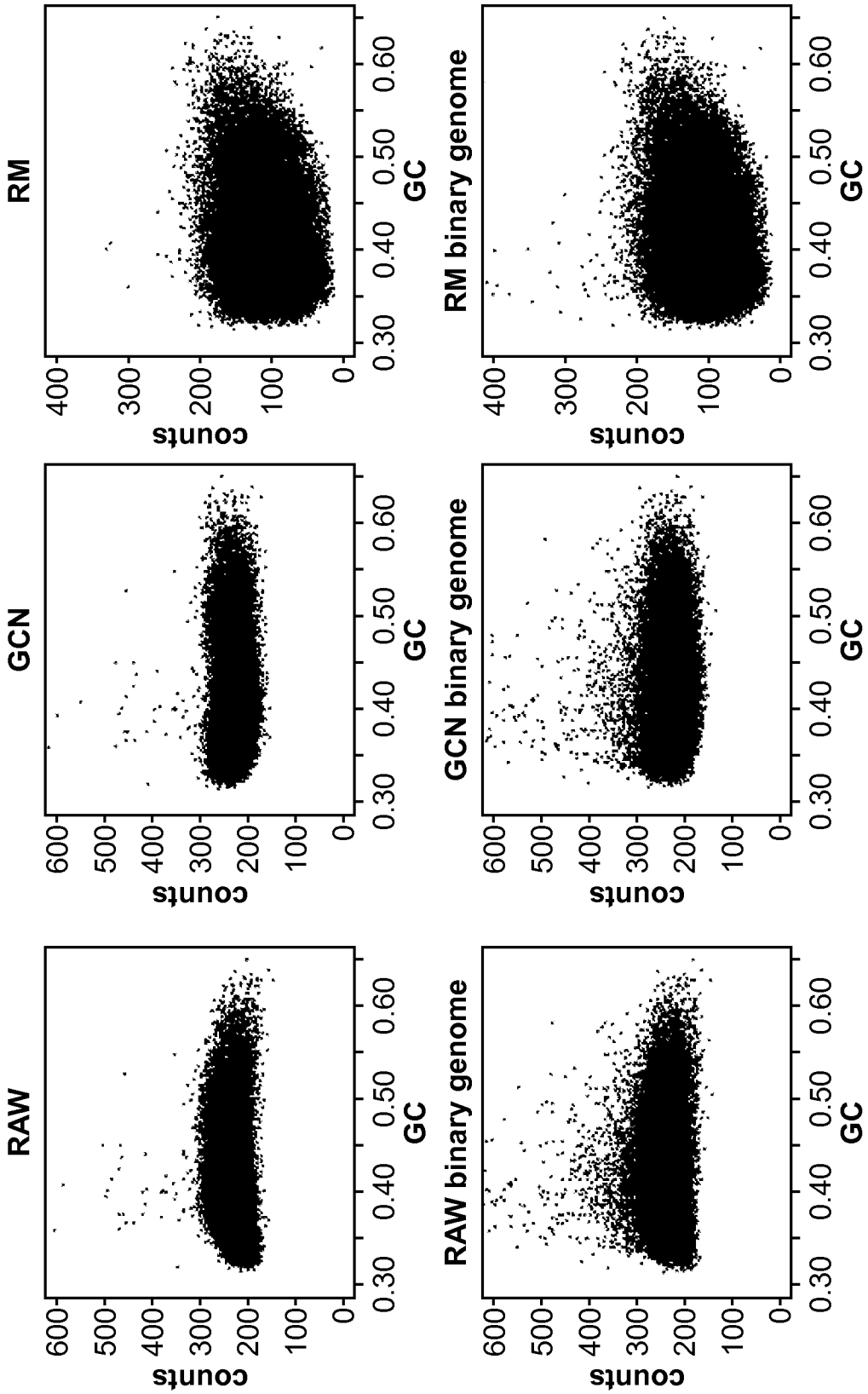

RM
RM binary genome
GCN
GCN binary genome
RAW
RAW binary genome
counts
GC
0
100
200
300
400
500
600
0.30
0.40
0.50
0.60

FIG. 137

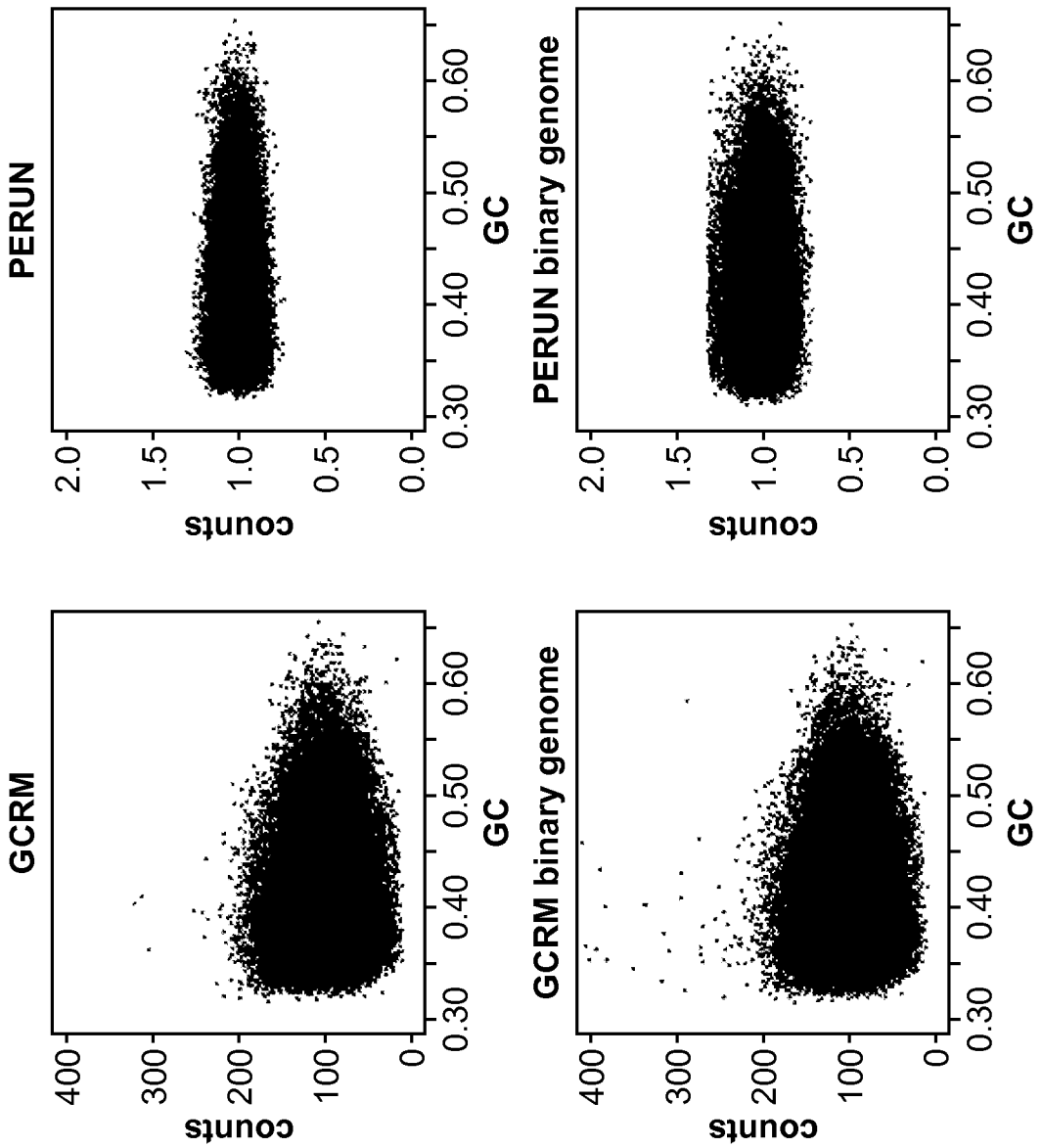

PERUN
counts
GC
PERUN binary genome
counts
GC
GCRM
counts
GC
GCRM binary genome
counts
GC

FIG. 137 (Cont.)

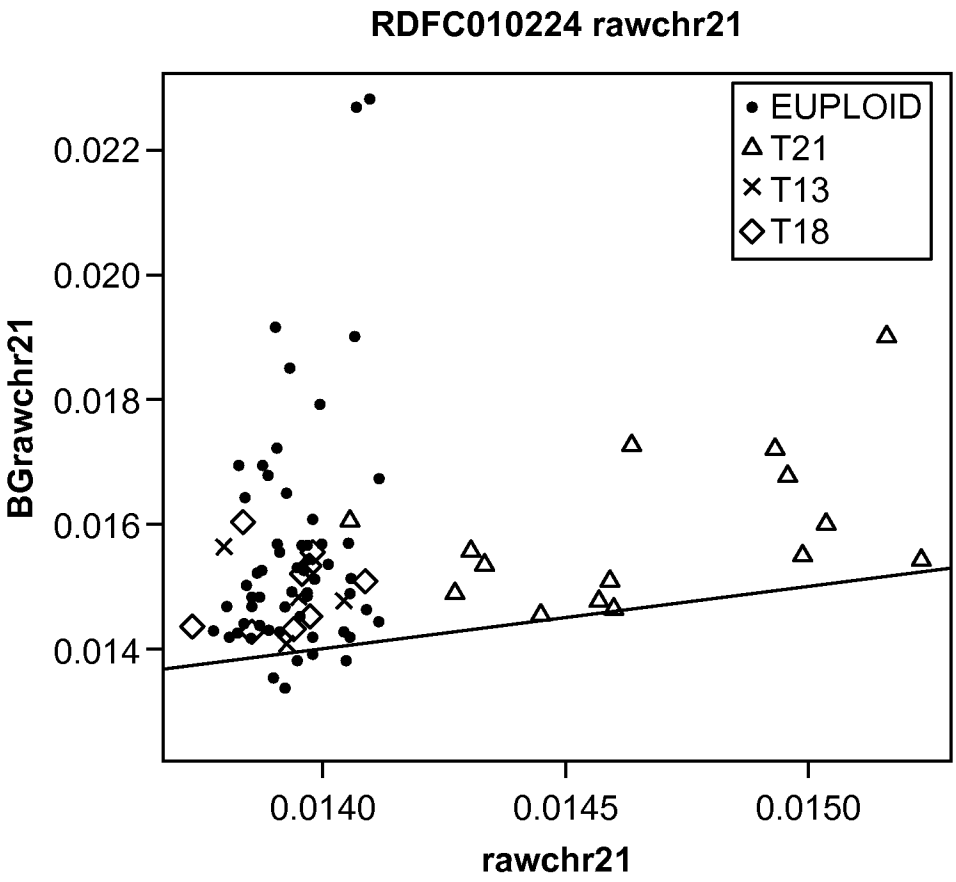

RDFC010224 rawchr21
EUPLOID
T21
T13
T18
0.022
0.020
0.018
0.016
0.014
BGrawchr21
0.0140
0.0145
0.0150
rawchr21

FIG. 138

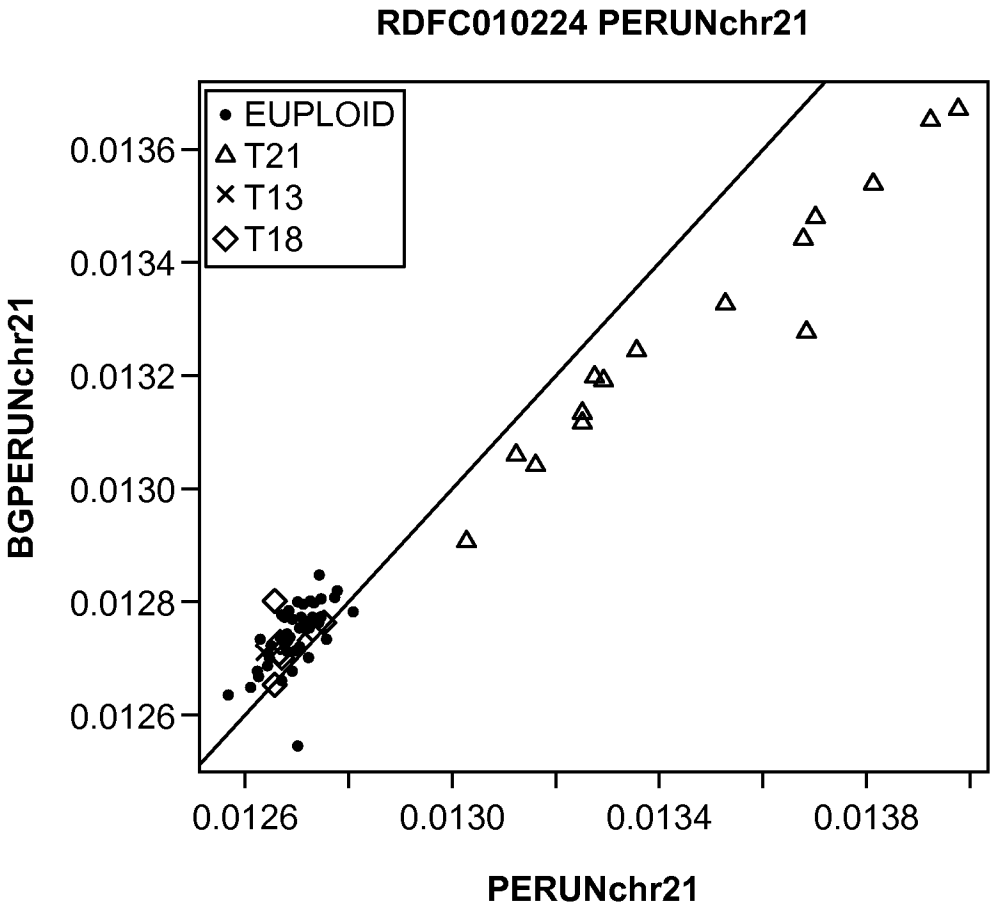

RDFC010224 PERUNchr21
EUPLOID
T21
T13
T18
0.0136
0.0134
0.0132
0.0130
0.0128
0.0126
BGPERUNchr21
0.0126
0.0130
0.0134
0.0138
PERUNchr21

FIG. 139

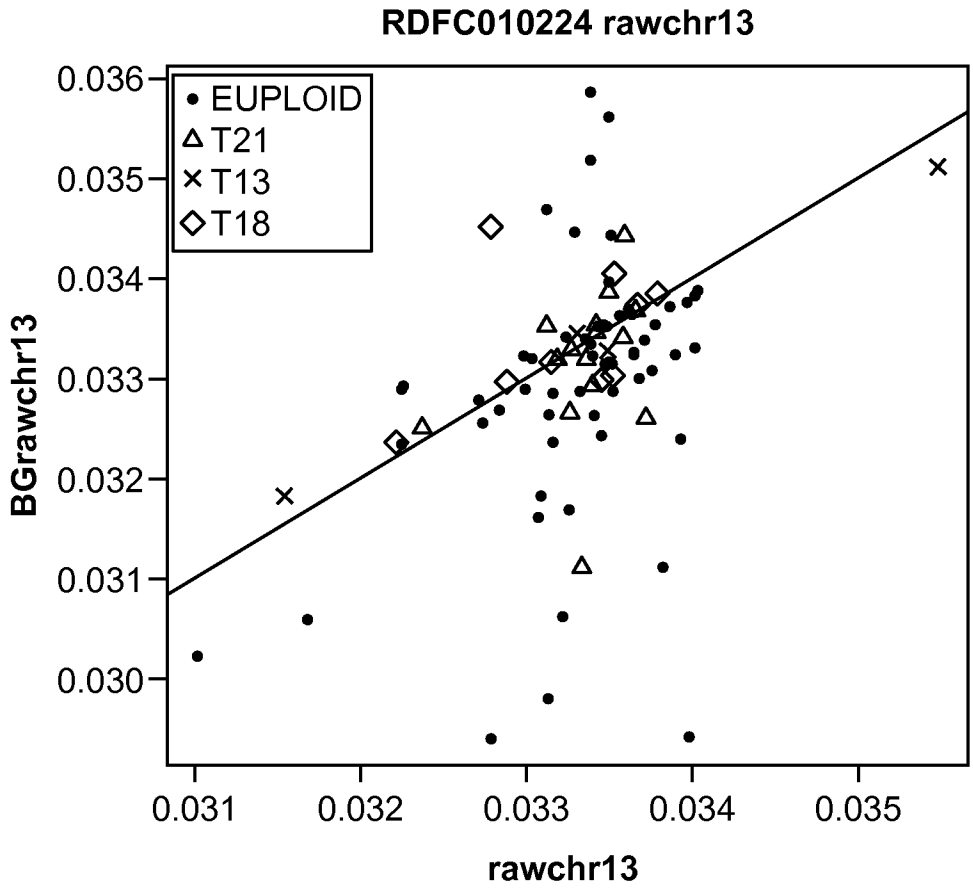

RDFC010224 rawchr13
EUPLOID
T21
T13
T18
BGrawchr13
rawchr13
0.036
0.035
0.034
0.033
0.032
0.031
0.030
0.031
0.032
0.033
0.034
0.035

FIG. 140

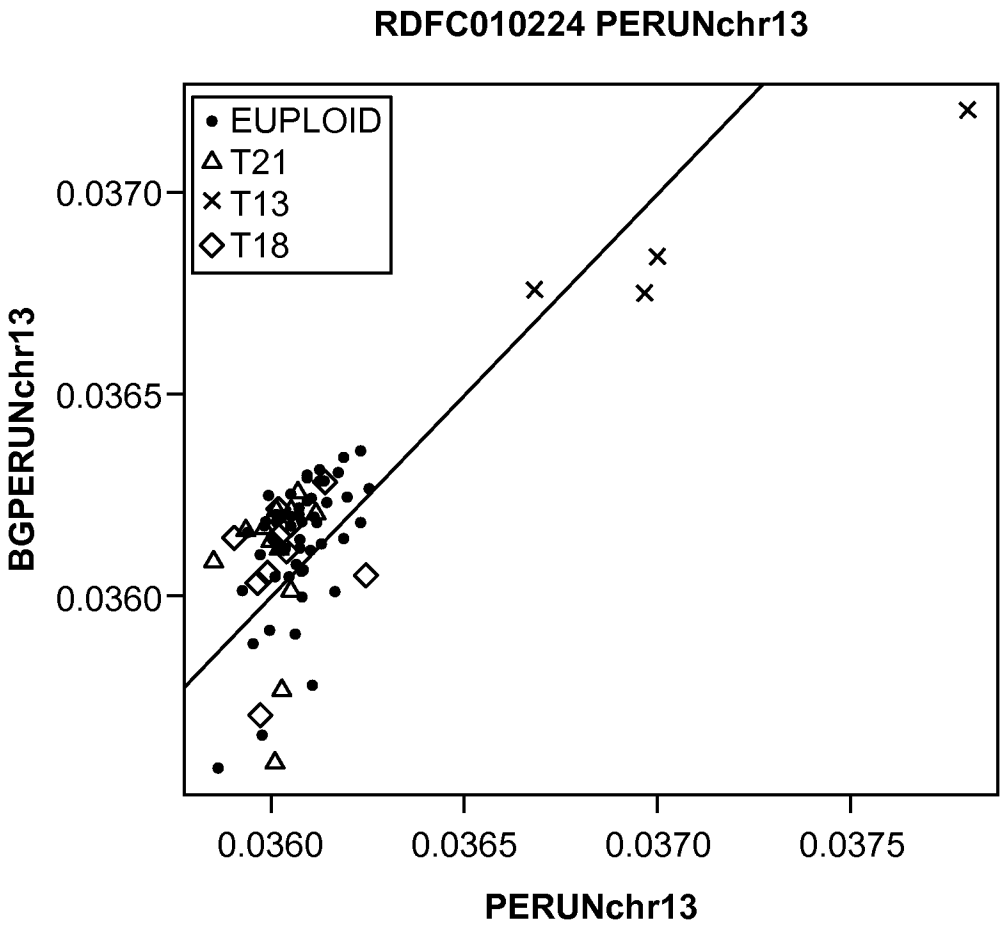

RDFC010224 PERUNchr13
EUPLOID
T21
T13
T18
0.0370
0.0365
0.0360
BGPERUNchr13
0.0360
0.0365
0.0370
0.0375
PERUNchr13

FIG. 141

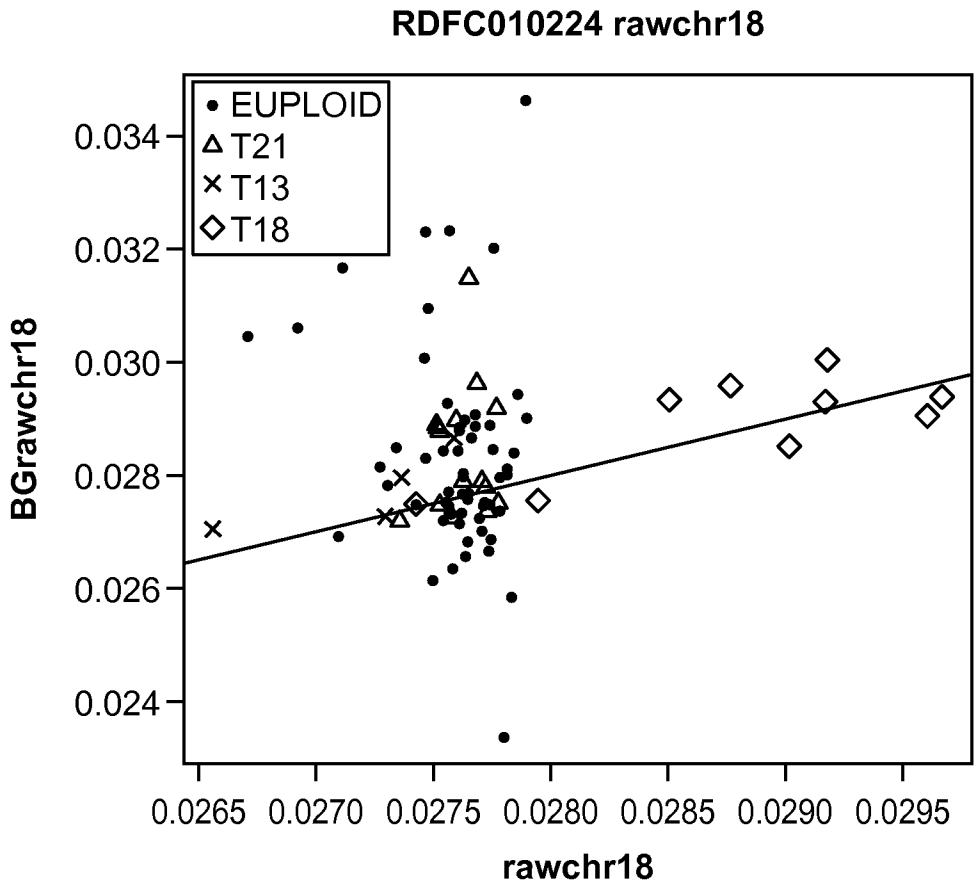

RDFC010224 rawchr18
EUPLOID
T21
T13
T18
BGrawchr18
0.034
0.032
0.030
0.028
0.026
0.024
0.0265
0.0270
0.0275
0.0280
0.0285
0.0290
0.0295
rawchr18

FIG. 142

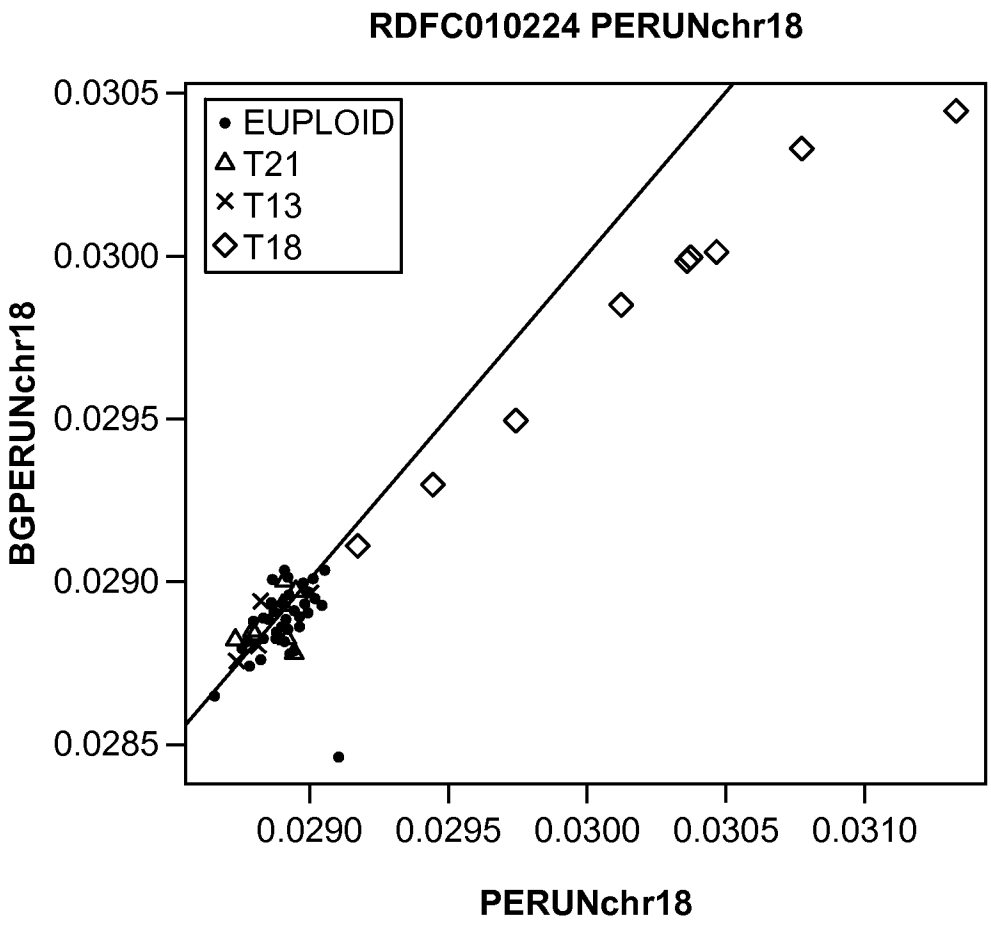

RDFC010224 PERUNchr18
EUPLOID
T21
T13
T18
BGPERUNchr18
0.0305
0.0300
0.0295
0.0290
0.0285
0.0290
0.0295
0.0300
0.0305
0.0310
PERUNchr18

FIG. 143

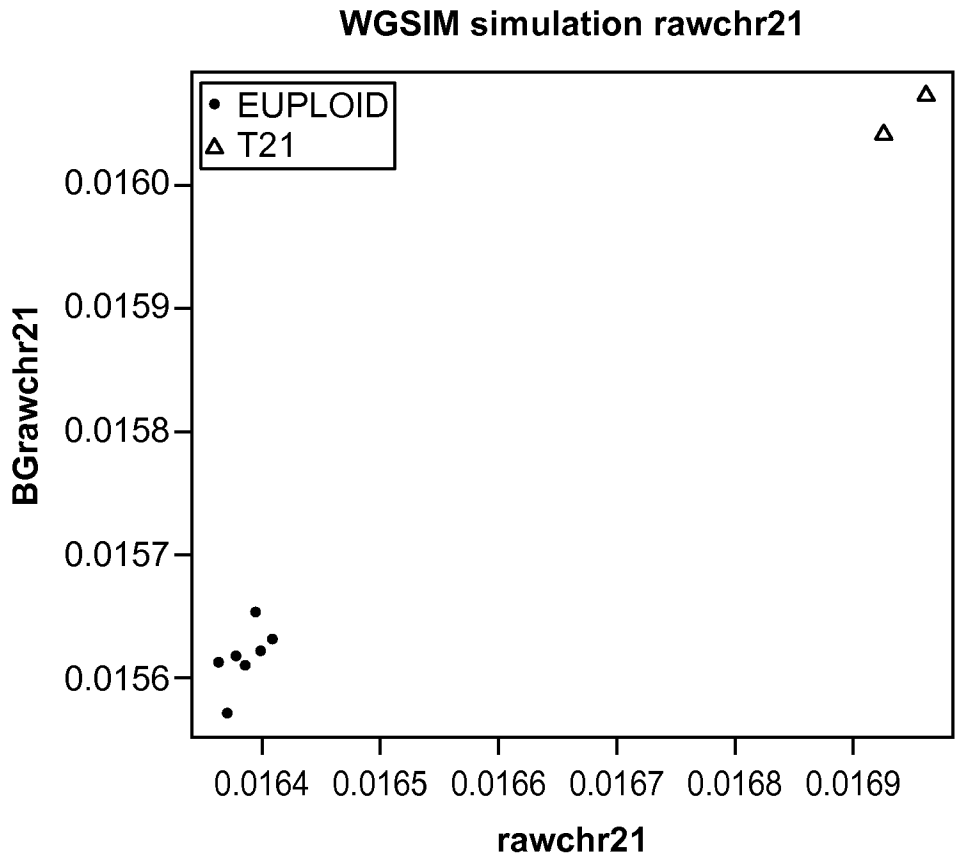

WGSIM simulation rawchr21
EUPLOID
T21
0.0160
0.0159
0.0158
0.0157
0.0156
BGrawchr21
0.0164
0.0165
0.0166
0.0167
0.0168
0.0169
rawchr21

FIG. 144

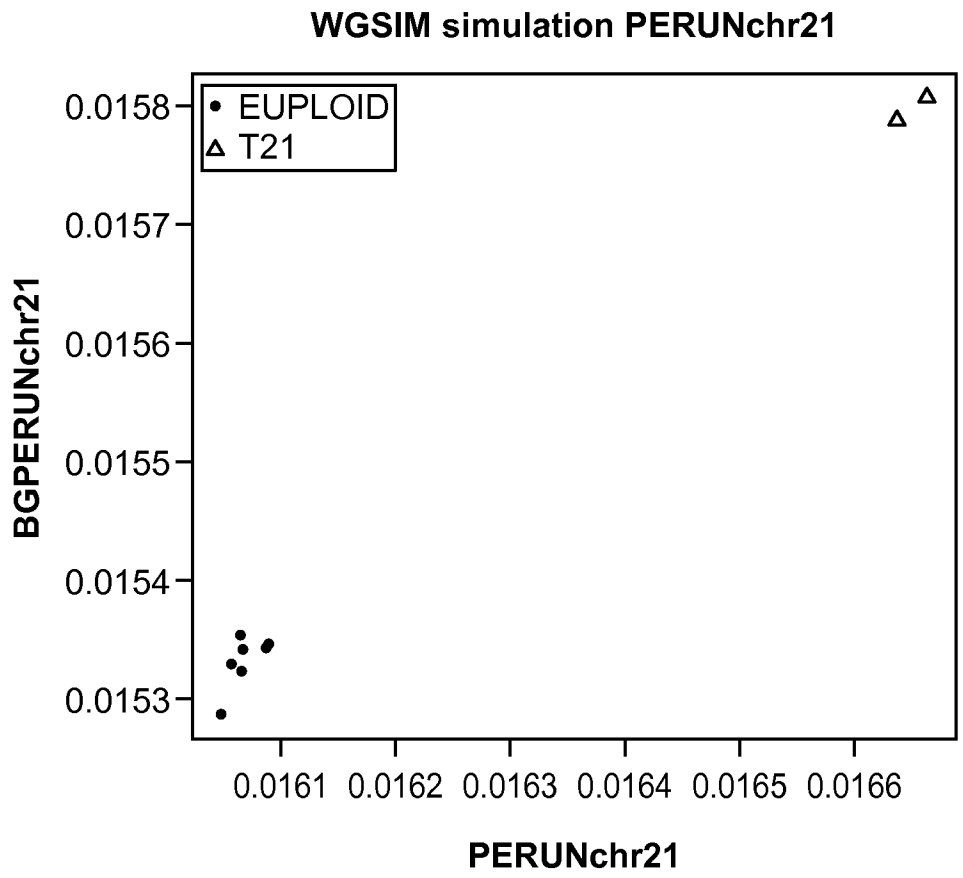

WGSIM simulation PERUNchr21
EUPLOID
T21
0.0158
0.0157
0.0156
0.0155
0.0154
0.0153
BGPERUNchr21
0.0161
0.0162
0.0163
0.0164
0.0165
0.0166
PERUNchr21

FIG. 145

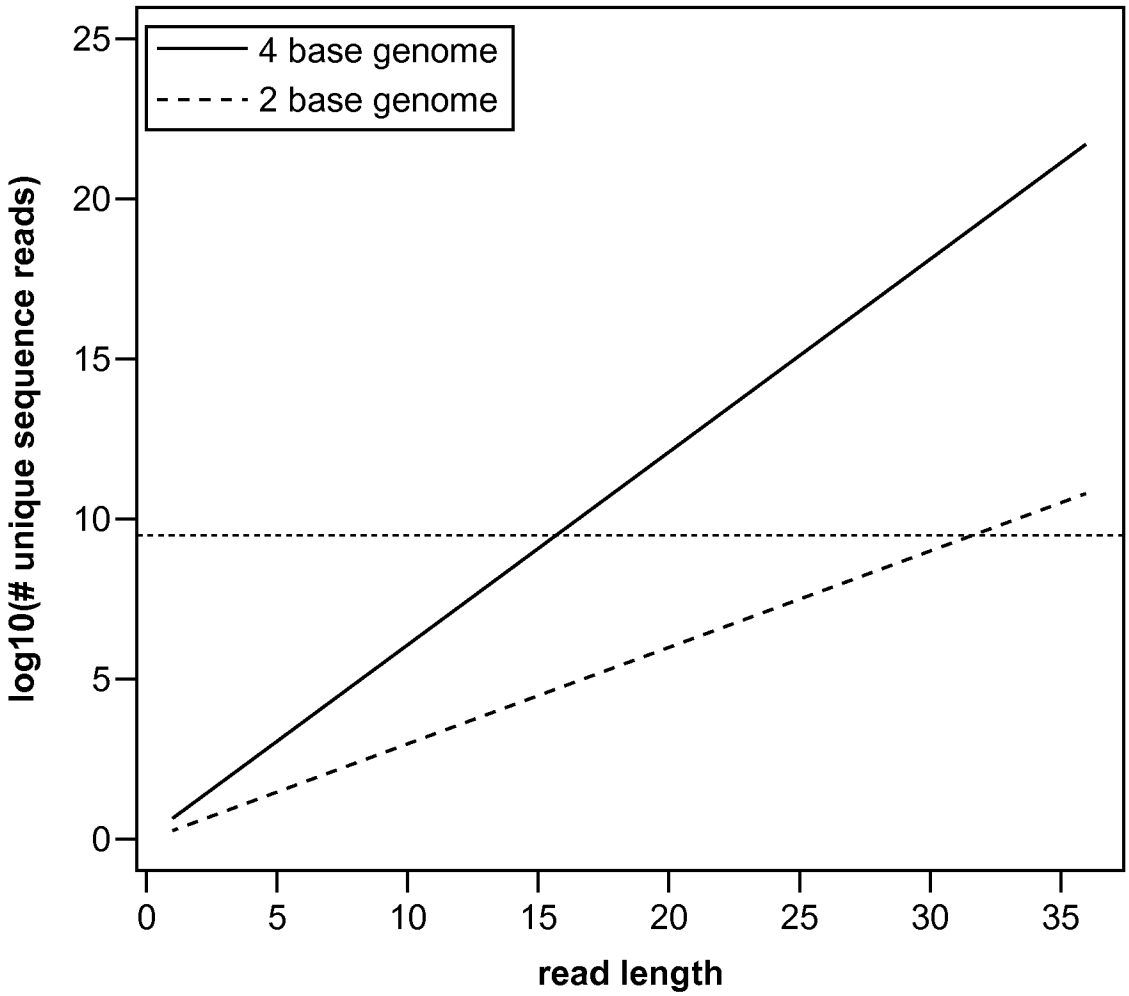

4 base genome
2 base genome
log10(# unique sequence reads)
read length
0
5
10
15
20
25
30
35

FIG. 146

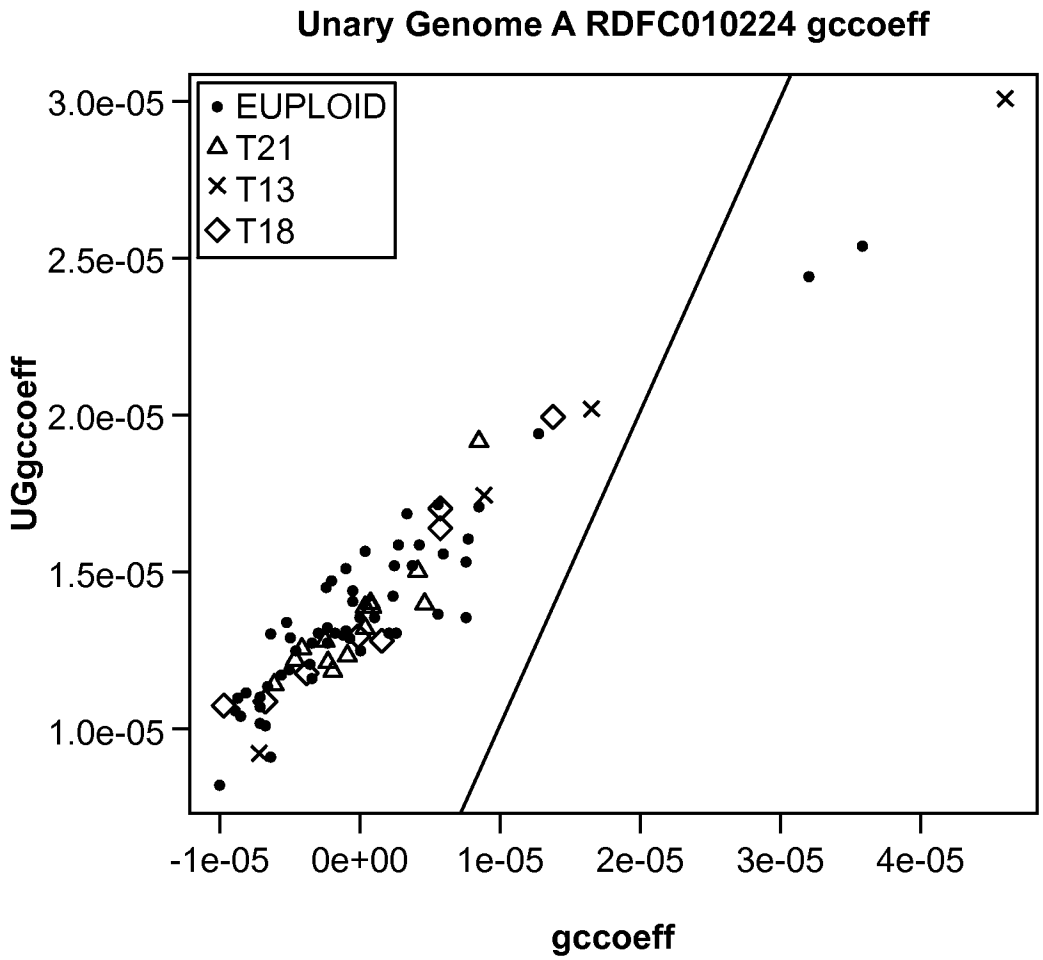

Unary Genome A RDFC010224 gccoeff
EUPLOID
T21
T13
T18
UGgccoeff
3.0e-05
2.5e-05
2.0e-05
1.5e-05
1.0e-05
-1e-05
0e+00
1e-05
2e-05
3e-05
4e-05
gccoeff

FIG. 147

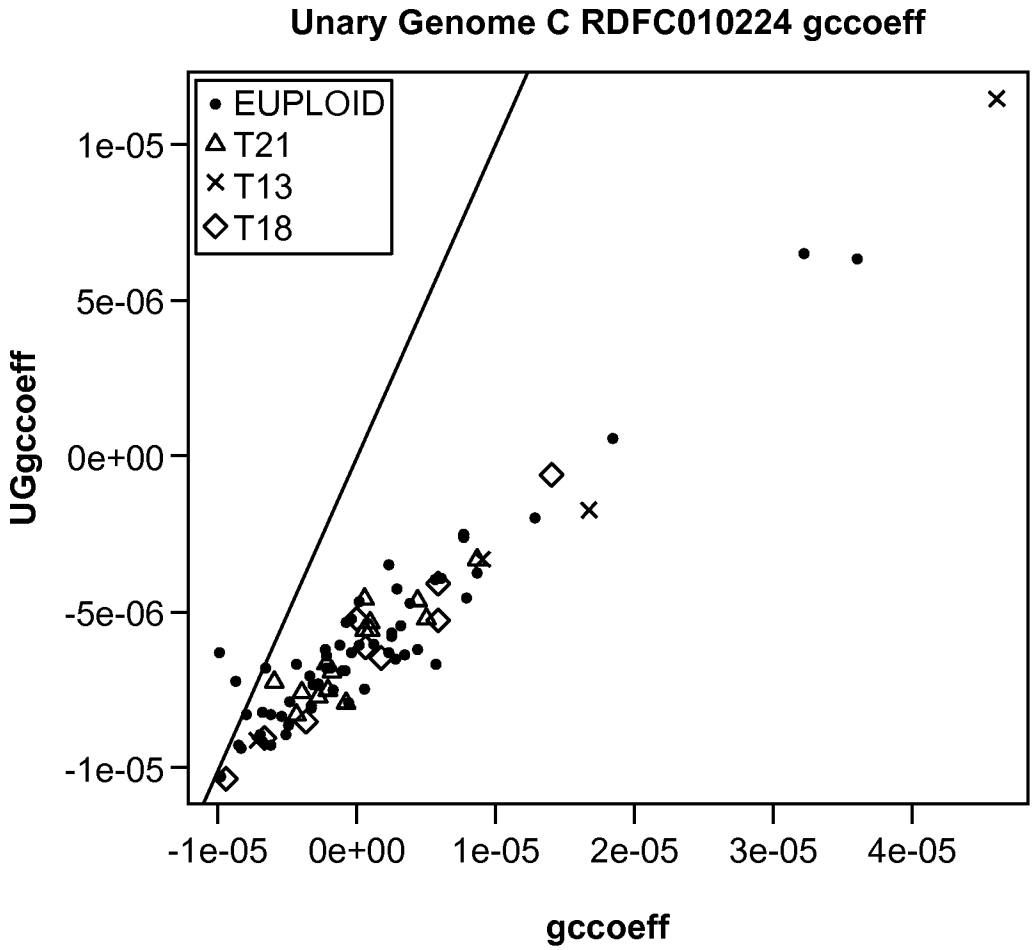

Unary Genome C RDFC010224 gccoeff
EUPLOID
T21
T13
T18
1e-05
5e-06
0e+00
-5e-06
-1e-05
UGgccoeff
-1e-05
0e+00
1e-05
2e-05
3e-05
4e-05
gccoeff

FIG. 148

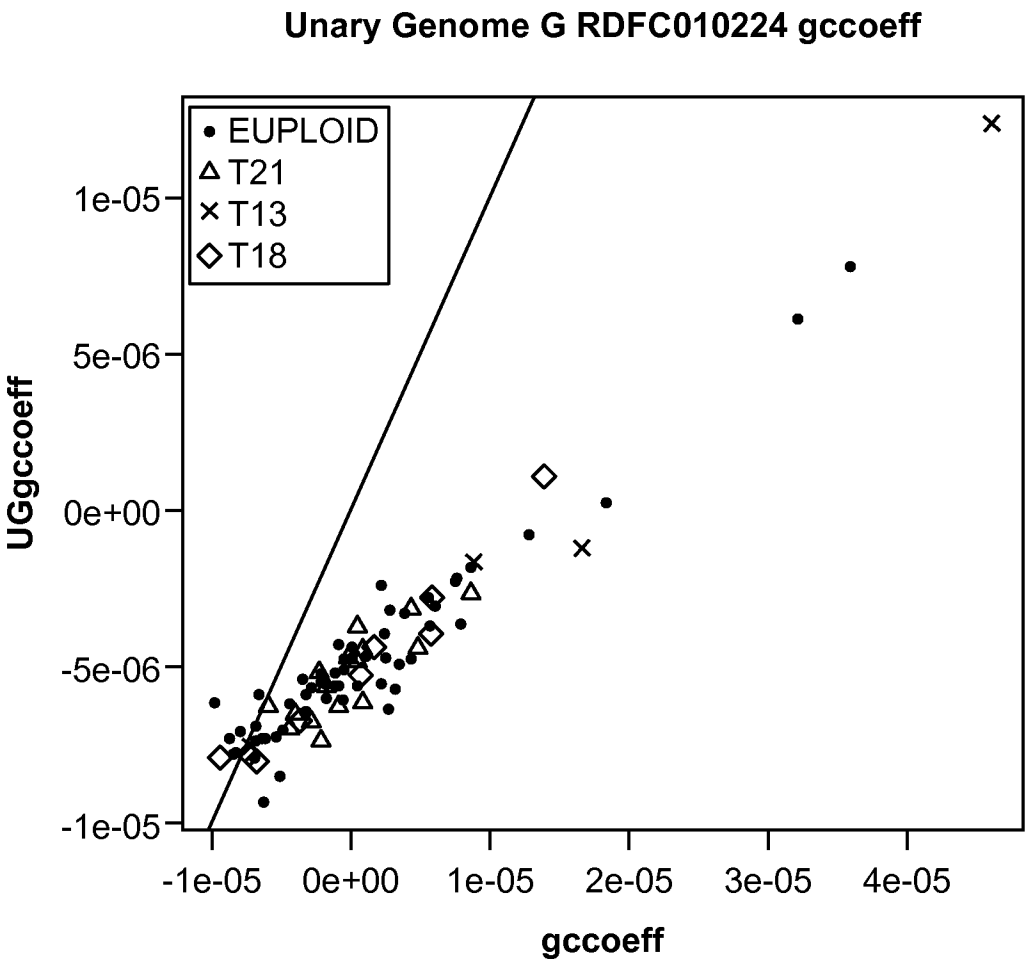

Unary Genome G RDFC010224 gccoeff
EUPLOID
T21
T13
T18
1e-05
5e-06
0e+00
-5e-06
-1e-05
UGgccoeff
-1e-05
0e+00
1e-05
2e-05
3e-05
4e-05
gccoeff

FIG. 149

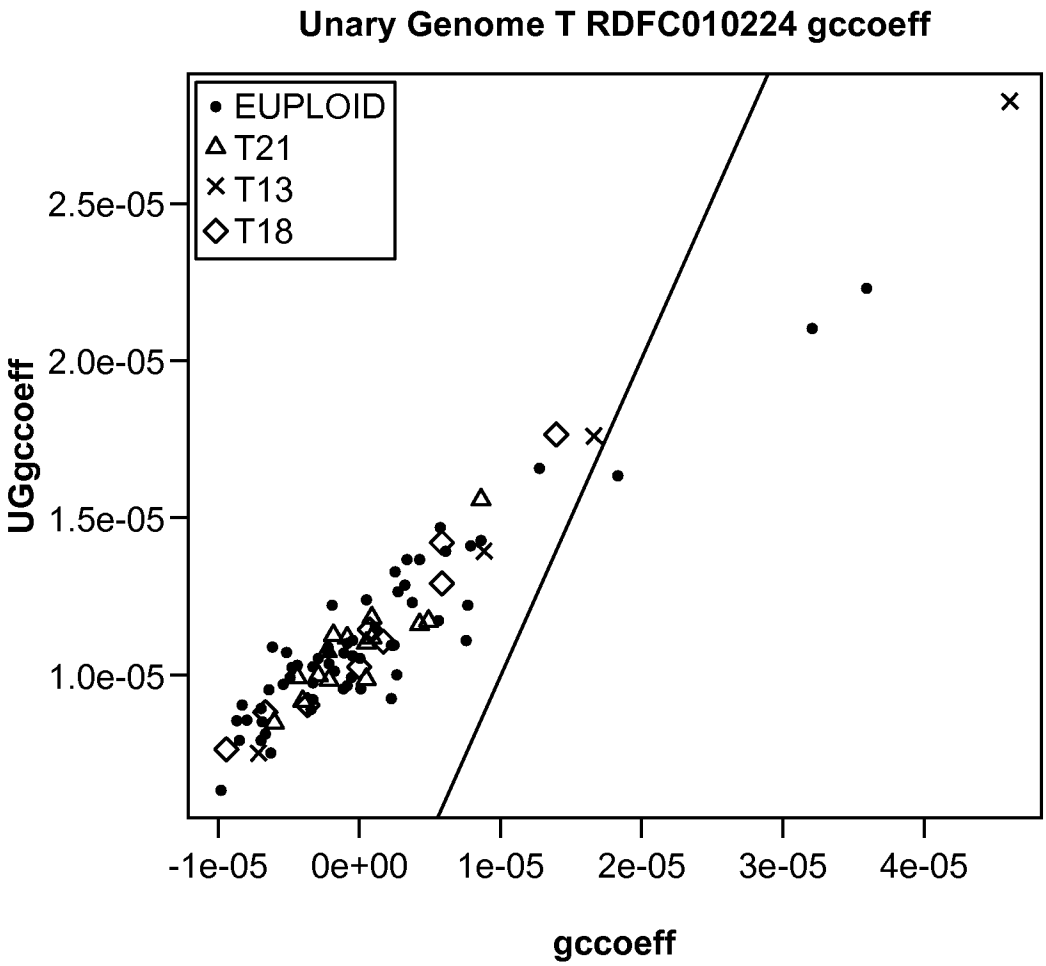

Unary Genome T RDFC010224 gccoeff
EUPLOID
T21
T13
T18
UGgccoeff
2.5e-05
2.0e-05
1.5e-05
1.0e-05
-1e-05
0e+00
1e-05
2e-05
3e-05
4e-05
gccoeff

FIG. 150

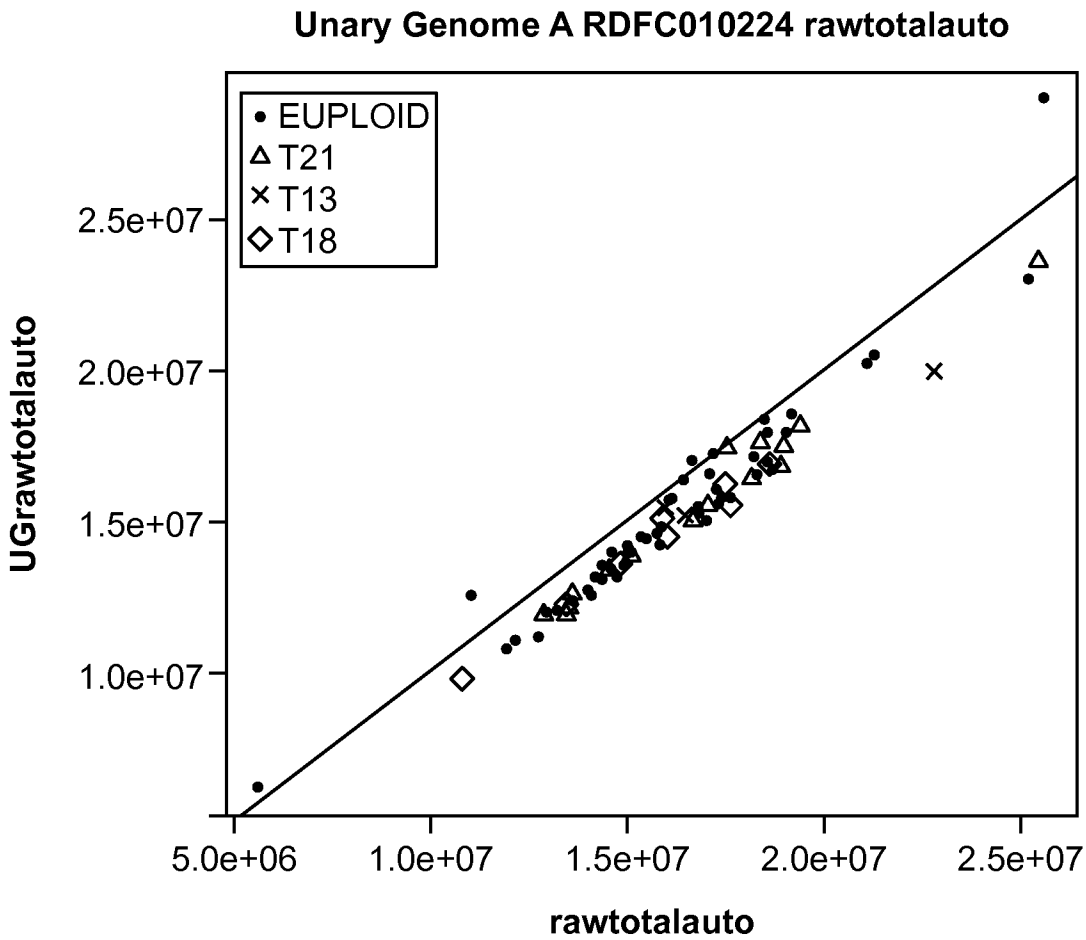

Unary Genome A RDFC010224 rawtotalauto
EUPLOID
T21
T13
T18
UGrawtotalauto
2.5e+07
2.0e+07
1.5e+07
1.0e+07
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
rawtotalauto

FIG. 151

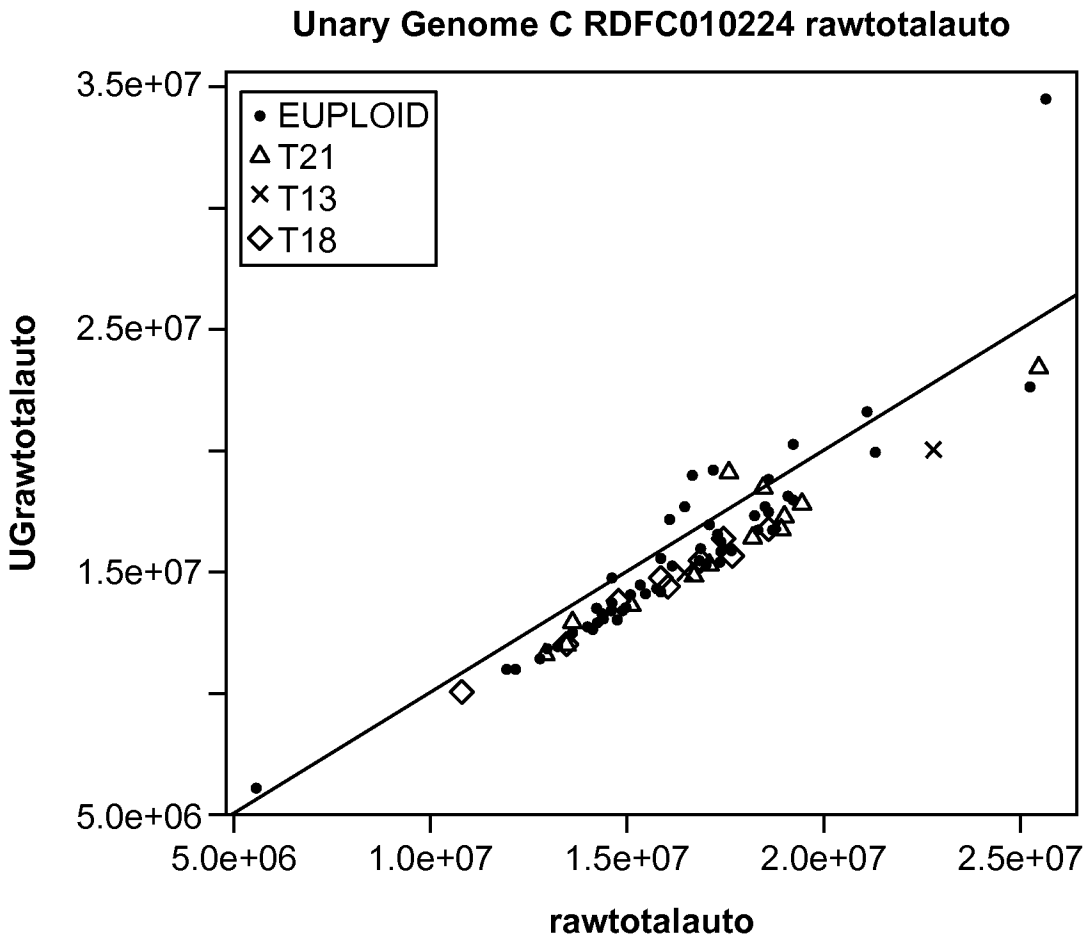

Unary Genome C RDFC010224 rawtotalauto
EUPLOID
T21
T13
T18
UGrawtotalauto
3.5e+07
2.5e+07
1.5e+07
5.0e+06
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
rawtotalauto

FIG. 152

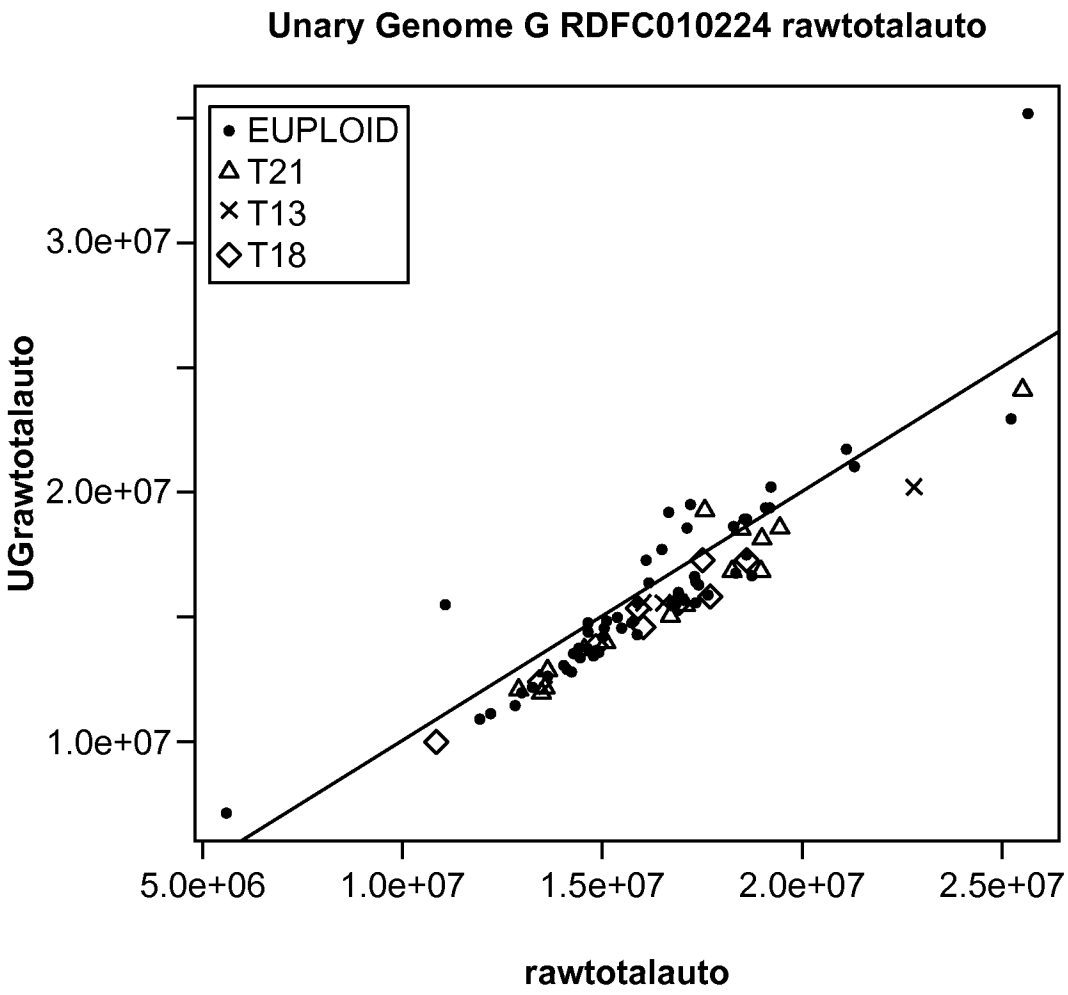

Unary Genome G RDFC010224 rawtotalauto
EUPLOID
T21
T13
T18
UGrawtotalauto
3.0e+07
2.0e+07
1.0e+07
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
rawtotalauto

FIG. 153

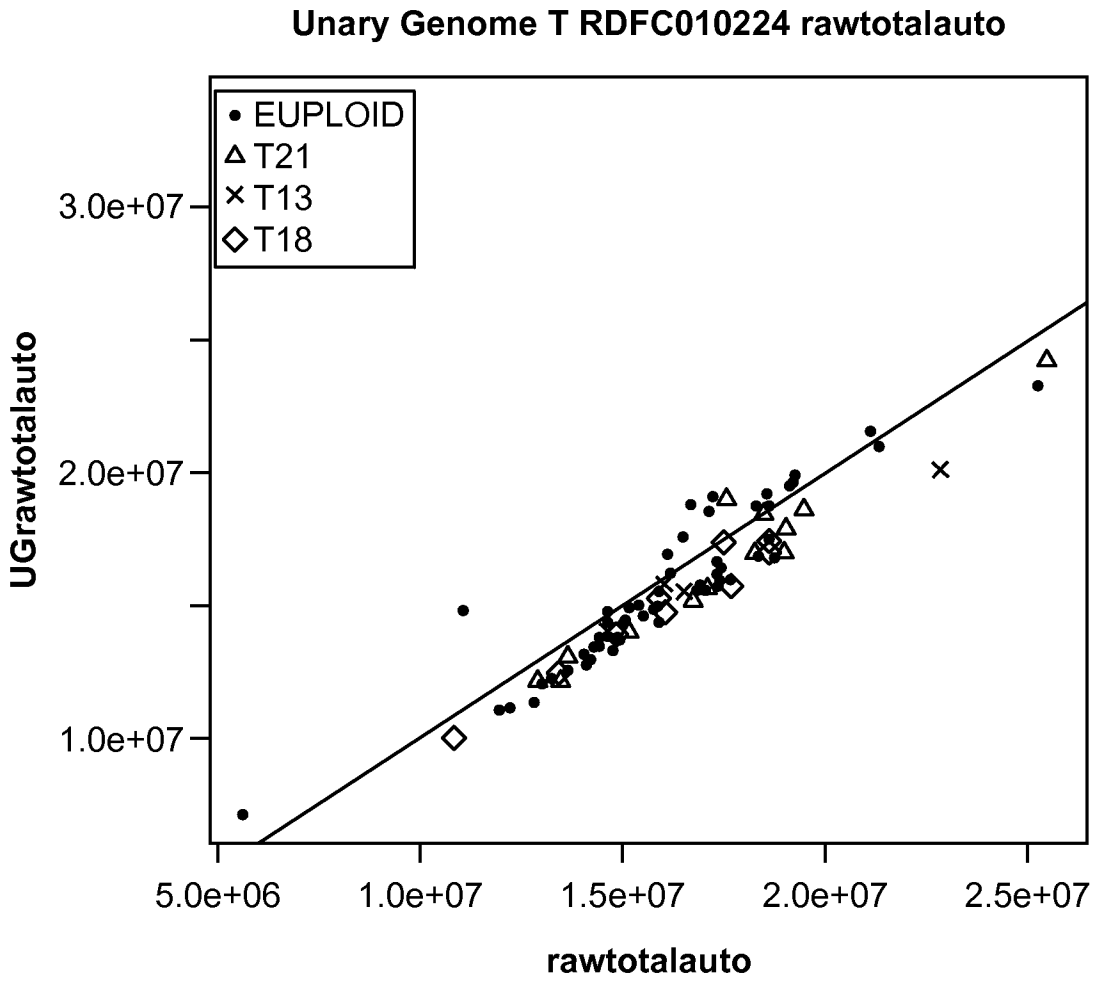

Unary Genome T RDFC010224 rawtotalauto
EUPLOID
T21
T13
T18
3.0e+07
2.0e+07
1.0e+07
UGrawtotalauto
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
rawtotalauto

FIG. 154

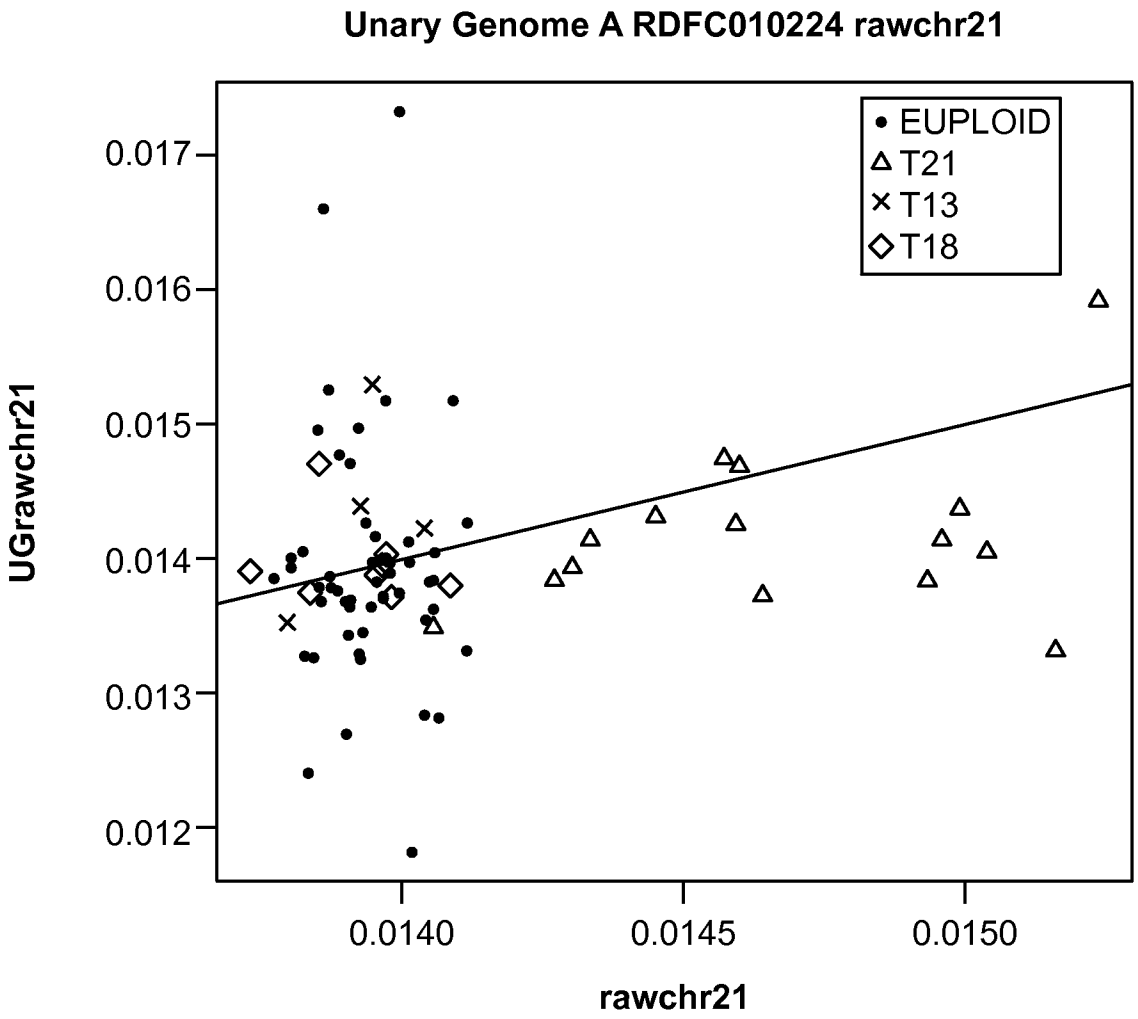

Unary Genome A RDFC010224 rawchr21
EUPLOID
T21
T13
T18
UGrawchr21
0.017
0.016
0.015
0.014
0.013
0.012
0.0140
0.0145
0.0150
rawchr21

FIG. 155

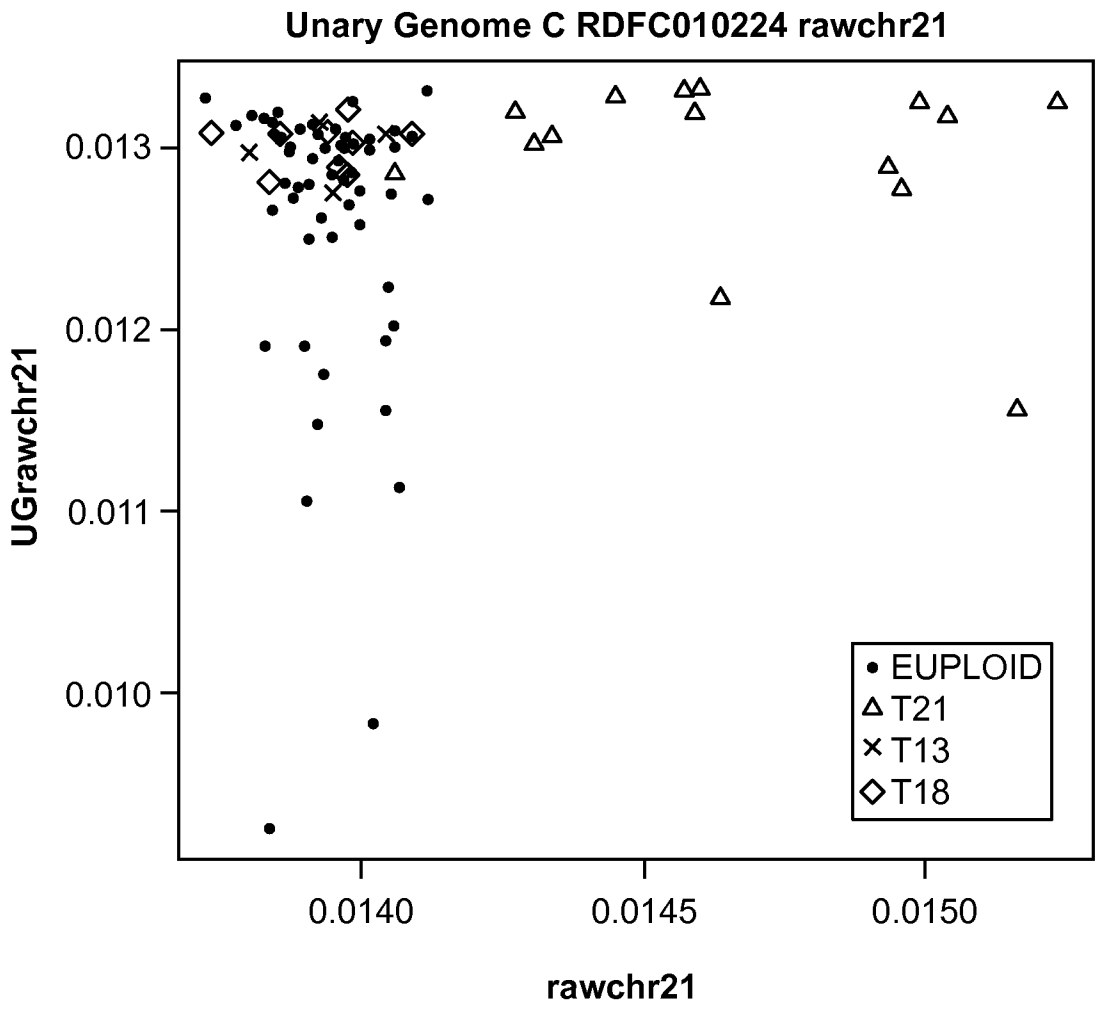

Unary Genome C RDFC010224 rawchr21
UGrawchr21
0.013
0.012
0.011
0.010
0.0140
0.0145
0.0150
rawchr21
EUPLOID
T21
T13
T18

FIG. 156

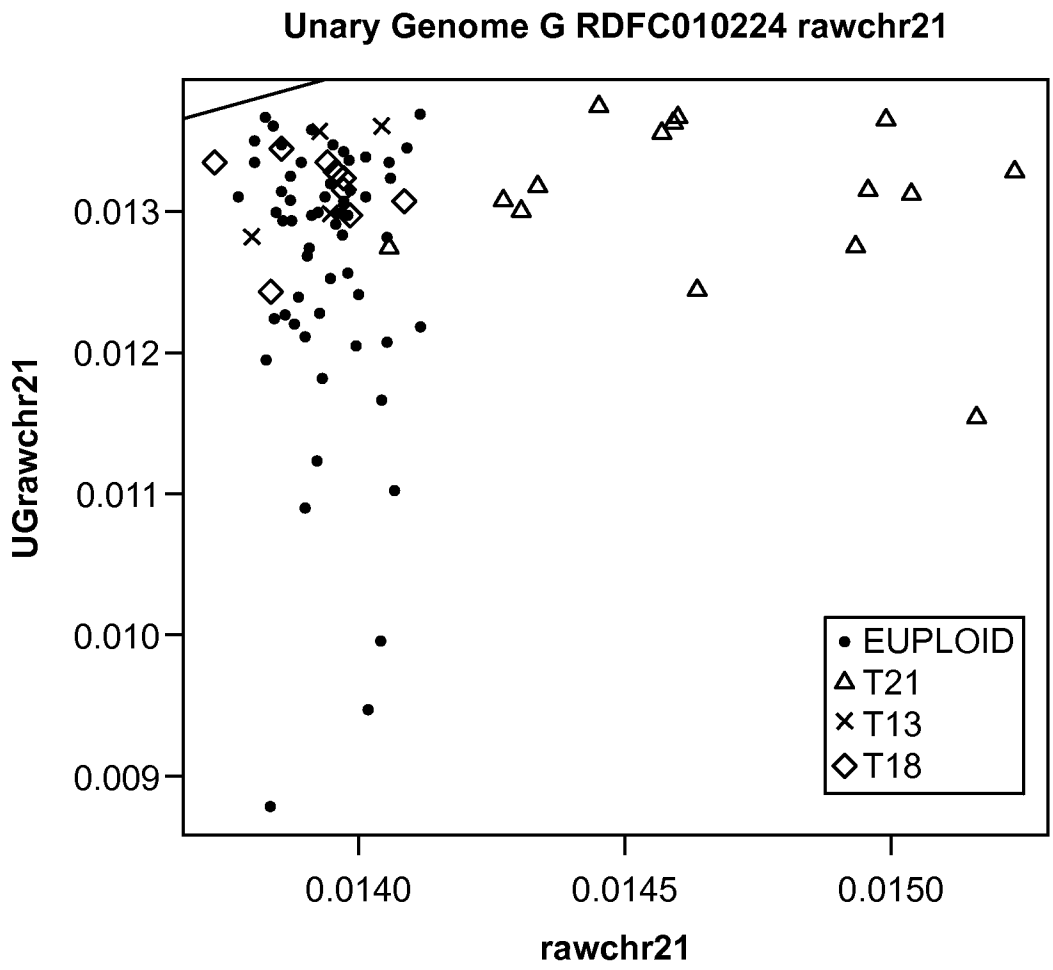

Unary Genome G RDFC010224 rawchr21
UGrawchr21
0.013
0.012
0.011
0.010
0.009
0.0140
0.0145
0.0150
rawchr21
EUPLOID
T21
T13
T18

FIG. 157

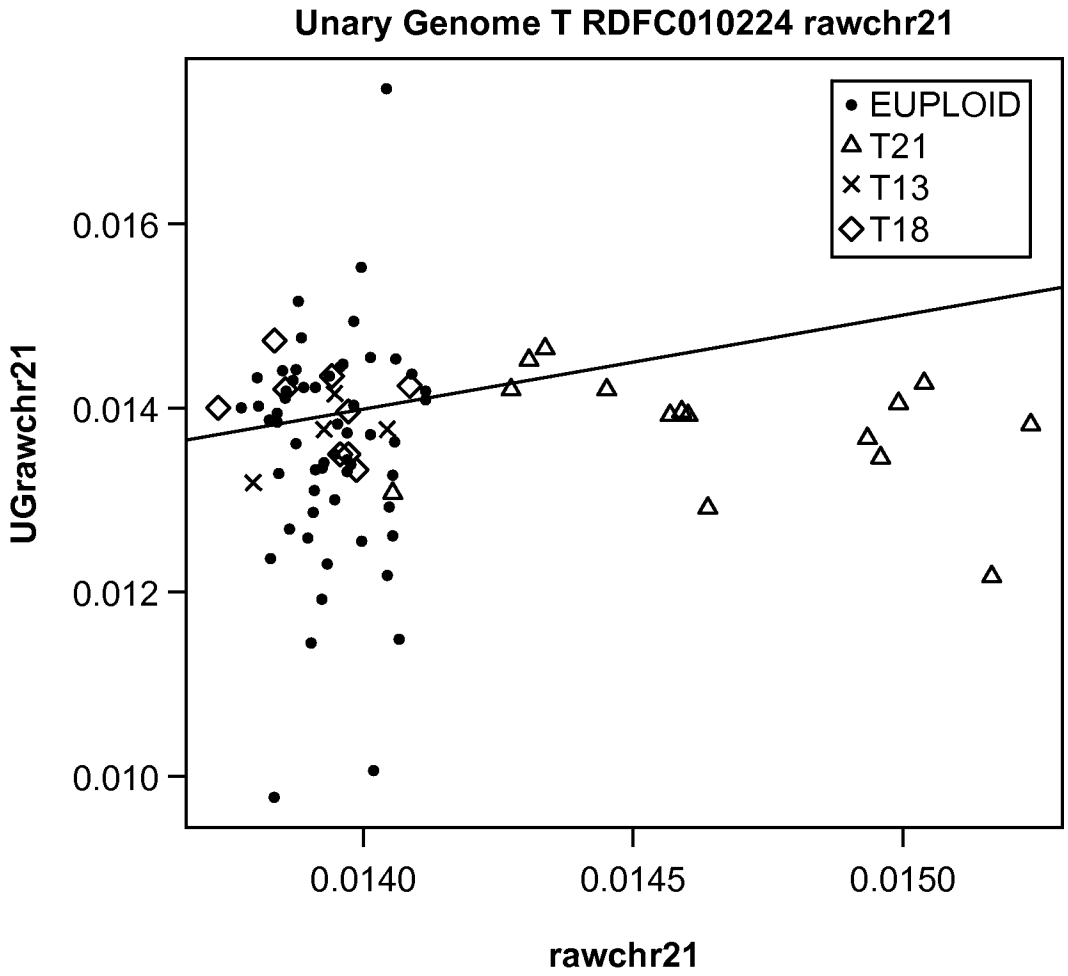

Unary Genome T RDFC010224 rawchr21
EUPLOID
T21
T13
T18
0.016
0.014
0.012
0.010
UGrawchr21
0.0140
0.0145
0.0150
rawchr21

FIG. 158

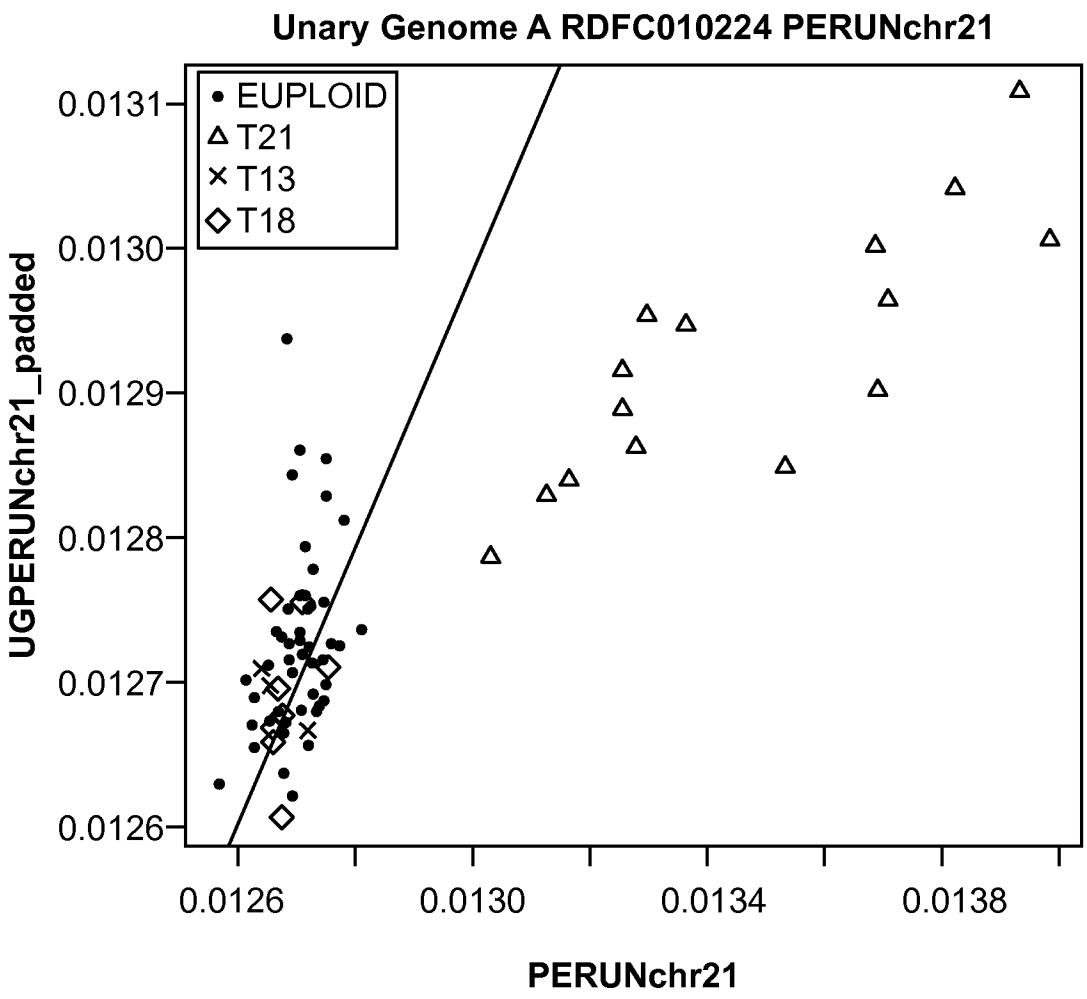

Unary Genome A RDFC010224 PERUNchr21
EUPLOID
T21
T13
T18
UGPERUNchr21_padded
0.0131
0.0130
0.0129
0.0128
0.0127
0.0126
0.0126
0.0130
0.0134
0.0138
PERUNchr21

FIG. 159

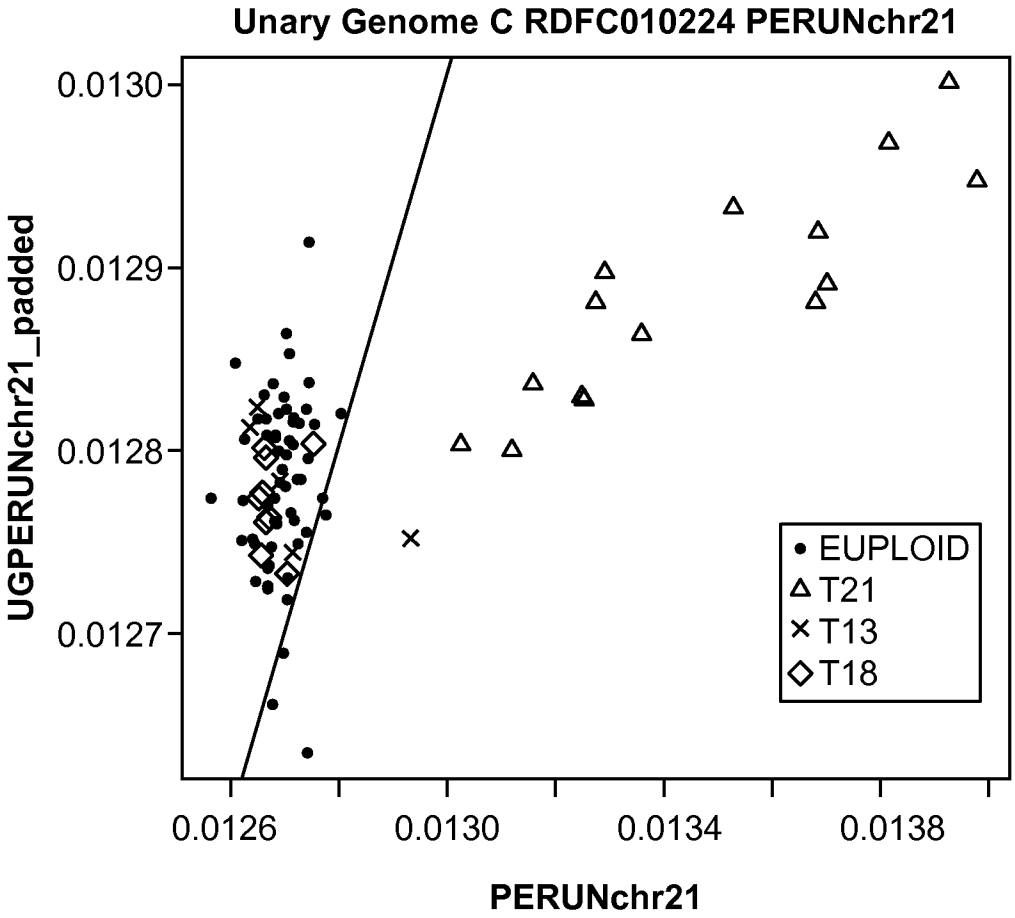

Unary Genome C RDFC010224 PERUNchr21
UGPERUNchr21_padded
0.0130
0.0129
0.0128
0.0127
0.0126
0.0130
0.0134
0.0138
PERUNchr21
EUPLOID
T21
T13
T18

FIG. 160

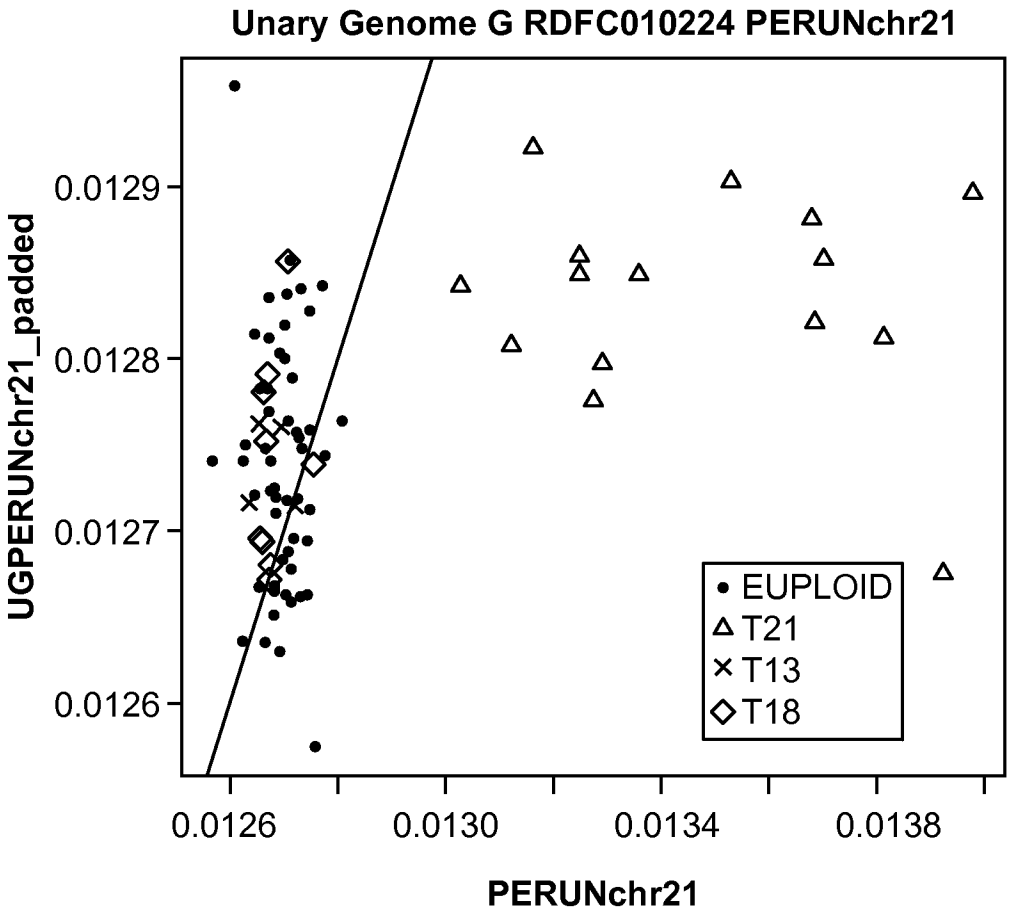

Unary Genome G RDFC010224 PERUNchr21
UGPERUNchr21_padded
0.0129
0.0128
0.0127
0.0126
0.0126
0.0130
0.0134
0.0138
PERUNchr21
EUPLOID
T21
T13
T18

FIG. 161

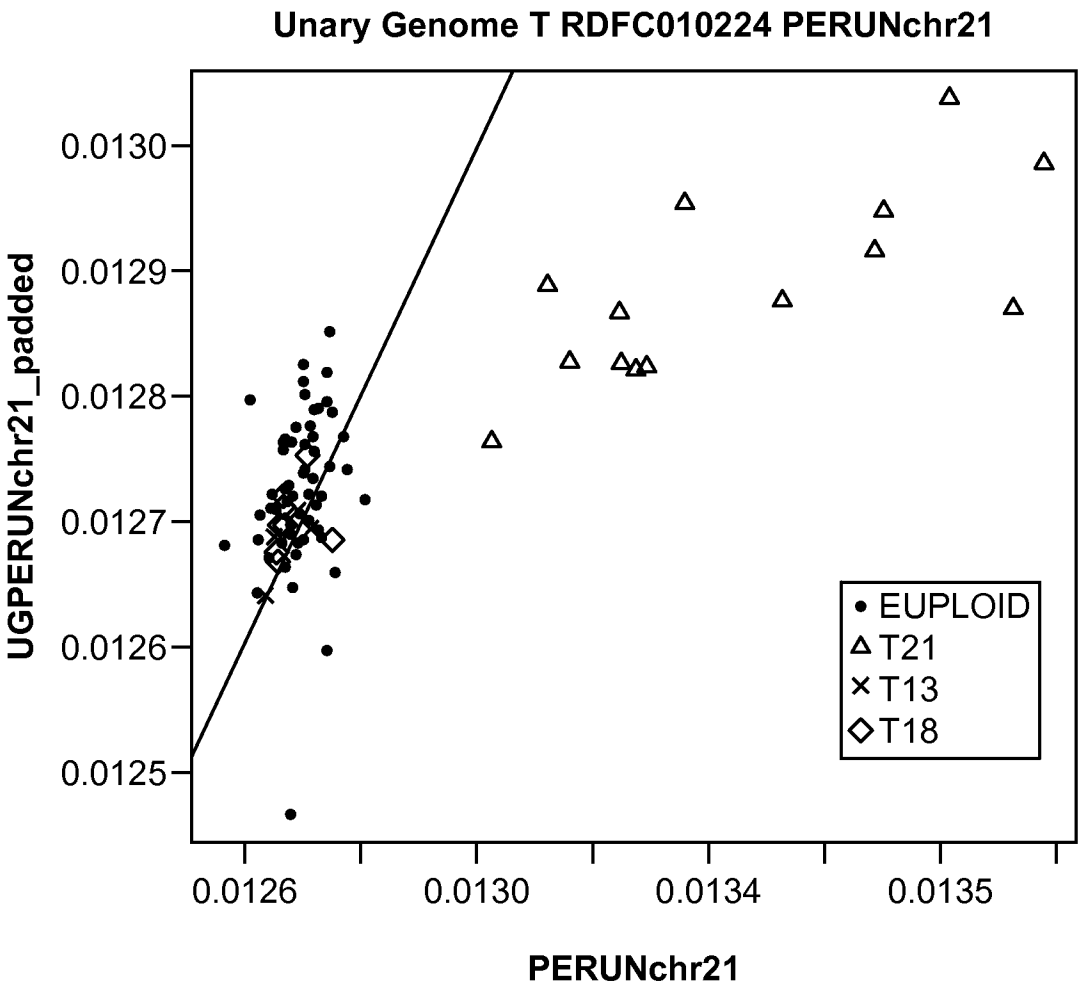

Unary Genome T RDFC010224 PERUNchr21
UGPERUNchr21_padded
0.0130
0.0129
0.0128
0.0127
0.0126
0.0125
0.0126
0.0130
0.0134
0.0135
PERUNchr21
EUPLOID
T21
T13
T18

FIG. 162

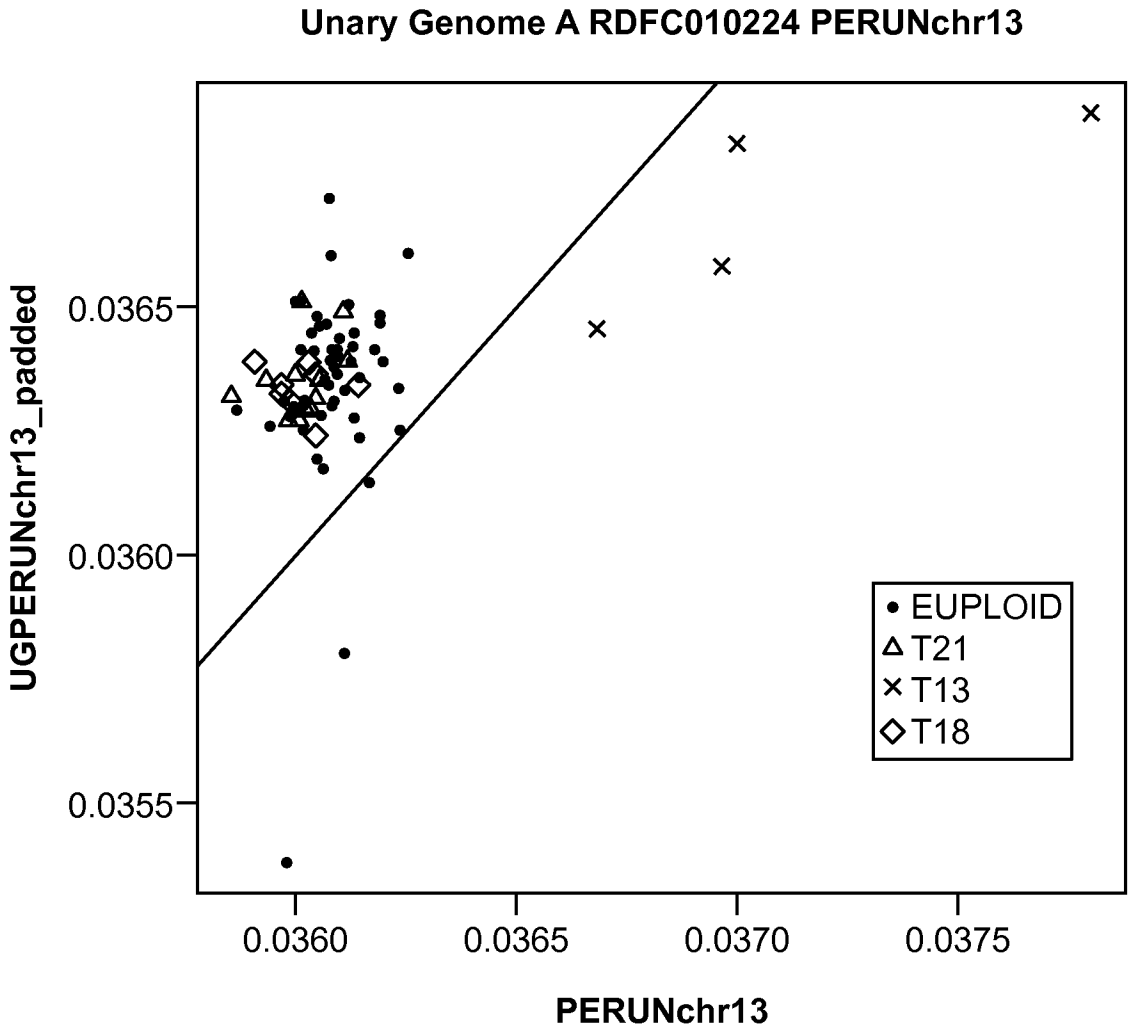

Unary Genome A RDFC010224 PERUNchr13
UGPERUNchr13_padded
0.0365
0.0360
0.0355
0.0360
0.0365
0.0370
0.0375
PERUNchr13
EUPLOID
T21
T13
T18

FIG. 163

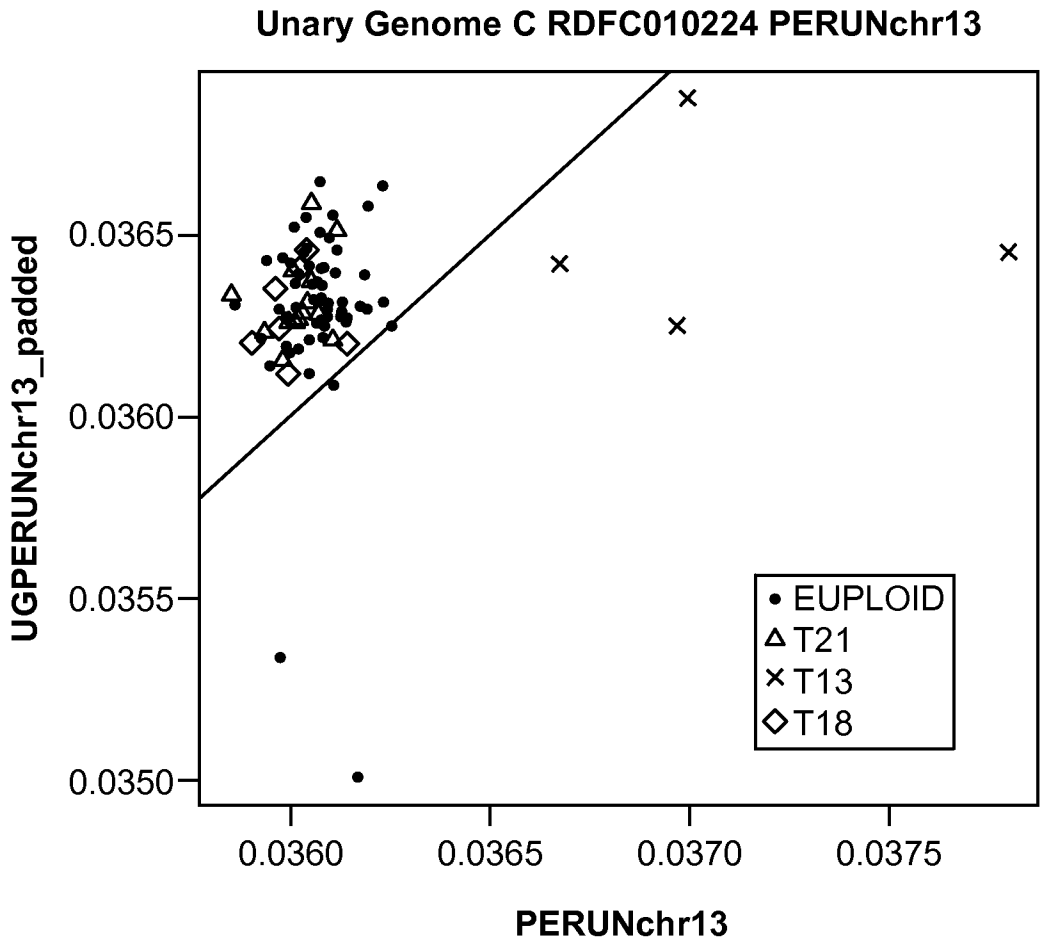

Unary Genome C RDFC010224 PERUNchr13
UGPERUNchr13_padded
0.0365
0.0360
0.0355
0.0350
0.0360
0.0365
0.0370
0.0375
PERUNchr13
EUPLOID
T21
T13
T18

FIG. 164

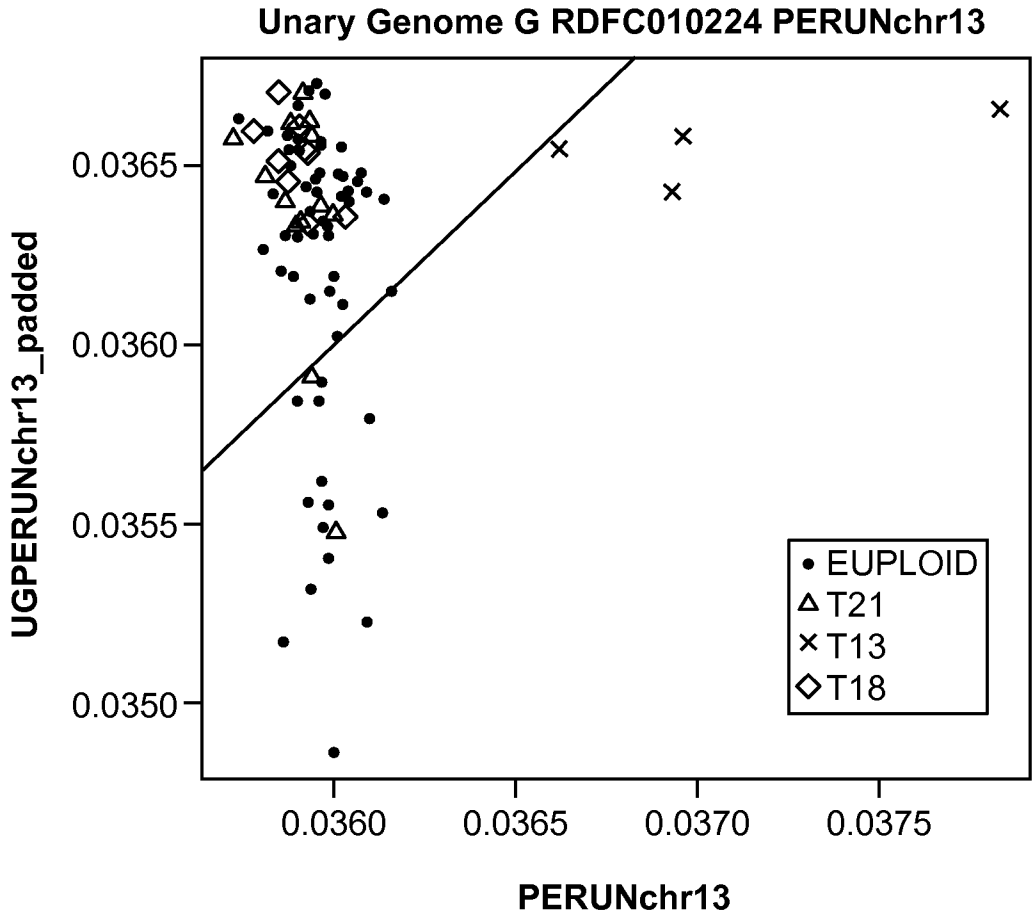

Unary Genome G RDFC010224 PERUNchr13
UGPERUNchr13_padded
0.0365
0.0360
0.0355
0.0350
0.0360
0.0365
0.0370
0.0375
PERUNchr13
EUPLOID
T21
T13
T18

FIG. 165

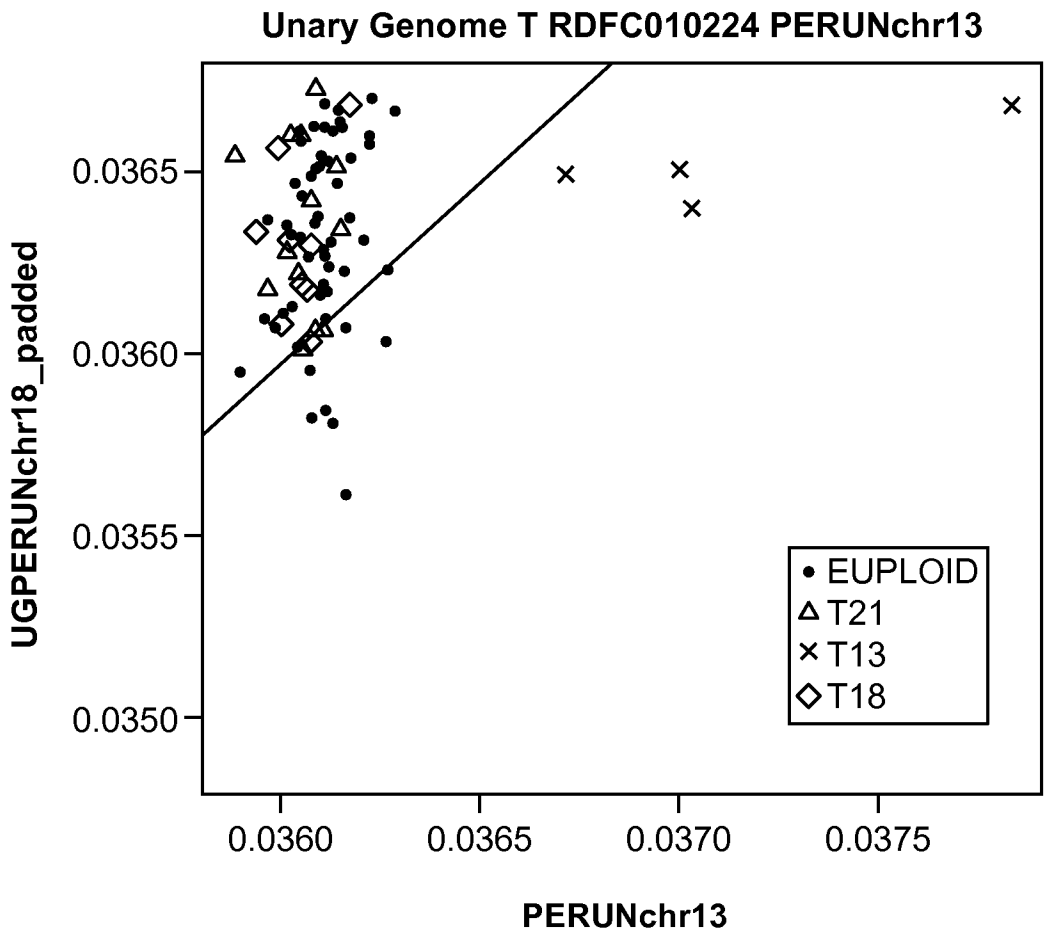

Unary Genome T RDFC010224 PERUNchr13
UGPERUNchr18_padded
0.0365
0.0360
0.0355
0.0350
0.0360
0.0365
0.0370
0.0375
PERUNchr13
EUPLOID
T21
T13
T18

FIG. 166

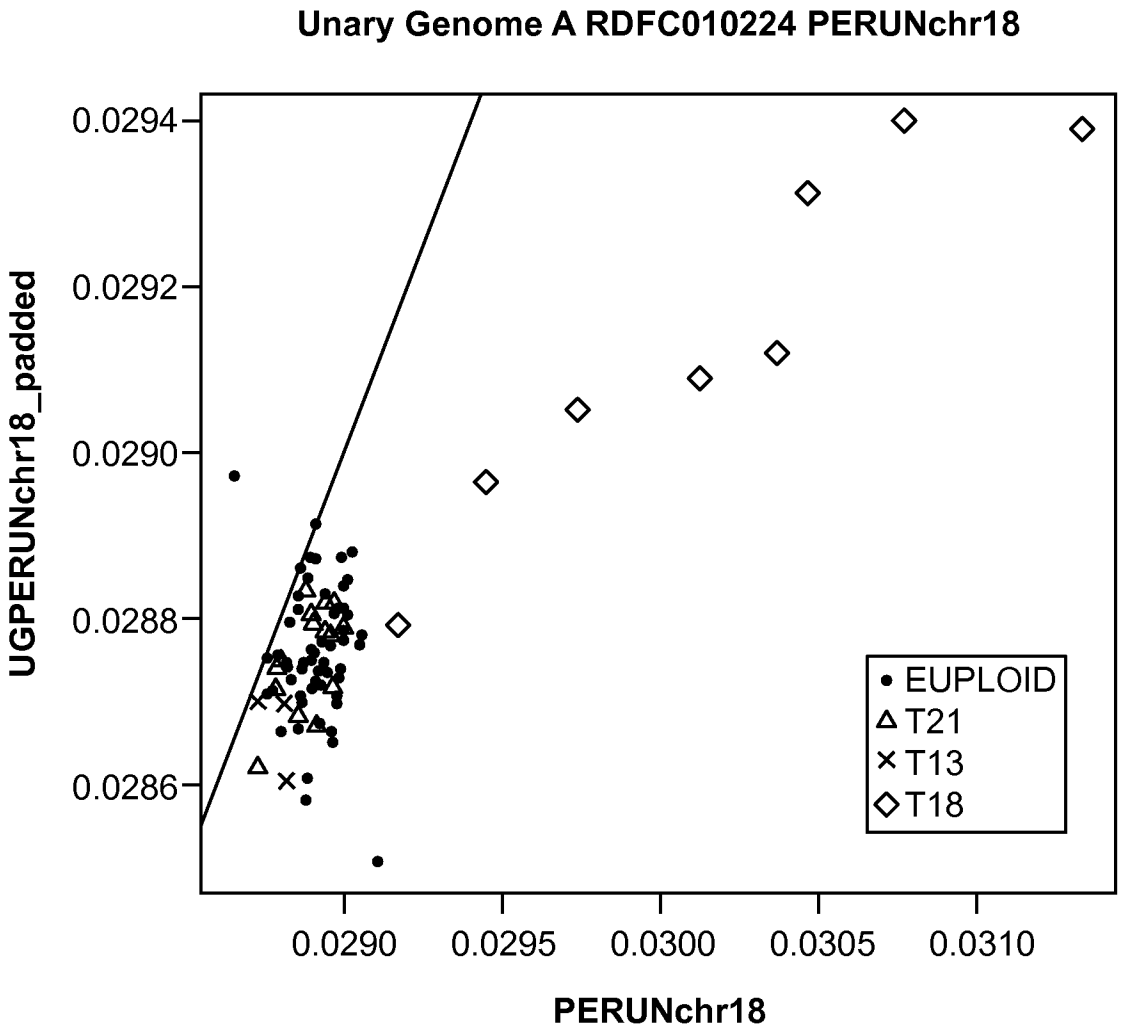

Unary Genome A RDFC010224 PERUNchr18
UGPERUNchr18_padded
0.0294
0.0292
0.0290
0.0288
0.0286
0.0290
0.0295
0.0300
0.0305
0.0310
PERUNchr18
EUPLOID
T21
T13
T18

FIG. 167

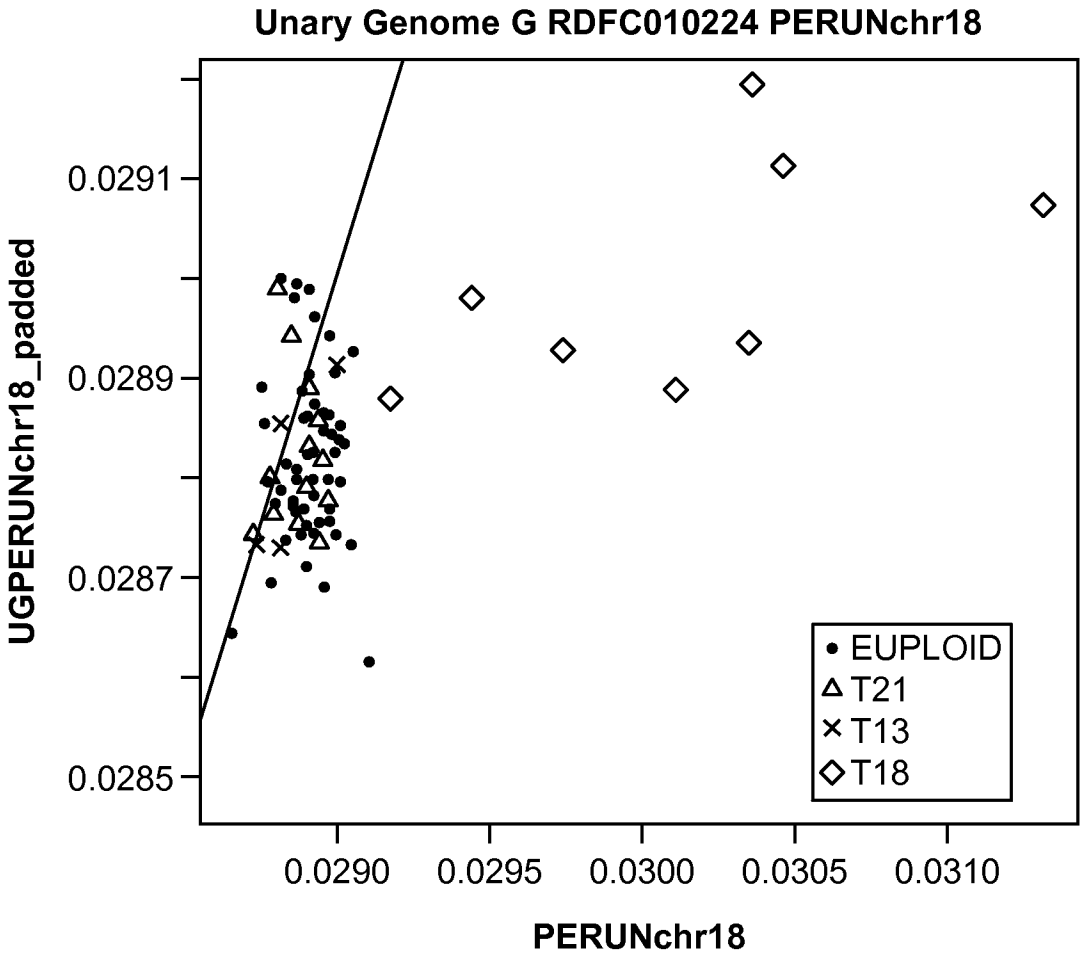

Unary Genome G RDFC010224 PERUNchr18
UGPERUNchr18_padded
0.0291
0.0289
0.0287
0.0285
0.0290
0.0295
0.0300
0.0305
0.0310
PERUNchr18
EUPLOID
T21
T13
T18

FIG. 168

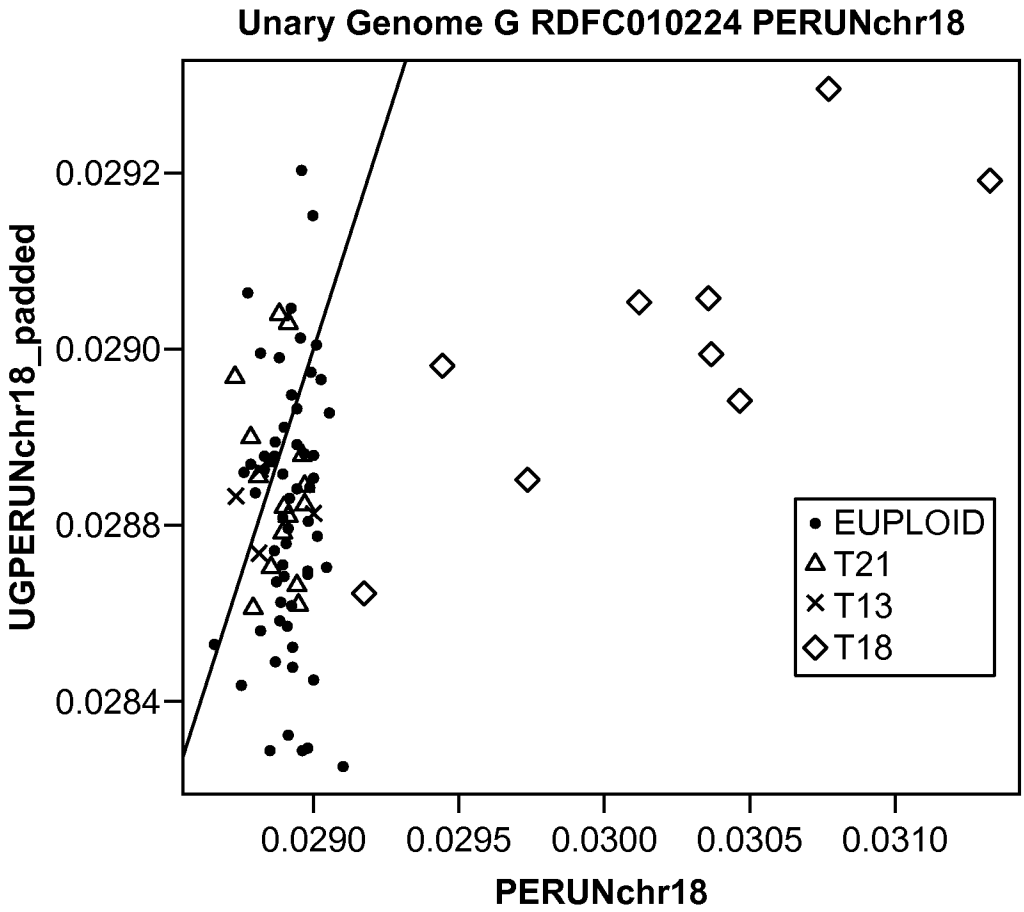

Unary Genome G RDFC010224 PERUNchr18
UGPERUNchr18_padded
0.0292
0.0290
0.0288
0.0284
0.0290
0.0295
0.0300
0.0305
0.0310
PERUNchr18
EUPLOID
T21
T13
T18

FIG. 169

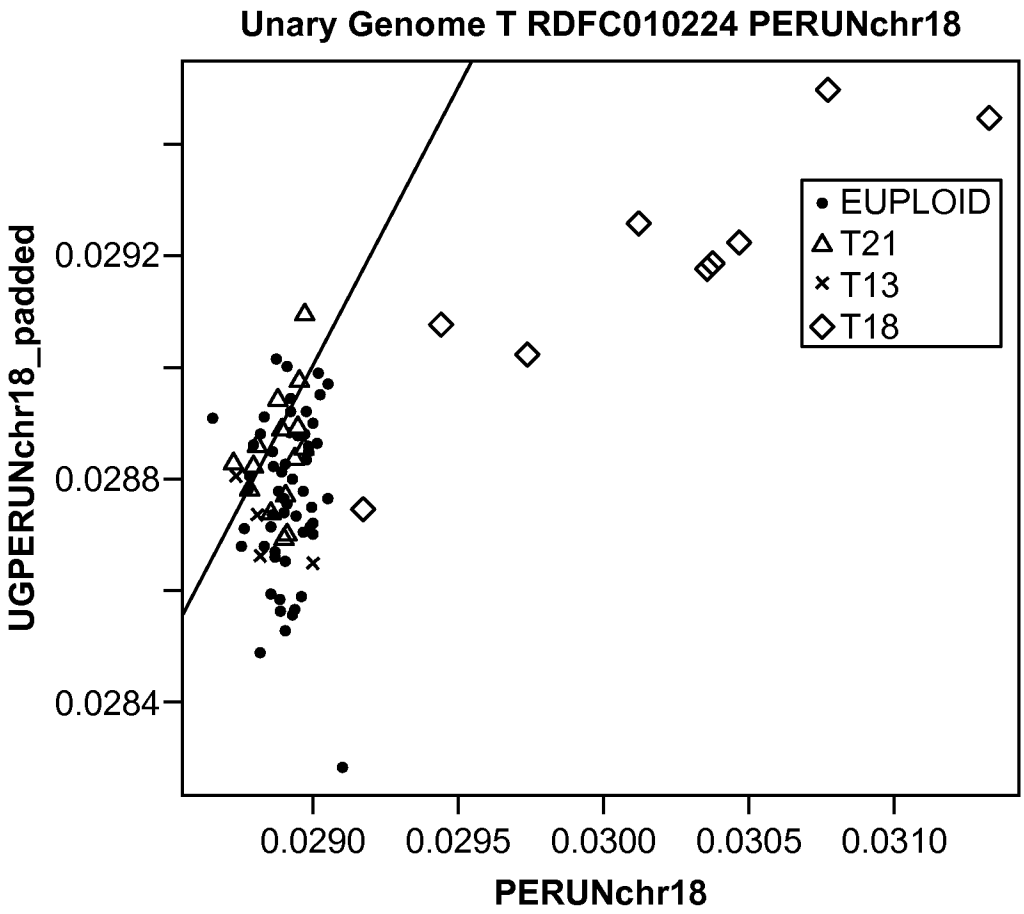

Unary Genome T RDFC010224 PERUNchr18
UGPERUNchr18_padded
0.0292
0.0288
0.0284
0.0290
0.0295
0.0300
0.0305
0.0310
PERUNchr18
EUPLOID
T21
T13
T18

FIG. 170

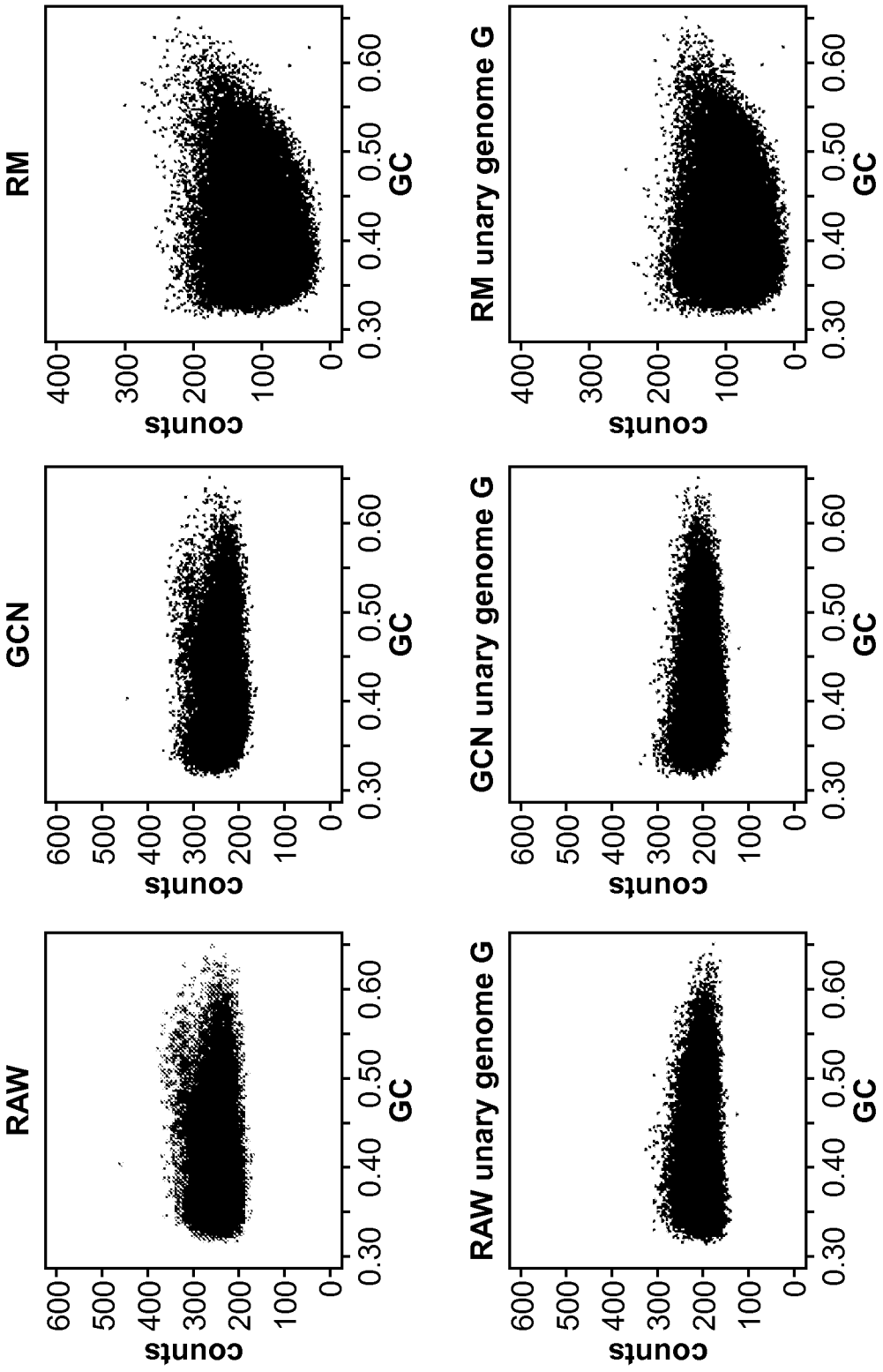

RM
RM unary genome G
GCN
GCN unary genome G
RAW
RAW unary genome G
counts
GC
0
100
200
300
400
500
600
0.30
0.40
0.50
0.60

FIG. 171

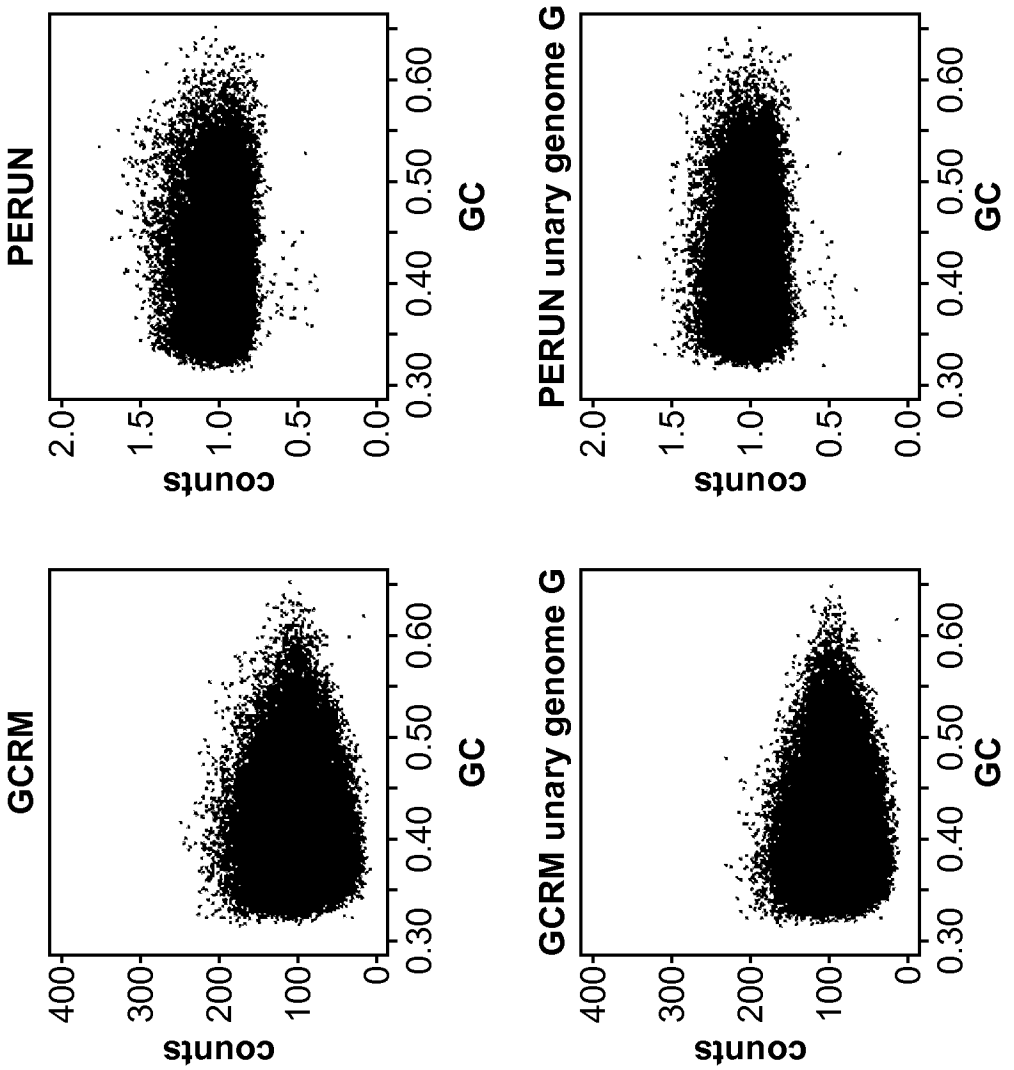

PERUN
counts
GC
PERUN unary genome G
counts
GC
GCRM
counts
GC
GCRM unary genome G
counts
GC

FIG. 171 (Cont.)

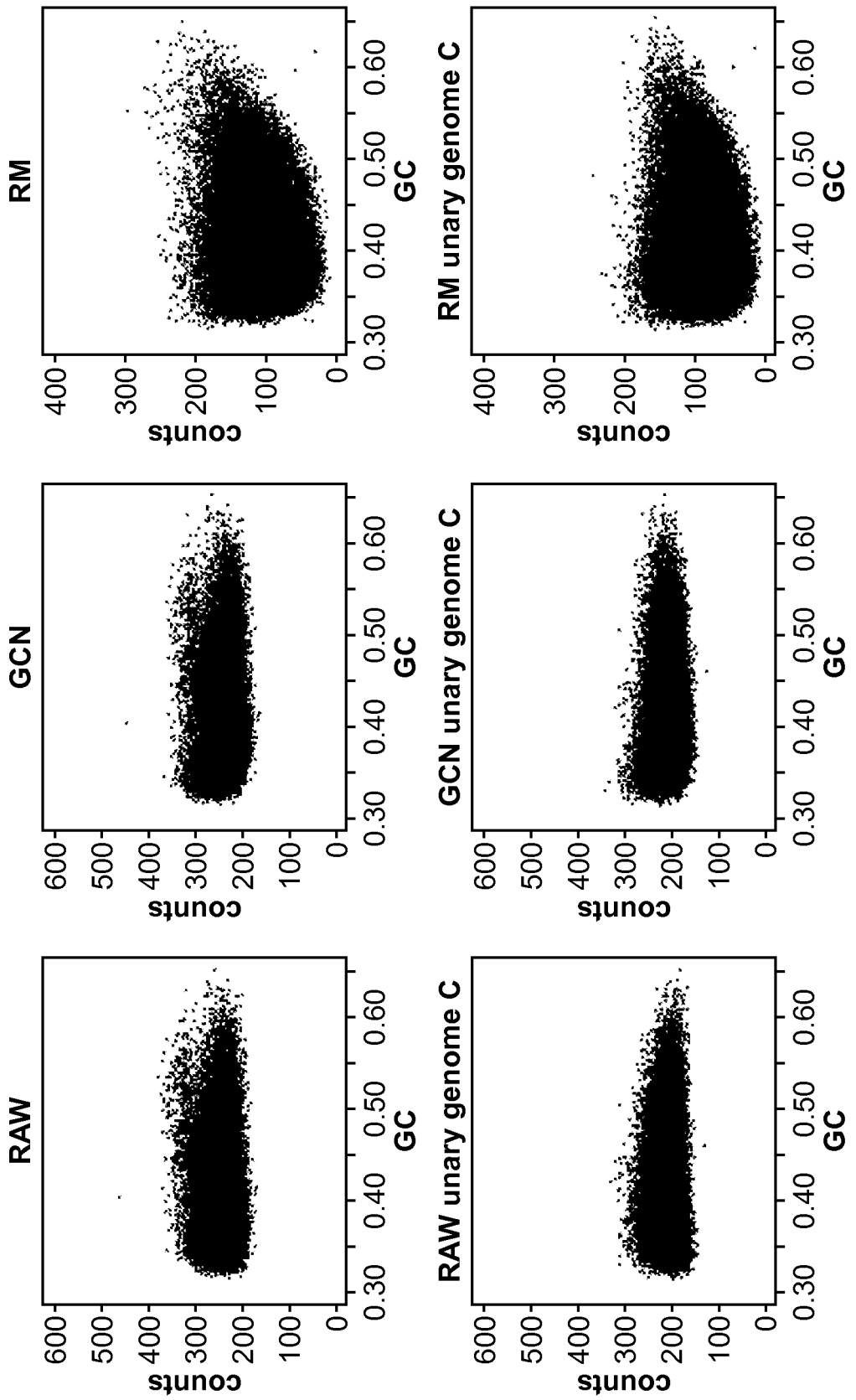

RAW
GCN
RM
RAW unary genome C
GCN unary genome C
RM unary genome C
counts
GC
0
100
200
300
400
500
600
0.30
0.40
0.50
0.60

FIG. 172

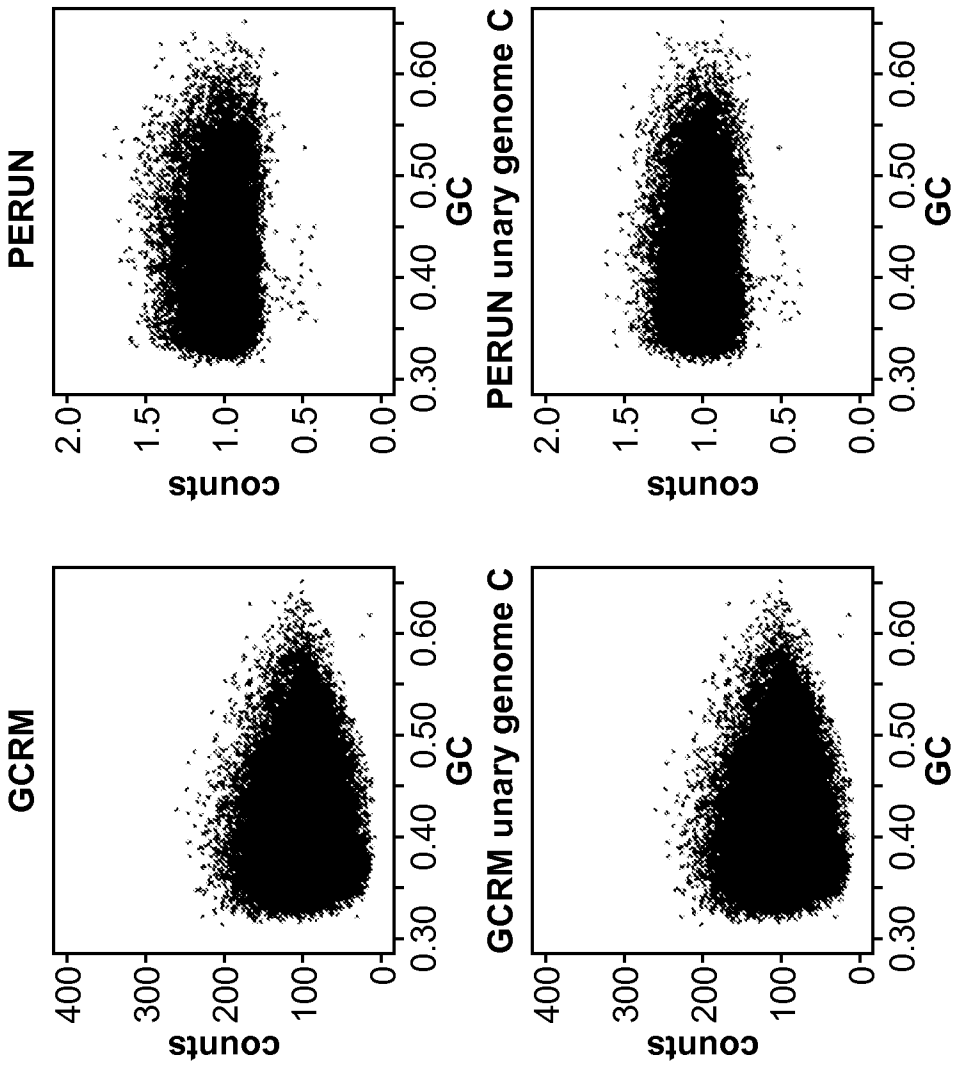

PERUN
PERUN unary genome C
GCRM
GCRM unary genome C
counts
GC
0.0
0.5
1.0
1.5
2.0
0
100
200
300
400
0.30
0.40
0.50
0.60

FIG. 172 (Cont.)

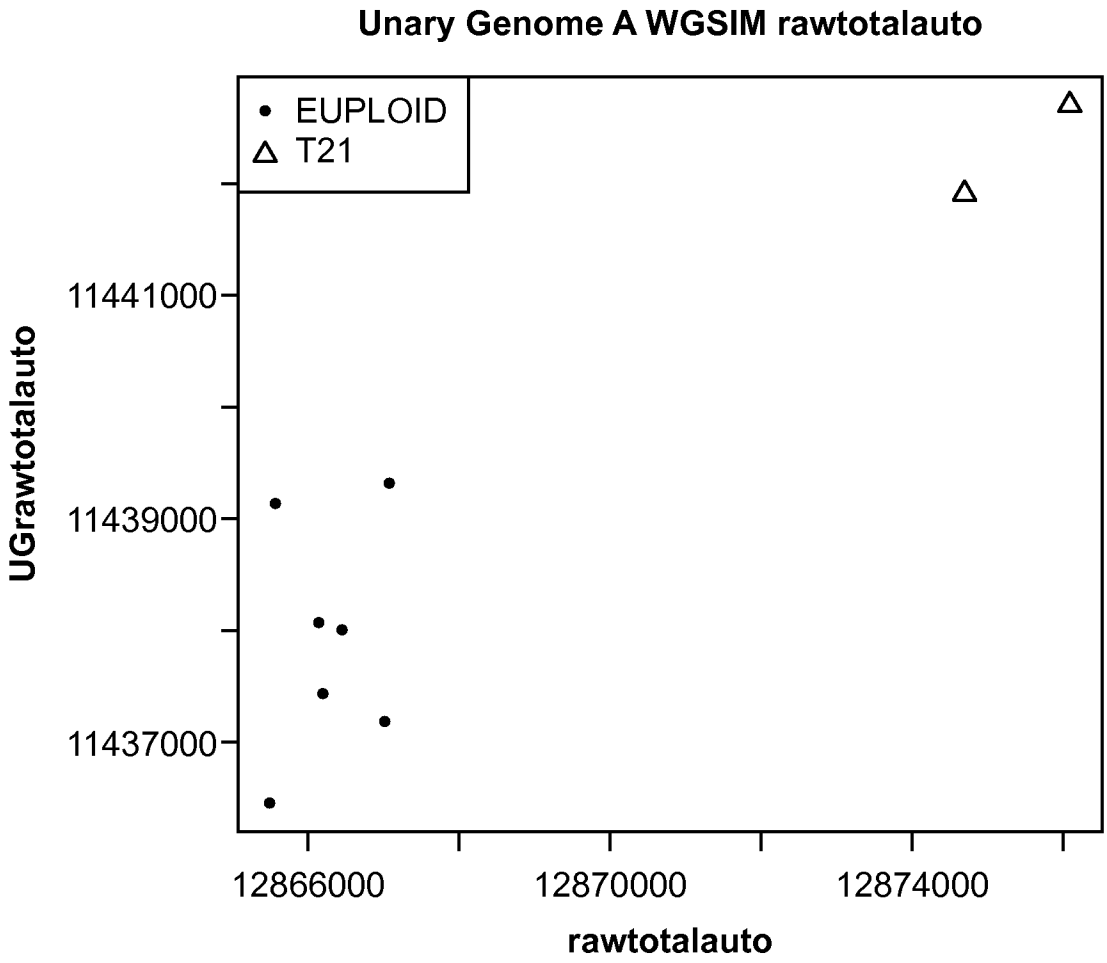

Unary Genome A WGSIM rawtotalauto
EUPLOID
T21
11441000
11439000
11437000
UGrawtotalauto
12866000
12870000
12874000
rawtotalauto

FIG. 173

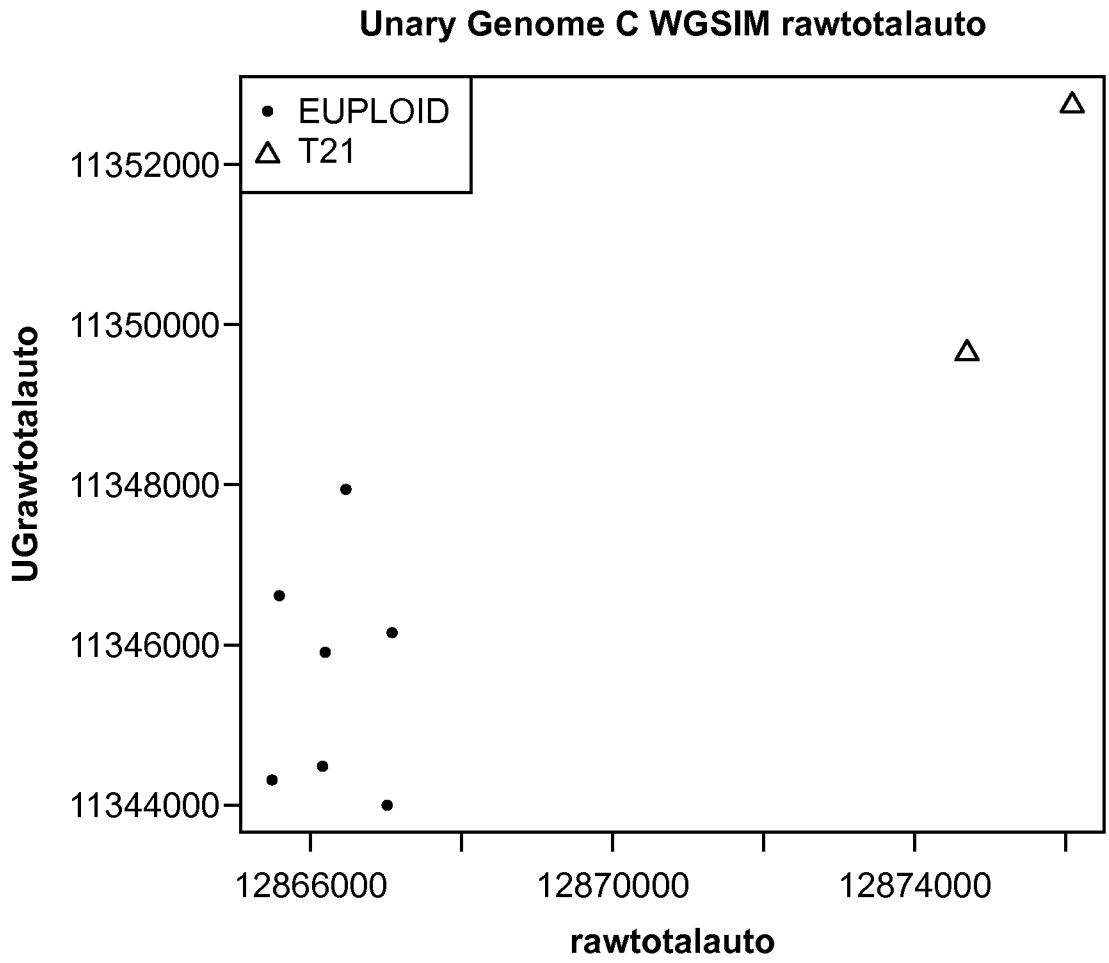

Unary Genome C WGSIM rawtotalauto
EUPLOID
T21
11352000
11350000
11348000
11346000
11344000
UGrawtotalauto
12866000
12870000
12874000
rawtotalauto

FIG. 174

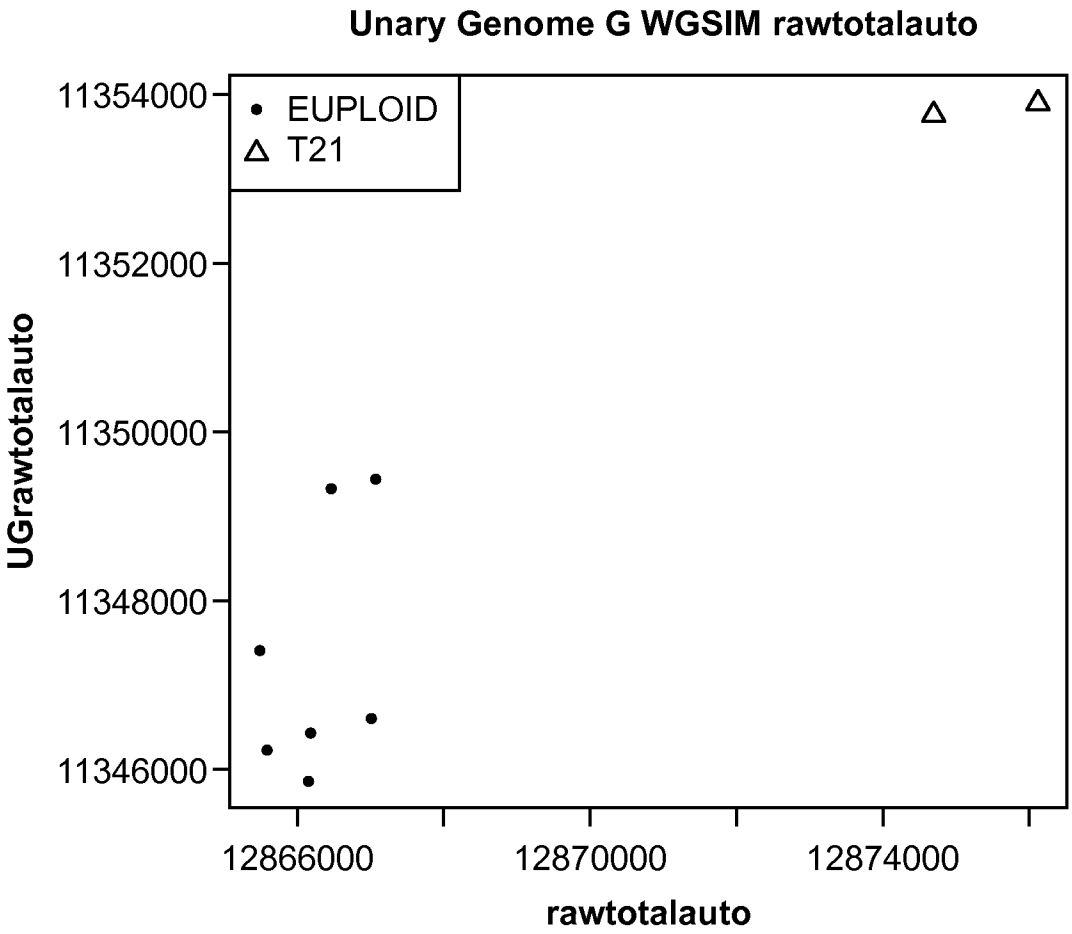

Unary Genome G WGSIM rawtotalauto
EUPLOID
T21
11354000
11352000
11350000
11348000
11346000
UGrawtotalauto
12866000
12870000
12874000
rawtotalauto

FIG. 175

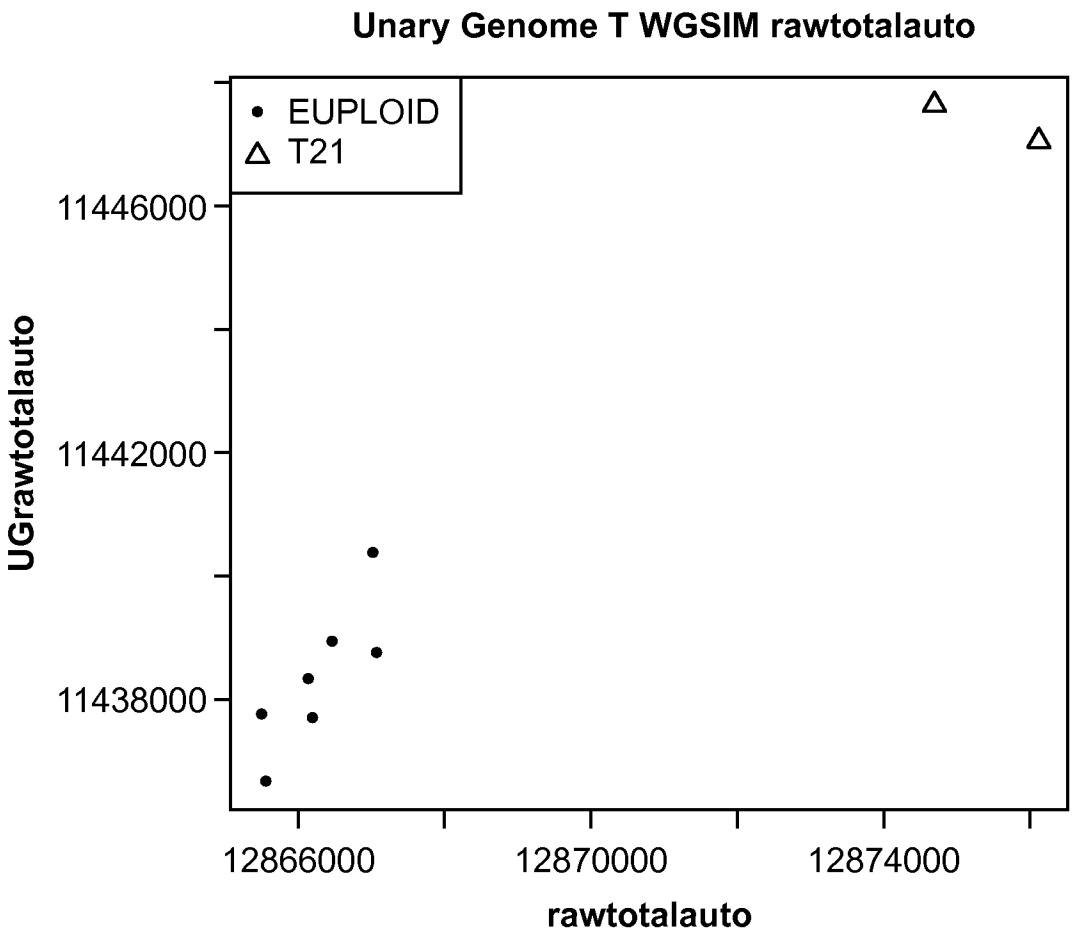

Unary Genome T WGSIM rawtotalauto
EUPLOID
T21
11446000
11442000
11438000
UGrawtotalauto
12866000
12870000
12874000
rawtotalauto

FIG. 176

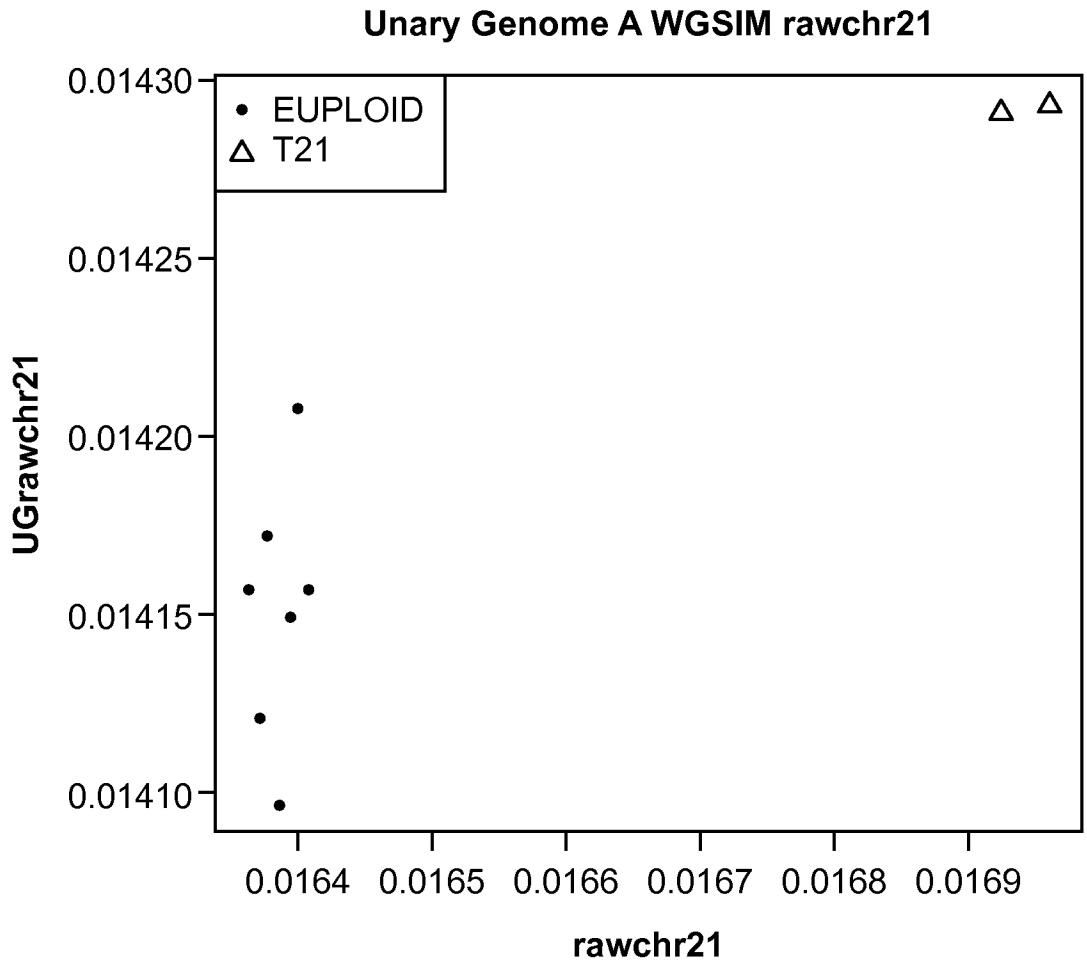

Unary Genome A WGSIM rawchr21
EUPLOID
T21
UGrawchr21
0.01430
0.01425
0.01420
0.01415
0.01410
0.0164
0.0165
0.0166
0.0167
0.0168
0.0169
rawchr21

FIG. 177

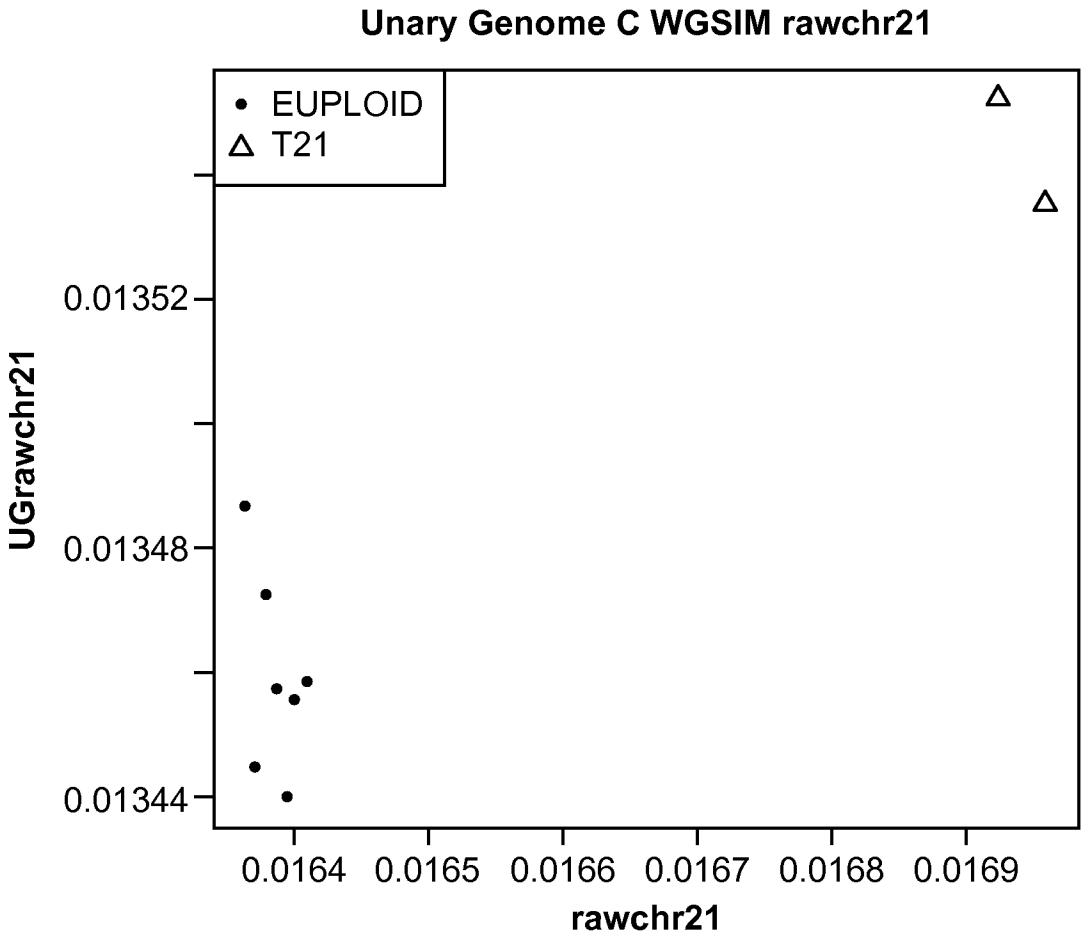

Unary Genome C WGSIM rawchr21
EUPLOID
T21
0.01352
0.01348
0.01344
UGrawchr21
0.0164
0.0165
0.0166
0.0167
0.0168
0.0169
rawchr21

FIG. 178

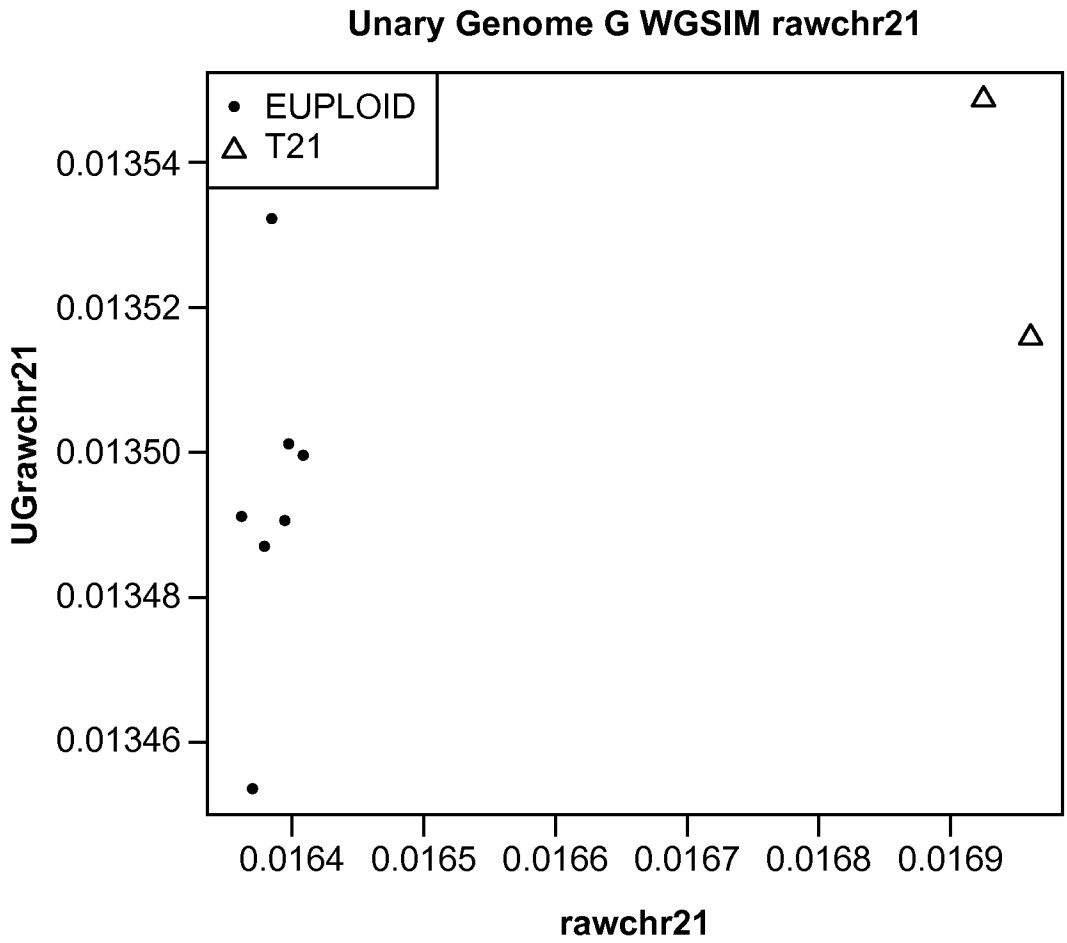

Unary Genome G WGSIM rawchr21
EUPLOID
T21
0.01354
0.01352
0.01350
0.01348
0.01346
UGrawchr21
0.0164
0.0165
0.0166
0.0167
0.0168
0.0169
rawchr21

FIG. 179

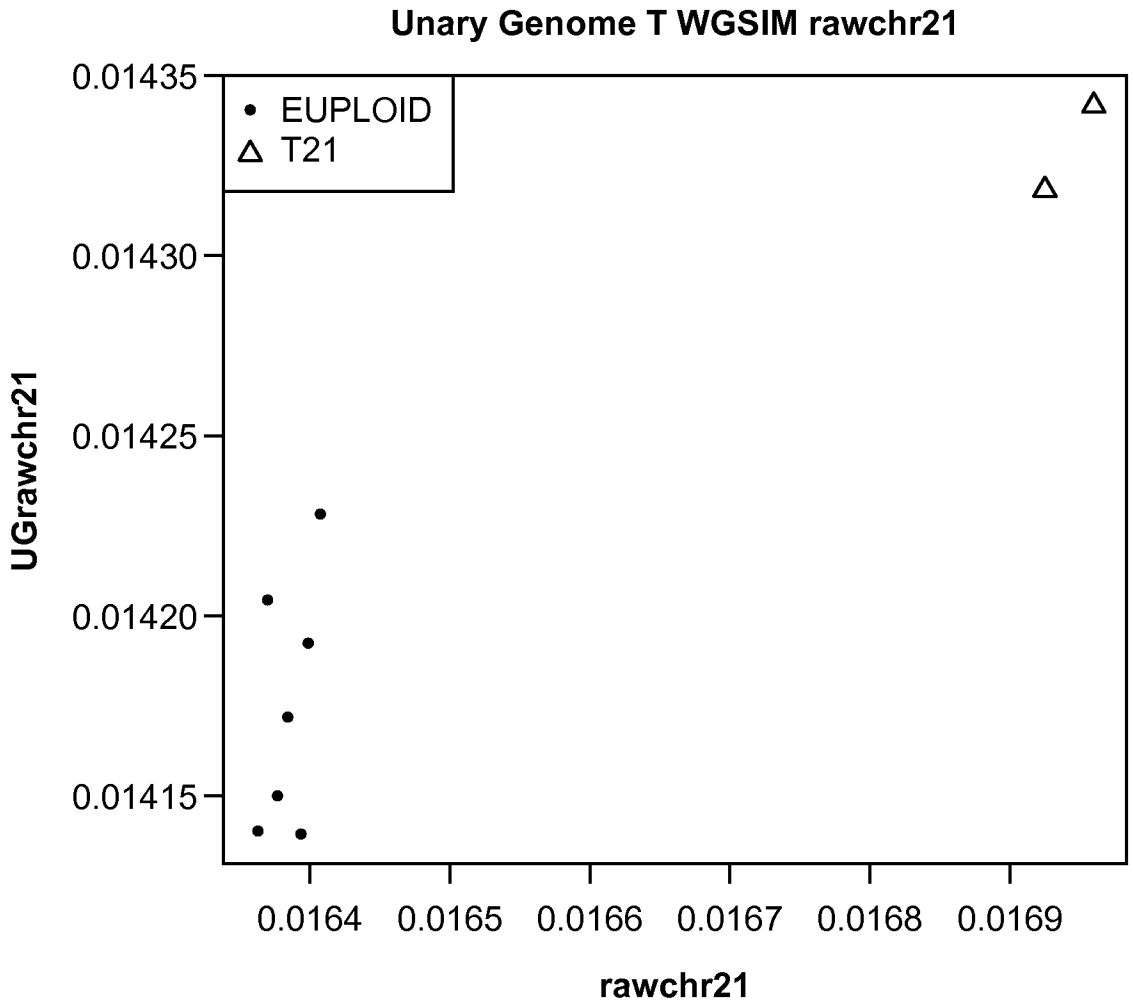

Unary Genome T WGSIM rawchr21
EUPLOID
T21
0.01435
0.01430
0.01425
0.01420
0.01415
UGrawchr21
0.0164
0.0165
0.0166
0.0167
0.0168
0.0169
rawchr21

FIG. 180

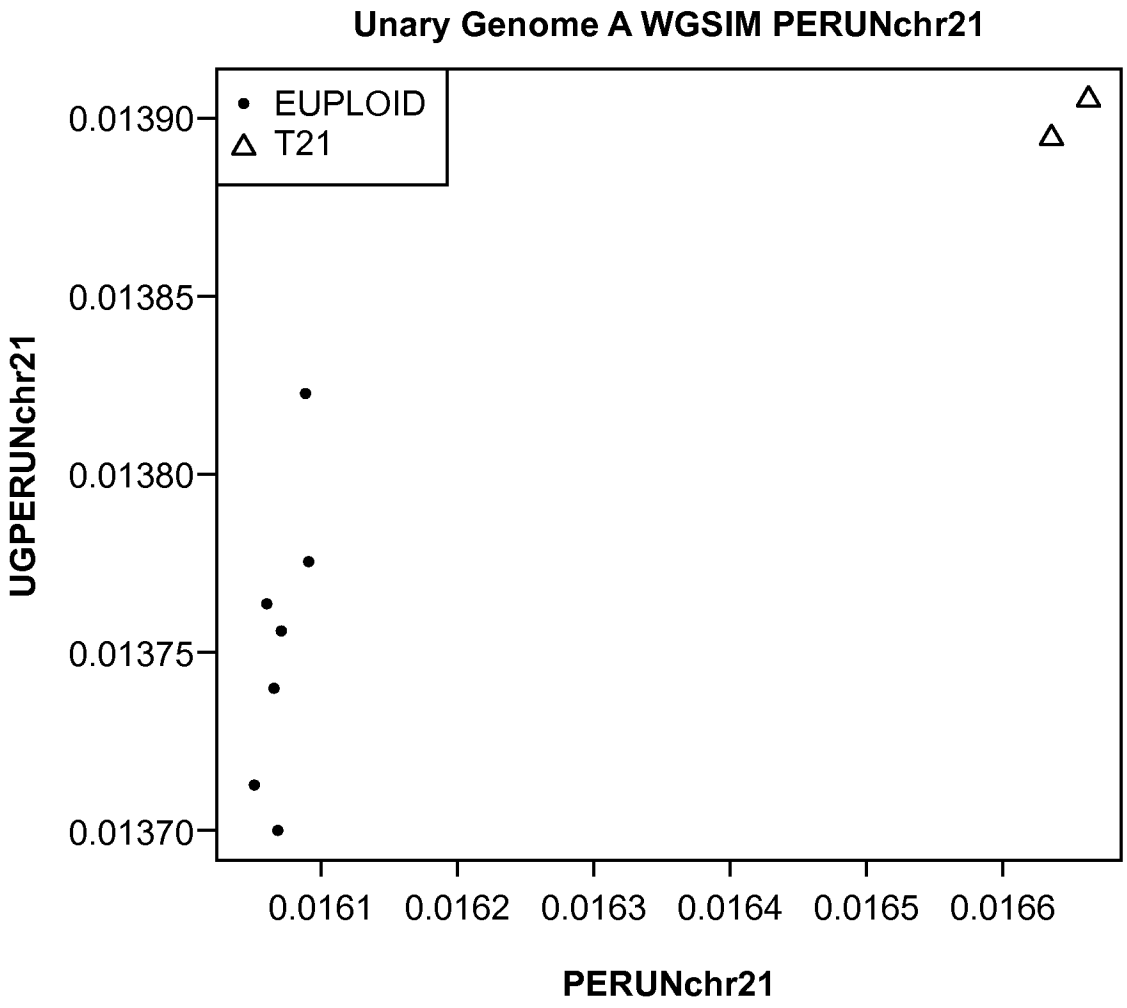

Unary Genome A WGSIM PERUNchr21
EUPLOID
T21
0.01390
0.01385
0.01380
0.01375
0.01370
UGPERUNchr21
0.0161
0.0162
0.0163
0.0164
0.0165
0.0166
PERUNchr21

FIG. 181

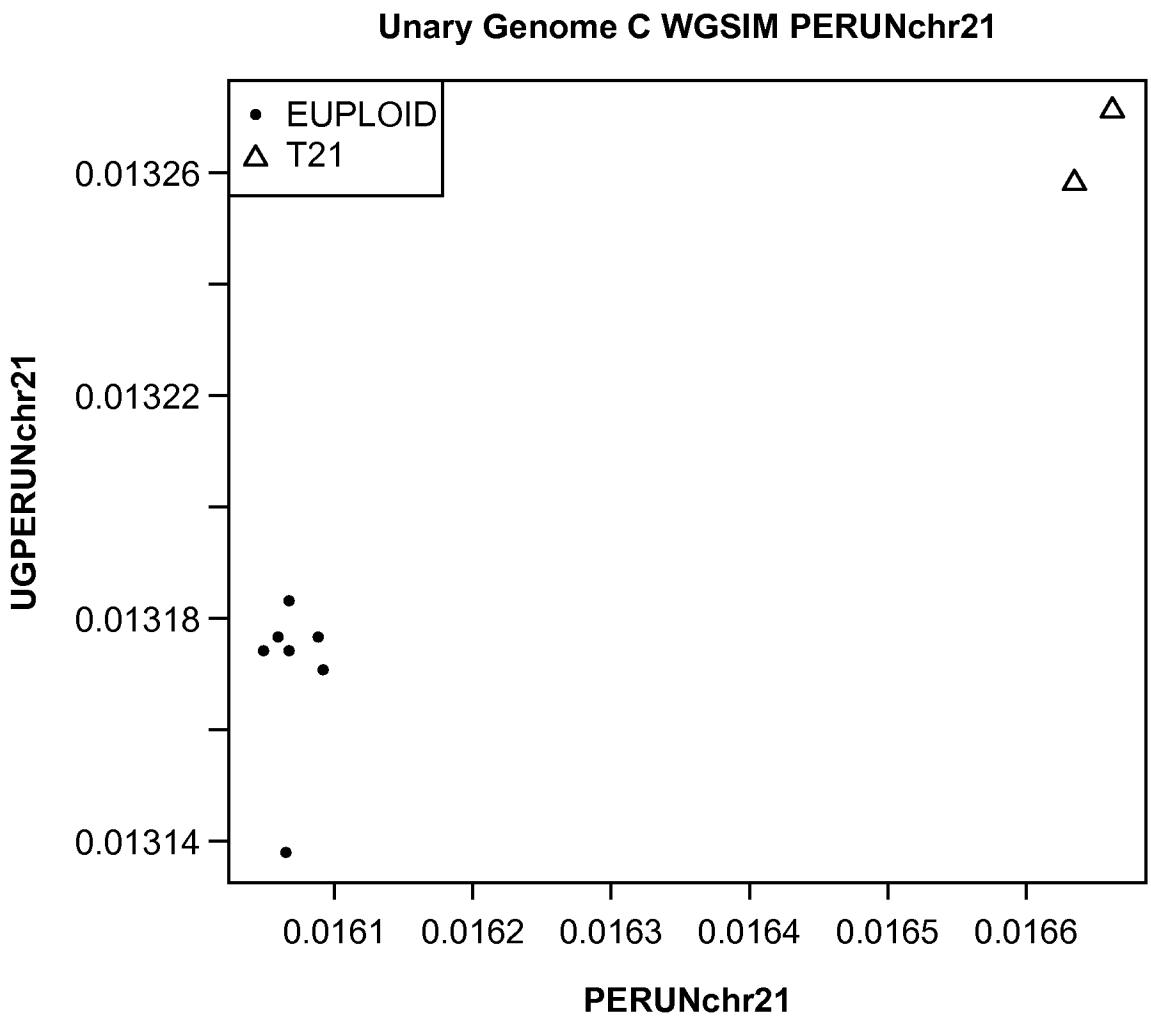

Unary Genome C WGSIM PERUNchr21
EUPLOID
T21
0.01326
0.01322
0.01318
0.01314
UGPERUNchr21
0.0161
0.0162
0.0163
0.0164
0.0165
0.0166
PERUNchr21

FIG. 182

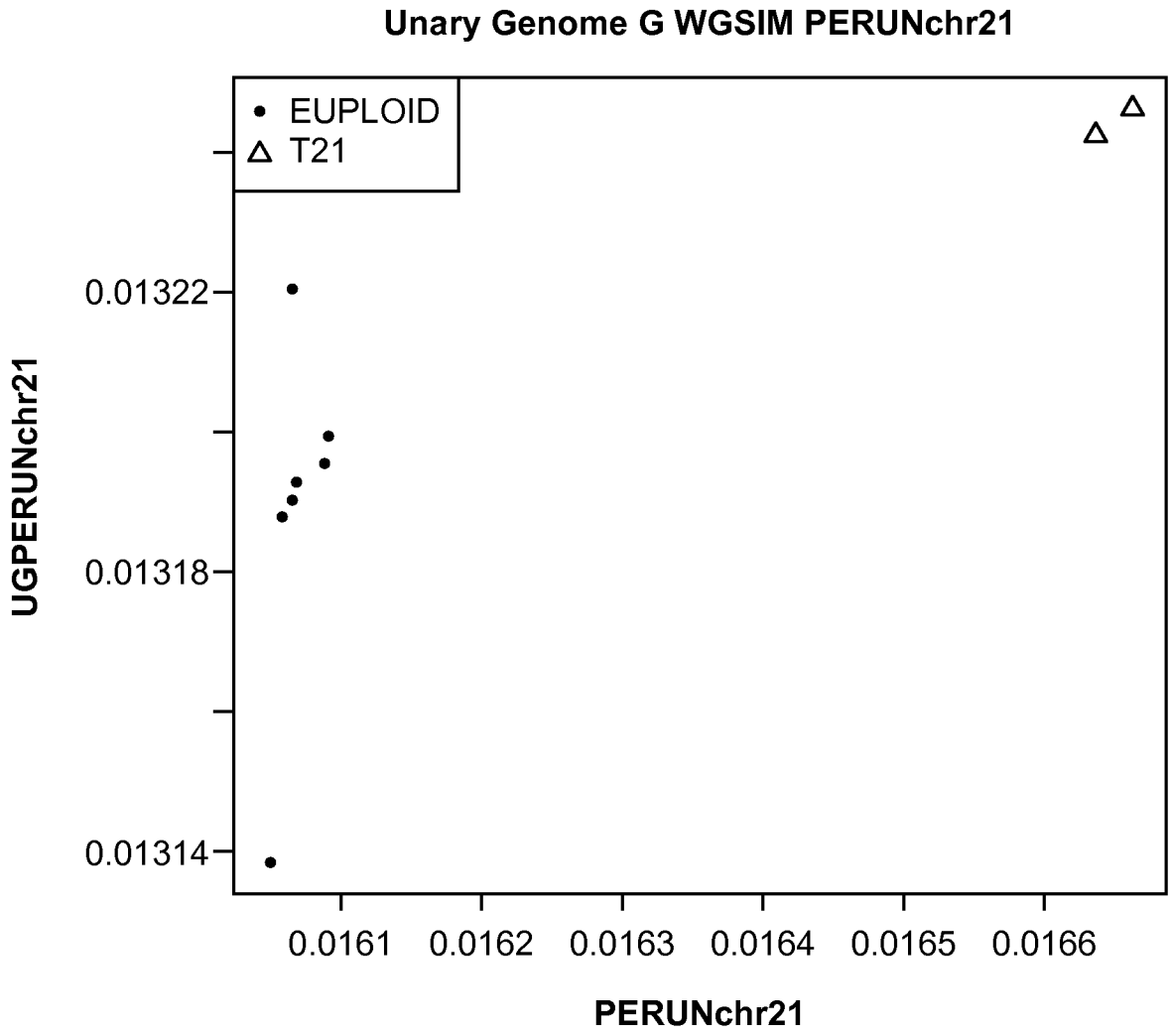

Unary Genome G WGSIM PERUNchr21
EUPLOID
T21
0.01322
0.01318
0.01314
UGPERUNchr21
0.0161
0.0162
0.0163
0.0164
0.0165
0.0166
PERUNchr21

FIG. 183

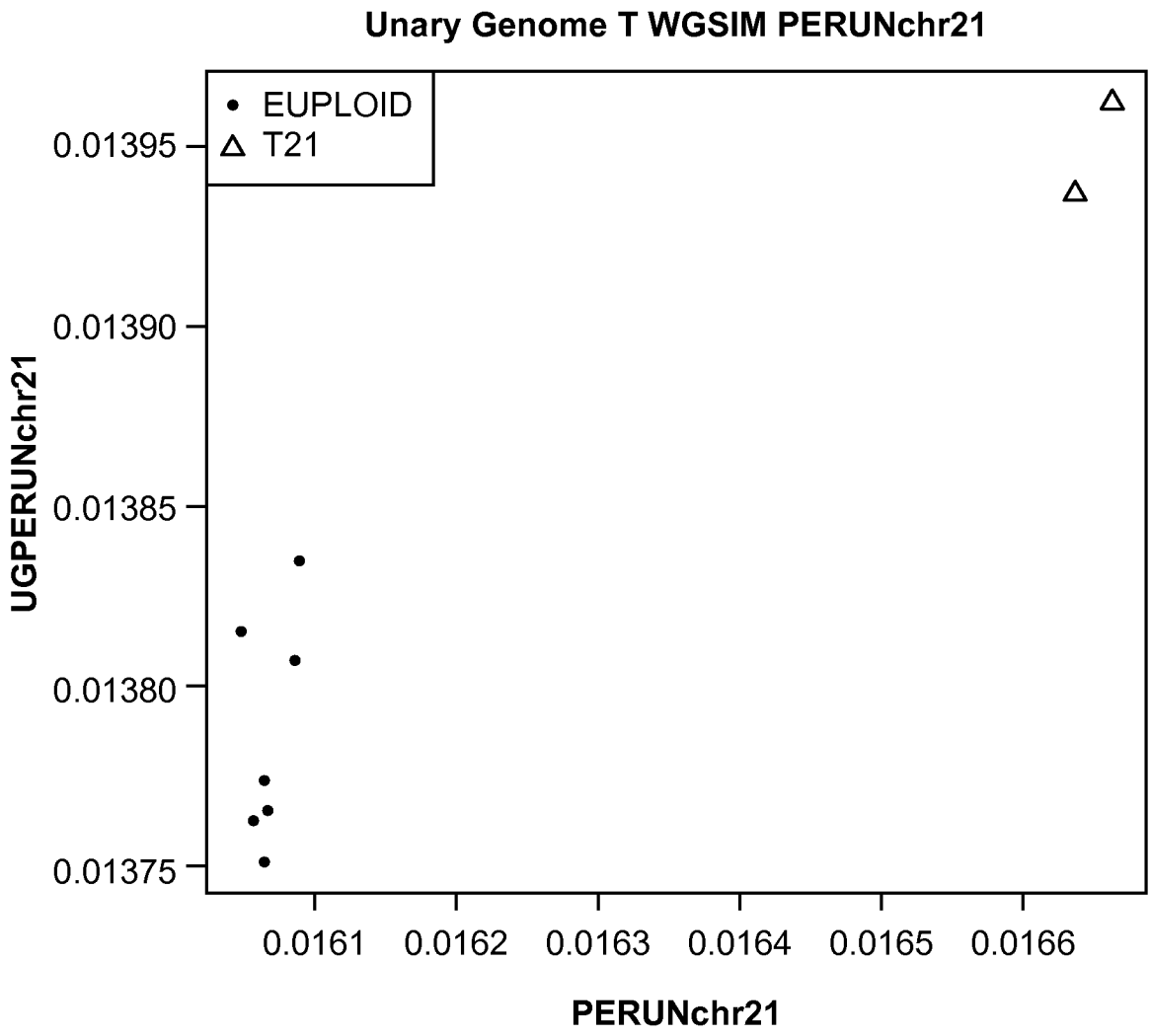

Unary Genome T WGSIM PERUNchr21
EUPLOID
T21
0.01395
0.01390
0.01385
0.01380
0.01375
UGPERUNchr21
0.0161
0.0162
0.0163
0.0164
0.0165
0.0166
PERUNchr21

FIG. 184