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Abstract

Abstract: This document provides methods and materials for identifying chromosomal anomalies that can be used to identify a mammal as having a disease (e.g., cancer or congenital abnormality). For example, this document provides methods and materials for evaluating sequencing data to identify a mammal as having a disease associated with one or more chromosomal anomalies (e.g., cancer or congenital abnormalities). For example, this document provides methods and materials for evaluating sequencing data that can be used in cancer diagnostics, non-invasive prenatal testing (NIPT), preimplantation genetic diagnosis and evaluation of congenital abnormalities.

RAPID ANEUPLOIDY DETECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 62/849,662, filed on May 17, 2019; U.S. Provisional Application Serial No. 62/905,327, filed on September 24, 2019 and U.S. Provisional Application Serial No. 62/971,050, filed on February 6, 2020. The disclosures of the prior applications are considered part of (and are incorporated by reference herein) the disclosure of this application.

STATEMENT REGARDING FEDERAL FUNDING

This invention was made with government support under grants CA230691 and CA230400 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

1. Technical Field

This document provides methods and materials for identifying chromosomal anomalies that can be used in cancer diagnostics, non-invasive prenatal testing (NIPT), preimplantation genetic diagnosis, and evaluation of congenital abnormalities. For example, this document provides methods and materials for evaluating sequencing data to identify a mammal as having a disease associated with one or more chromosomal anomalies (e.g., cancer or congenital abnormality). Additionally or alternatively, this document provides methods and materials for evaluating sequencing data that can be used in cancer diagnostics, non-invasive prenatal testing (NIPT), preimplantation genetic diagnosis, and evaluation of congenital abnormalities.

2. Background Information

Aneuploidy is defined as an abnormal chromosome number. It was the first genomic abnormality identified in cancers (Boveri 2008 *Journal of cell science* 121(Supplement 1):1-84; and Nowell 1976 *Science* 194(4260):23-28), and it has been estimated to be present in >90% of cancers of most histopathologic types (Knouse et al. 2017 *Annual Review of Cancer Biology* 1:335-354). Aneuploidy in cancers was first detected by karyotypic studies, later

evaluated through microarrays, Sanger sequencing, and most recently, massively parallel sequencing methods (Wang et al. 2002 *Proceedings of the National Academy of Sciences* 99(25):16156-16161). Recent sequencing methods include those employing circular binary segmentation, hidden Markov models, expectation maximization and mean-shift (as reviewed in (Zhao et al. 2013 *BMC bioinformatics* 14(11):S1)). In addition to their application to cancer genomes, these technologies form the basis for the non-invasive prenatal detection of fetuses with Downs' Syndrome and other trisomies (Bianchi et al. 2015 *JAMA* 314(2):162-169; Zhao et al. 2015 *Clinical chemistry* 61(4):608-616).

SUMMARY

This disclosure relates to methods and materials for identifying one or more chromosomal anomalies (e.g., aneuploidy). In some embodiments, this disclosure provides methods and materials for using amplicon-based sequencing data to identify a mammal as having a disease or disorder associated with one or more chromosomal anomalies. For example, methods and materials described herein can be applied to a sample obtained from a mammal to identify the mammal as having one or more chromosomal anomalies. For example, a mammal can be identified as having a disease or disorder based, at least in part, on the presence of one or more aneuploidies. In some embodiments, a single primer pair is used to amplify genomic elements throughout the genome. For example, a single primer pair described herein can be used to amplify ~1,000,000 unique repetitive elements (e.g., amplicons). In some embodiments, the amplified unique repetitive elements average less than 100 basepairs (bp) in size. In some embodiments, an approach (called WALDO for **W**ithin-**S**ample-**A**neup**L**oidy-**D**etecti**O**n) can be used to evaluate the sequencing data obtained from amplicons to identify the presence of one or more chromosomal anomalies (e.g., aneuploidy). As described herein, assessment of aneuploidy in 1,348 plasma samples from healthy people and 883 plasma samples from cancer patients detected aneuploidy in 49% of the plasma samples from cancer patients.

In one aspect, provided herein is a method of testing for the presence of aneuploidy in a genome of a mammal. The method comprises amplifying a plurality of chromosomal sequences in a DNA sample with a pair of primers complementary to the chromosomal sequences to form a plurality of amplicons; determining at least a portion of the nucleic acid sequence of one or more of the plurality of amplicons; mapping the sequenced amplicons to a

reference genome; dividing the DNA sample into a plurality of genomic intervals; quantifying a plurality of features for the amplicons mapped to the genomic intervals; comparing the plurality of features of amplicons in a first genomic interval with the plurality of features of amplicons in one or more different genomic intervals; and wherein at least 100,000 amplicons are formed in the step of amplifying (e.g., the plurality of amplicons can include ~745,000 amplicons).

In some embodiments, the method is performed in vitro. In some embodiments, the plurality of amplicons comprise about 1,000,000 amplicons, e.g., about 1,000,000-10,000 amplicons; about 1,000,000-50,000 amplicons; about 1,000,000-100,000 amplicons; about 1,000,000-200,000 amplicons; about 1,000,000—300,000 amplicons; about 1,000,000-400,000 amplicons; about 1,000,000-500,000 amplicons; about 1,000,000-600,000 amplicons; about 1,000,000-700,000 amplicons; about 1,000,000-800,000 amplicons; about 1,000,000-900,000 amplicons; about 900,000-10,000 amplicons; about 800,000-10,000 amplicons; about 700,000-10,000 amplicons; about 600,000-10,000 amplicons; about 500,000-10,000 amplicons; about 400,000-10,000 amplicons; about 300,000-10,000 amplicons; about 200,000-10,000 amplicons; about 100,000-10,000 amplicons or about 50,000-10,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 50,000 amplicons; about 100,000 amplicons; about 150,000 amplicons; about 200,000 amplicons; about 250,000 amplicons; about 300,000 amplicons; about 350,000 amplicons; about 400,000 amplicons; about 450,000 amplicons; about 500,00 amplicons; about 550,000 amplicons; about 600,000 amplicons; about 650,000 amplicons; about 700,000 amplicons; about 750,000 amplicons; about 800,000 amplicons; about 850,000 amplicons; about 900,000 amplicons; about 950,000 amplicons; or about 1,000,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 750,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 350,000 amplicons.

In some embodiments, the number of repetitive elements, e.g., amplicons, amplified by the single primer pair disclosed herein is a function of: the number of repetitive elements present in a sample and/or the length of a repetitive element present in a sample. For example, in some samples, the number of repetitive elements, e.g., amplicons, that can be

detected with the single primer pair is about ~750,000 amplicons. In some embodiments, in other samples, the number of repetitive elements, e.g., amplicons, that can be detected with the single primer pair is about ~350,000 amplicons.

In some embodiments, the DNA sample is a plurality of euploid DNA samples. In some embodiments, the DNA sample is a plurality of test DNA samples. In some embodiments, the DNA sample is a plurality of test DNA samples. In some embodiments, the DNA sample is from plasma. In some embodiments, the DNA sample is from serum. In some embodiments, the DNA sample comprises cell fetal DNA. In some embodiments, the DNA sample comprises at least 3 picograms of DNA. In some embodiments, the mammal is a human. In some embodiments the pair of primers comprises a first primer comprising SEQ ID NO: 1 and a second primer comprising SEQ ID NO: 10. In some embodiments, the methods provide herein include one or more additional pairs of primers. In some embodiments, the amplicons include repetitive elements (e.g., one or more types of repetitive elements shown in Table 1). In some embodiments, the amplicons include unique short interspersed nucleotide elements (SINEs). In some embodiments, the amplicons include unique long interspersed nucleotide elements (LINEs).

In some embodiments, the average length of the amplicons is about 100 basepairs or less. In some embodiments, the average length of the amplicons is less than about 110 bp, e.g., about 10-110bp, about 10-105bp, about 10-100bp, about 10-99bp, about 10-98bp, about 10-97bp, about 10-96bp, about 10-95bp, about 10-94bp, about 10-93bp, about 10-92bp, about 10-91bp, about 10-90bp, about 10-89bp, about 10-87bp, about 10-86bp, about 10-85bp, about 10-84bp, about 10-83bp, about 10-82bp, about 10-81bp, about 10-80bp, about 10-79bp, about 10-78bp, about 10-77bp, about 10-76bp, about 10-75bp, about 10-74bp, about 10-73bp, about 10-72bp, about 10-71bp, about 10-70bp, about 10-65bp, about 10-60bp, about 10-55bp, about 10-50bp, about 10-40bp, about 10-30bp, about 10-20bp, about 15-110bp, about 20-110bp, about 25-110bp, about 30-110bp, about 35-110bp, about 40-110bp, about 45-110bp, about 50-110bp, about 55-110bp about 60-110bp, about 65-110bp, about 70-110bp, about 75-110bp, about 80-110bp, about 85-110bp, about 90-110bp, about 95-110bp, about 100-110bp, or about 105-110bp.

In some embodiments, the average length of the amplicons is about 10 bp; about 20 bp; about 30bp; about 40bp; about 45 bp; about 50 bp; about 60bp; about 65 bp; about 70bp;

about 75bp; about 80bp; about 85bp; about 90bp; about 95bp; about 100bp; about 105bp or about 110bp.

In some embodiments, the amplicons comprise one or more long amplicons where the average length is 1000 basepairs or greater. In some embodiments, the long amplicons comprise DNA from a contaminating cell. In some embodiments, the contaminating cell is a leukocyte. In some embodiments, the genomic intervals comprise from about 100 nucleotides to about 125,000,000 nucleotides (e.g., the genomic intervals can include about 500,000 nucleotides).

In another aspect, the disclosure provides a method of evaluating a subject for the presence of, or the risk of developing, any of a plurality of, *e.g.*, any of at least four, cancers in the subject comprising:

(i) acquiring, e.g., directly acquiring or indirectly acquiring, a value for, e.g., detecting, the presence of one or more genetic biomarkers, *e.g.*, one or more mutations (*e.g.*, one or more driver gene mutations), in each of one or more genes (*e.g.*, one or more driver genes, *e.g.*, in at least four driver genes), and optionally wherein, each gene, *e.g.*, driver gene, is associated with the presence, or risk, of a cancer of the plurality of cancers;

(ii) acquiring, e.g., directly acquiring or indirectly acquiring, a value for, e.g., detecting, the level of each of a plurality of, *e.g.*, at least four, protein biomarkers, and optionally wherein, the level of each protein biomarker of the plurality is associated with the presence, or risk, of a cancer of the plurality of cancers; or

(iii) acquiring, e.g., directly acquiring or indirectly acquiring, a value for, e.g., detecting, aneuploidy, wherein the aneuploidy value is a function of the copy number or length of a genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family), wherein the RE family comprises:

(a) a RE Family other than a long interspersed nucleotide element (LINE);

(b) a RE Family which when amplified with a primer moiety complementary to its repeated terminal elements, provides amplicons having an average length of less than X nts, wherein X is 100, 105, or 110,

(c) a RE family which is less than about 700bp long; or

(d) a RE family which is present in at least 100 copies per genome;

and optionally wherein, the aneuploidy is associated with the presence, or risk, of a cancer of the plurality of cancers;

thereby evaluating the subject for the presence of or risk of developing, any of the plurality of, *e.g.*, any of at least four, cancers.

In an embodiment, one of (i), (ii) and (iii) is directly acquired. In an embodiment, (i) and (ii) are directly acquired. In an embodiment, (i) and (iii) are directly acquired. In an embodiment, (ii) and (iii) are directly acquired. In an embodiment, all of (i), (ii) and (iii) are directly acquired.

In an embodiment, one of (i), (ii) and (iii) is indirectly acquired. In an embodiment, (i) and (ii) are indirectly acquired. In an embodiment, (i) and (iii) are indirectly acquired. In an embodiment, (ii) and (iii) are indirectly acquired. In an embodiment, all of (i), (ii) and (iii) are indirectly acquired.

In an embodiment, the method comprises sequencing one or more subgenomic intervals or amplicons comprising the genetic biomarkers. In an embodiment, the method comprises analyzing one or more genomic sequences for aneuploidy. In an embodiment, the method comprises, contacting a protein biomarker with a detection reagent. In an embodiment, the method comprises: (1) sequencing one or more subgenomic intervals or amplicons comprising the genetic biomarkers; (2) analyzing one or more genomic sequences for aneuploidy, and/or (3) contacting a protein biomarker with a detection reagent.

In an embodiment, the aneuploidy value is a function of the copy number of the genomic sequence disposed between at least two terminal repeated elements of a RE Family. In an embodiment, the aneuploidy value is a function of the length of the genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family).

In some embodiments, the method is performed in vitro.

In an embodiment, a sample, e.g., a biological sample, obtained from the subject is evaluated for one, two or all of (i)-(iii). In an embodiment, the biological sample comprises a liquid sample, e.g., a blood sample. In an embodiment, the biological sample comprises a cell-free DNA sample, a plasma sample or a serum sample. In an embodiment, the biological sample comprises cell-free DNA, e.g., circulating tumor DNA. In an embodiment, the biological sample comprises cells and/or tissue. In an embodiment, the biological sample comprises cells (e.g., normal or cancer cells) and cell-free DNA.

In an embodiment of any of the methods disclosed herein, specificity of detection of the cancer in the plurality of cancers with (i), (ii) and (iii) is substantially the same as, e.g., not substantially lower than, the specificity of detection of the cancer in the plurality of cancers with: (i); (ii); (iii); (i) and (ii); (i) and (iii); or (ii) and (iii).

In an embodiment of any of the methods disclosed herein, sensitivity of detection of the cancer in the plurality of cancers with (i), (ii) and (iii) is higher, e.g., about 1.1, 1.2, 1.3, 1.4, 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 10 fold higher, than the sensitivity of detection of the cancer in the plurality of cancers with: (i); (ii); (iii); (i) and (ii); (i) and (iii); or (ii) and (iii). In an embodiment, an increased sensitivity of detection, e.g., about 1.1, 1.2, 1.3, 1.4, 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 10 fold increase in sensitivity of detection at a specified specificity, e.g., at a predetermined specificity, e.g., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% specificity.

In some embodiments, the plurality of amplicons comprise about 1,000,000 amplicons, e.g., about 1,000,000-10,000 amplicons; about 1,000,000-50,000 amplicons; about 1,000,000-100,000 amplicons; about 1,000,000-200,000 amplicons; about 1,000,000—300,000 amplicons; about 1,000,000-400,000 amplicons; about 1,000,000-500,000 amplicons; about 1,000,000-600,000 amplicons; about 1,000,000-700,000 amplicons; about 1,000,000-800,000 amplicons; about 1,000,000-900,000 amplicons; about 900,000-10,000 amplicons; about 800,000-10,000 amplicons; about 700,000-10,000 amplicons; about 600,000-10,000 amplicons; about 500,000-10,000 amplicons; about 400,000-10,000 amplicons; about 300,000-10,000 amplicons; about 200,000-10,000 amplicons; about 100,000-10,000 amplicons or about 50,000-10,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 50,000 amplicons; about 100,000 amplicons; about 150,000 amplicons; about 200,000 amplicons; about 250,000 amplicons; about 300,000 amplicons; about 350,000 amplicons; about 400,000 amplicons; about 450,000 amplicons; about 500,00 amplicons; about 550,000 amplicons; about 600,000 amplicons; about 650,000 amplicons; about 700,000 amplicons; about 750,000 amplicons; about 800,000 amplicons; about 850,000 amplicons; about 900,000 amplicons; about 950,000 amplicons; or about 1,000,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 750,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 350,000 amplicons.

In some embodiments, the number of repetitive elements, e.g., amplicons, amplified by the single primer pair disclosed herein is a function of: the number of repetitive elements present in a sample and/or the length of a repetitive element present in a sample. For example, in some samples, the number of repetitive elements, e.g., amplicons, that can be detected with the single primer pair is about ~750,000 amplicons. In some embodiments, in other samples, the number of repetitive elements, e.g., amplicons, that can be detected with the single primer pair is about ~350,000 amplicons.

In some embodiments, the average length of the amplicons is about 100 basepairs or less. In some embodiments, the average length of the amplicons is less than about 110 bp, e.g., about 10-110bp, about 10-105bp, about 10-100bp, about 10-99bp, about 10-98bp, about 10-97bp, about 10-96bp, about 10-95bp, about 10-94bp, about 10-93bp, about 10-92bp, about 10-91bp, about 10-90bp, about 10-89bp, about 10-87bp, about 10-86bp, about 10-85bp, about 10-84bp, about 10-83bp, about 10-82bp, about 10-81bp, about 10-80bp, about 10-79bp, about 10-78bp, about 10-77bp, about 10-76bp, about 10-75bp, about 10-74bp, about 10-73bp, about 10-72bp, about 10-71bp, about 10-70bp, about 10-65bp, about 10-60bp, about 10-55bp, about 10-50bp, about 10-40bp, about 10-30bp, about 10-20bp, about 15-110bp, about 20-110bp, about 25-110bp, about 30-110bp, about 35-110bp, about 40-110bp, about 45-110bp, about 50-110bp, about 55-110bp about 60-110bp, about 65-110bp, about 70-110bp, about 75-110bp, about 80-110bp, about 85-110bp, about 90-110bp, about 95-110bp, about 100-110bp, or about 105-110bp.

In some embodiments, the average length of the amplicons is about 10 bp; about 20 bp; about 30bp; about 40bp; about 45 bp; about 50 bp; about 60bp; about 65 bp; about 70bp; about 75bp; about 80bp; about 85bp; about 90bp; about 95bp; about 100bp; about 105bp or about 110bp.

In some embodiments, the method further comprises subjecting the subject to a radiologic scan, e.g., a PET-CT scan, of an organ or body region. In some embodiments, the radiologic scanning of an organ or body region characterizes the cancer. In some embodiments, the radiologic scanning of an organ or body region identifies the location of the cancer. In some embodiments, the radiologic scan is a PET-CT scan. In some

embodiments, the radiologic scanning is performed after the subject is evaluated for the presence of each of a plurality of cancers.

In another aspect, the disclosure provides a method of testing for the presence of aneuploidy in a genome of a mammal. The method comprises:

a) amplifying a plurality of chromosomal sequences in a DNA sample with a primer moiety, e.g., a primer or pair of primers complementary to the chromosomal sequences to form a plurality of amplicons, e.g., wherein the primer moiety amplifies a sufficient number of sequences to allow aneuploidy detection;
b) determining at least a portion of the nucleic acid sequence of one or more of the plurality of amplicons;
c) mapping the sequenced amplicons to a reference genome;
d) dividing the DNA sample into a plurality of genomic intervals;
e) quantifying a plurality of features for the amplicons mapped to the genomic intervals;
f) comparing the plurality of features of amplicons in a first genomic interval with the plurality of features of amplicons in one or more different genomic intervals; and

wherein a number of amplicons sufficient to detect aneuploidy, e.g., at least 10,000, 20,000, 50,000, or 100,000 amplicons are formed in the step of amplifying.

In some embodiments, the method is performed in vitro.

In an embodiment of any of the methods disclosed herein, increase in sensitivity of detection of the cancer in the plurality of cancers does not affect, e.g., reduce or substantially reduce, the specificity of detection of the cancer in the plurality of cancer. In an embodiment, the specificity of detection of the cancer in the plurality of cancers is at a plateau, e.g., the specificity of detection is not altered by detection of additional biomarkers.

In another aspect, provided herein is a method of detecting aneuploidy in a sample comprising low input DNA, using any of the methods disclosed herein.

In some embodiments, the sample comprises about 0.01 picogram (pg) to 500 pg of DNA. In some embodiments, the sample comprises about 0.01-500pg, 0.05-400pg, 0.1-300pg, 0.5-200pg, 1-100pg, 10-90pg, or 20-50pg DNA. In some embodiments, the sample

comprises at least 0.01 pg, at least .01 pg, at least 0.1 pg, at least 1 pg. at least 2 pg, at least 3 pg, at least 4 pg, at least 5 pg, at least 6 pg, at least 7 pg, at least 8 pg, at least 9 pg at least 10pg, at least 11 pg, at least 12 pg, at least 13 pg, at least 14 pg, at least 15 pg, at least 16 pg, at least 17 pg, at least 18 pg , at least 19 pg, at least 20 pg, at least 21 pg, at least 22 pg, at least 23 pg, at least 24 pg, at least 25 pg, at least 26 pg, at least 27 pg, at least 28 pg, at least 29 pg, at least 30 pg, at least 31 pg, at least 32 pg, at least 33 pg, at least 34 pg, at least 35 pg, at least 36 pg, at least 37 pg, at least 38 pg, at least 39 pg, at least 40 pg, at least 50 pg, at least 60 pg, at least 70 pg, at least 80 pg, at least 90 pg, at least 100pg, at least 150pg, at least 200 pg, at least 300 pg, at least 350 pg, at least 400 pg, at least 450 pg, or at least 500 pg DNA.

In some embodiments, the sample comprises 1 pg DNA. In some embodiments, the sample comprises 2 pg DNA. In some embodiments, the sample comprises 3 pg DNA. In some embodiments, the sample comprises 4 pg DNA. In some embodiments, the sample comprises 5 pg DNA. In some embodiments, the sample comprises 10 pg DNA.

In some embodiments, the sample is a biological sample from a subject. In an embodiment, the biological sample comprises a liquid sample, e.g., a blood sample. In an embodiment, the biological sample comprises a cell-free DNA sample, a plasma sample or a serum sample. In an embodiment, the biological sample comprises cell-free DNA, e.g., circulating tumor DNA. In an embodiment, the biological sample comprises cells and/or tissue. In an embodiment, the biological sample comprises cells (e.g., normal or cancer cells) and cell-free DNA.

In some embodiments, the sample is a trisomy 21 sample. In some embodiments, the sample is a forensic sample. In some embodiments, the sample is from an embryo, e.g., preimplantation embryo.

In some embodiments, the sample is a biobank sample, e.g., as described in Example 3.

In some embodiments, the method is used for diagnostics, e.g., preimplantation diagnostics.

In some embodiments, the method is used for forensics.

In some embodiments, the method is an in vitro method.

In another aspect, provided herein is a method of identifying or distinguishing a sample using any of the methods disclosed herein.

In some embodiments, the sample, e.g., first sample, from a subject (e.g., first subject) is distinguished from a second sample from a second subject. In some embodiments, the sample, e.g., first sample, is identified as being from the first subject based on a polymorphism (e.g., a plurality of polymorphisms, e.g., common polymorphisms). In some embodiments, the second sample is identified as being from the second subject based on a polymorphism (e.g., a plurality of polymorphisms, e.g., common polymorphisms). In some embodiments, a common polymorphism is present in a repetitive element, e.g., as described herein. In some embodiments, methods disclosed in Example 8 can be used to identify and/or distinguish the sample.

In another aspect, provided herein is a reaction mixture comprising: at least 2, 3,4, 5, 6 ,7, 8, 9 or 10 detection reagents, wherein a detection reagent mediates a readout that is a value of the level or presence of: (i) one or more genetic biomarkers referred to herein; (ii) one or more protein biomarkers referred to herein; and/or (iii) the copy number or length, e.g., aneuploidy, of a genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family) referred to herein.

In yet another aspect, the disclosure provides a kit comprising: (a) at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 detection reagents, wherein a detection reagent mediates a readout that is a value of the level or presence of: (i) one or more genetic biomarkers referred to herein; (ii) one or more protein biomarkers referred to herein; and/or (iii) the copy number or length, e.g., aneuploidy, of a genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family) referred to herein; and (b) instructions for using said kit.

In some embodiments of any of the methods disclosed herein, quantifying amplicons mapped to genomic intervals comprises identifying a plurality of genomic intervals with one or more shared amplicon features. In some embodiments, the shared amplicon feature is the number of the mapped amplicons.

In some embodiments of any of the methods disclosed herein, the shared amplicon feature is the average length of the mapped amplicons. In some embodiments, the plurality of genomic intervals with shared amplicon features are grouped into clusters. In some embodiments, each cluster includes about two hundred genomic intervals. In some embodiments, the clusters comprise predefined clusters. In some embodiments, the comparison of the genomic intervals further comprises matching one or more genomic intervals from test samples to predefined clusters. In some embodiments, matching genomic intervals from test samples to predefined clusters further comprises identifying one or more genomic intervals with shared amplicon features outside a predetermined significance threshold for a predefined cluster. In some embodiments, the method comprises supervised machine learning. In some embodiments, the supervised machine learning employs a support vector machine model.

In some embodiments of any of the methods disclosed herein, a single pair of primers is used for the amplification of a plurality of amplicons from a DNA sample comprising a first primer comprising a sequence that is at least 80% identical to SEQ ID NO: 1 and a second primer comprising a sequence that is at least 80% identical to SEQ ID NO: 10. In some embodiments, the sequence of the first primer is at least 90% identical to SEQ ID NO. 1. In some embodiments, the sequence of the first primer is at least 95% identical to SEQ ID NO. 1. In some embodiments, the sequence of the first primer is 100% identical to SEQ ID NO. 1. In some embodiments, the sequence of the second primer is at least 90% identical to SEQ ID NO. 10. In some embodiments, the sequence of the second primer is at least 95% identical to SEQ ID NO. 10. In some embodiments, the sequence of the second primer is 100% identical to SEQ ID NO. 10. In some embodiments, a kit comprising a pair of primers is used to amplify a plurality of amplicons from a DNA sample, wherein a first primer of the primer pair comprises SEQ ID NO: 1 or a sequence at least 80% identical thereto, and a second primer of the primer pair comprises SEQ ID NO: 10, or a sequence at least 80% identical thereto.

In another aspect, the disclosure provides a method of testing for the presence of cancer of a mammal. The method includes: a) amplifying a plurality of chromosomal sequences in a DNA sample with a pair of primers complementary to the chromosomal sequences to form a plurality of amplicons; b) determining at least a portion of the nucleic

acid sequence of one or more of the plurality of amplicons; c) mapping the sequenced amplicons to a reference genome; d) dividing the DNA sample into a plurality of genomic intervals; e) quantifying a plurality of features for the amplicons mapped to the genomic intervals; f) comparing the plurality of features of amplicons in a first genomic interval with the plurality of features of amplicons in one or more different genomic intervals; and g) determining the presence of cancer in the mammal when the plurality of features of amplicons in a first genomic interval is different from the plurality of features of amplicons in one or more different genomic intervals. In some embodiments, the method can include at least 100,000 amplicons formed in the step of amplifying. In some embodiments, the cancer can be a Stage I cancer. In some embodiments, the cancer can be a liver cancer, an ovarian cancer, an esophageal cancer, a stomach cancer, a pancreatic cancer, a colorectal cancer, a lung cancer, a breast cancer, or a prostate cancer.

In some embodiments, the method is an in vitro method.

In some embodiments of any of the methods, reaction mixtures or kits disclosed herein, the plurality of amplicons comprise about 1,000,000 amplicons, e.g., about 1,000,000-10,000 amplicons; about 1,000,000-50,000 amplicons; about 1,000,000-100,000 amplicons; about 1,000,000-200,000 amplicons; about 1,000,000—300,000 amplicons; about 1,000,000-400,000 amplicons; about 1,000,000-500,000 amplicons; about 1,000,000-600,000 amplicons; about 1,000,000-700,000 amplicons; about 1,000,000-800,000 amplicons; about 1,000,000-900,000 amplicons; about 900,000-10,000 amplicons; about 800,000-10,000 amplicons; about 700,000-10,000 amplicons; about 600,000-10,000 amplicons; about 500,000-10,000 amplicons; about 400,000-10,000 amplicons; about 300,000-10,000 amplicons; about 200,000-10,000 amplicons; about 100,000-10,000 amplicons or about 50,000-10,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 50,000 amplicons; about 100,000 amplicons; about 150,000 amplicons; about 200,000 amplicons; about 250,000 amplicons; about 300,000 amplicons; about 350,000 amplicons; about 400,000 amplicons; about 450,000 amplicons; about 500,00 amplicons; about 550,000 amplicons; about 600,000 amplicons; about 650,000 amplicons; about 700,000 amplicons; about 750,000 amplicons; about 800,000 amplicons; about 850,000 amplicons; about 900,000 amplicons; about 950,000 amplicons; or about 1,000,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 750,000 amplicons.

In some embodiments, the plurality of amplicons comprises about 350,000 amplicons.

In some embodiments of any of the methods disclosed herein, the number of repetitive elements, e.g., amplicons, amplified by the single primer pair disclosed herein is a function of: the number of repetitive elements present in a sample and/or the length of a repetitive element present in a sample. For example, in some samples, the number of repetitive elements, e.g., amplicons, that can be detected with the single primer pair is about ~750,000 amplicons. In some embodiments, in other samples, the number of repetitive elements, e.g., amplicons, that can be detected with the single primer pair is about ~350,000 amplicons.

In some embodiments of any of the methods, reaction mixtures or kits disclosed herein, the average length of the amplicons is about 100 basepairs or less. In some embodiments, the average length of the amplicons is less than about 110 bp, e.g., about 10-110bp, about 10-105bp, about 10-100bp, about 10-99bp, about 10-98bp, about 10-97bp, about 10-96bp, about 10-95bp, about 10-94bp, about 10-93bp, about 10-92bp, about 10-91bp, about 10-90bp, about 10-89bp, about 10-87bp, about 10-86bp, about 10-85bp, about 10-84bp, about 10-83bp, about 10-82bp, about 10-81bp, about 10-80bp, about 10-79bp, about 10-78bp, about 10-77bp, about 10-76bp, about 10-75bp, about 10-74bp, about 10-73bp, about 10-72bp, about 10-71bp, about 10-70bp, about 10-65bp, about 10-60bp, about 10-55bp, about 10-50bp, about 10-40bp, about 10-30bp, about 10-20bp, about 15-110bp, about 20-110bp, about 25-110bp, about 30-110bp, about 35-110bp, about 40-110bp, about 45-110bp, about 50-110bp, about 55-110bp about 60-110bp, about 65-110bp, about 70-110bp, about 75-110bp, about 80-110bp, about 85-110bp, about 90-110bp, about 95-110bp, about 100-110bp, or about 105-110bp.

In some embodiments, the average length of the amplicons is about 10 bp; about 20 bp; about 30bp; about 40bp; about 45 bp; about 50 bp; about 60bp; about 65 bp; about 70bp; about 75bp; about 80bp; about 85bp; about 90bp; about 95bp; about 100bp; about 105bp or about 110bp.

Additional features of any of the methods disclosed herein include one or more of the following enumerated embodiments.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following enumerated embodiments.

ENUMERATED EMBODIMENTS

E1. A method of evaluating a subject for the presence of, or the risk of developing, any of a plurality of, *e.g.*, any of at least four, cancers in the subject comprising:

(i) acquiring, e.g., directly acquiring or indirectly acquiring, a value for, e.g., detecting, the presence of one or more genetic biomarkers, *e.g.*, one or more mutations (*e.g.*, one or more driver gene mutations), in each of one or more genes (*e.g.*, one or more driver genes, *e.g.*, in at least four driver genes), and optionally wherein, each gene, *e.g.*, driver gene, is associated with the presence, or risk, of a cancer of the plurality of cancers;

(ii) acquiring, e.g., directly acquiring or indirectly acquiring, a value for, e.g., detecting, the level of each of a plurality of, *e.g.*, at least four, protein biomarkers, and optionally wherein, the level of each protein biomarker of the plurality is associated with the presence, or risk, of a cancer of the plurality of cancers; or

(iii) acquiring, e.g., directly acquiring or indirectly acquiring, a value for, e.g., detecting, aneuploidy, wherein the aneuploidy value is a function of the copy number or length of a genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family), wherein the RE family comprises:

(a) a RE Family other than a long interspersed nucleotide element (LINE);

(b) a RE Family which when amplified with a primer moiety complementary to its repeated terminal elements, provides a plurality of amplicons having an average length of less than X nts, wherein X is 100, 105, or 110,

(c) a RE family which is less than about 700bp long; or

(d) a RE family which is present in at least 100 copies per genome;

and optionally wherein, the aneuploidy is associated with the presence, or risk, of a cancer of the plurality of cancers;

thereby evaluating the subject for the presence of or risk of developing, any of the plurality of, *e.g.*, any of at least four, cancers.

E2. The method of embodiment E1, wherein:

(a) one of (i), (ii) and (iii) is directly acquired;

(b) (i) and (ii) are directly acquired;

(c) (i) and (iii) are directly acquired;

(d) (ii) and (iii) are directly acquired; or

(e) all of (i), (ii) and (iii) are directly acquired.

E3. The method of embodiment E1, wherein:

(a) one of (i), (ii) and (iii) is indirectly acquired;

(b) (i) and (ii) are indirectly acquired;

(c) (i) and (iii) are indirectly acquired;

(d) (ii) and (iii) are indirectly acquired; or

(e) all of (i), (ii) and (iii) are indirectly acquired.

E4. The method of any one of embodiments E1-E3, comprising:

(1) sequencing one or more subgenomic intervals or amplicons comprising the genetic biomarkers;

(2) analyzing one or more genomic sequences for aneuploidy, and/or

(3) contacting a protein biomarker with a detection reagent.

E5. The method of any one of embodiments E1-E4, wherein the aneuploidy value is a function of:

(a) the copy number of the genomic sequence disposed between at least two terminal repeated elements of a RE Family; and/or

(b) the length of the genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family).

E6. The method of any one of embodiments E1-E5, wherein a biological sample obtained from the subject is evaluated for one, two or all of (i)-(iii).

E7. The method of embodiment E6, wherein the biological sample comprises a liquid sample, e.g., a blood sample.

E8. The method of embodiment E6 or E7, wherein the biological sample comprises a cell-free DNA sample, a plasma sample or a serum sample.

E9. The method of any one of embodiments E6-E8, wherein the biological sample comprises cell-free DNA, e.g., circulating tumor DNA.

E10. The method of any one of embodiment E1-E9, further comprising:

(i) acquiring a sequence for a subgenomic interval from cell-free DNA from a sample;

(ii) acquiring a leukocyte parameter, e.g., sequence of the subgenomic interval, from leukocyte DNA from the sample.

E11. The method of any one of embodiments E1-E10 further comprising:

(i) acquiring a sequence for a subgenomic interval for aneuploidy analysis from cell-free DNA from a sample;

(ii) acquiring a leukocyte parameter, e.g., a sequence for the subgenomic interval for aneuploidy analysis, from leukocyte DNA from the sample.

E12. The method of embodiment E10 or E11 further comprising

comparing (i) with (ii) to evaluate a genomic event, e.g., a mutation, found in the cell-free DNA subgenomic interval or cell-free DNA aneuploidy analysis sample.

E13. The method of any one of embodiments E10-E12, further classifying a genomic event, e.g., a mutation, in the subgenomic interval from cell-free DNA or from aneuploidy analysis of cell-free DNA, e.g., assigning the mutation to a first class or a second class.

E14. The method of any one of embodiments E10-E13, further comprising classifying a genomic event, e.g., a mutation, in the subgenomic interval from cell-free DNA or from aneuploidy analysis of cell-free DNA, as growth-deregulating, e.g., cancerous.

E15. The method of any one of embodiments E10-E13, further comprising classifying a genomic event, e.g., a mutation, in the subgenomic interval from cell-free DNA or from aneuploidy analysis of cell-free DNA, as other than growth-deregulating, e.g., as other than cancerous.

E16. The method of any one of embodiments E10-E14, wherein classifying a genomic event, e.g., a mutation, in the subgenomic interval from cell-free DNA or from aneuploidy analysis of cell-free DNA, as cancerous when:

(a) the subgenomic interval is aneuploid in cell-free DNA, and the subgenomic interval is not aneuploid in leukocytes; or

(b) the genomic event is present in the subgenomic interval of cell-free DNA, and the genomic event is not present in the subgenomic interval of leukocytes.

E17. The method of any one of embodiments E10-E13 or E15, wherein classifying a genomic event, e.g., a mutation, in the subgenomic interval from cell-free DNA or form aneuploidy analysis of cell-free DNA, as other than growth-deregulating when:

(a) the subgenomic interval is aneuploid in cell-free DNA, and the subgenomic interval is aneuploid in leukocytes; or

(b) the genomic event is present in the subgenomic interval of cell-free DNA and the genomic event is present in the subgenomic interval of leukocytes.

E18. The method of embodiment E17, wherein the genomic event is associated with clonal expansion of leukocytes, e.g., age-associated clonal hematopoiesis, e.g., clonal hematopoiesis of indeterminate potential (CHIP).

E19. The method of any one of embodiments E1-E18, wherein specificity of detection of the cancer in the plurality of cancers with (i), (ii) and (iii) is substantially the

same as, e.g., not substantially lower than, the specificity of detection of the cancer in the plurality of cancers with: (i); (ii); (iii); (i) and (ii); (i) and (iii); or (ii) and (iii).

E20. The method of any one of embodiments E1-E19, wherein sensitivity of detection of the cancer in the plurality of cancers with (i), (ii) and (iii) is higher, e.g., about 1.1, 1.2, 1.3, 1.4, 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 10 fold higher, than the sensitivity of detection of the cancer in the plurality of cancers with: (i); (ii); (iii); (i) and (ii); (i) and (iii); or (ii) and (iii).

E21. The method of any one of embodiments E1-E20, wherein (i), (ii) and (iii) result in an increased sensitivity of detection, e.g., about 1.1, 1.2, 1.3, 1.4, 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 10 fold increase in sensitivity of detection at a specified specificity, e.g., at a predetermined specificity, e.g., at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% specificity.

E22. The method of any one of embodiments E20-E21, wherein the increase in sensitivity of detection of the cancer in the plurality of cancers does not affect, e.g., reduce or substantially reduce, the specificity of detection of the cancer in the plurality of cancer.

E23. The method of embodiment E22, wherein the specificity of detection of the cancer in the plurality of cancers is at a plateau.

E24. The method of any one of embodiments E1-E23, wherein the RE family is other than a LINE.

E25. The method of any one of embodiments E1-E24, wherein the RE family comprises a repeated element which when amplified with a primer to its repeated terminal elements, provides a plurality of amplicons having an average length of less than about 110 bp, e.g., about 10-110bp, about 10-105bp, about 10-100bp, about 10-99bp, about 10-98bp, about 10-97bp, about 10-96bp, about 10-95bp, about 10-94bp, about 10-93bp, about 10-92bp, about 10-91bp, about 10-90bp, about 10-89bp, about 10-87bp, about 10-86bp, about 10-85bp, about 10-84bp, about 10-83bp, about 10-82bp, about 10-81bp, about 10-80bp,

about 10-79bp, about 10-78bp, about 10-77bp, about 10-76bp, about 10-75bp, about 10-74bp, about 10-73bp, about 10-72bp, about 10-71bp, about 10-70bp, about 10-65bp, about 10-60bp, about 10-55bp, about 10-50bp, about 10-40bp, about 10-30bp, about 10-20bp, about 15-110bp, about 20-110bp, about 25-110bp, about 30-110bp, about 35-110bp, about 40-110bp, about 45-110bp, about 50-110bp, about 55-110bp about 60-110bp, about 65-110bp, about 70-110bp, about 75-110bp, about 80-110bp, about 85-110bp, about 90-110bp, about 95-110bp, about 100-110bp, or about 105-110bp.

E26. The method of any one of embodiments E1-E25, wherein the RE family comprises one or more repetitive elements shown in Table 1.

E27. The method of any one of embodiments E1-E26, wherein the RE family comprises a SINE or a tandem repeat (e.g., microsatellite DNA, mini-satellite DNA, satellite DNA or DNA of genes with multiple copies (e.g., DNA encoding ribosomal RNA)).

E28. The method of embodiment E27, wherein the RE family is a SINE, e.g., an Alu family, a MIR or a MIR3, or a SINE described in Vassetzky and Kramerov (2013) *Nucleic Acids Res.* 41: D83-89.

E29. The method of any one of embodiments E1-E28, wherein the value for aneuploidy is further a function of the copy number or length of a genomic sequence disposed between the terminal repeated elements of a LINE repeated element.

E30. The method of any one of embodiments E1-E29, wherein the value for aneuploidy is further a function of the copy number or length of a plurality of genomic sequences disposed between the terminal repeated elements of a repeated element family which when amplified with a primer complementary to its repeated terminal elements, provides amplicons having an average length of more than 100 bp.

E31. The method of any one of embodiments E1-E30, wherein the value for aneuploidy is further a function of:

a) amplifying a plurality of chromosomal sequences in a DNA sample with a pair of primers complementary to the chromosomal sequences to form a plurality of amplicons;
b) determining at least a portion of the nucleic acid sequence of one or more of the plurality of amplicons;
c) mapping the sequenced amplicons to a reference genome;
d) dividing the DNA sample into a plurality of genomic intervals;
e) quantifying a plurality of features for the amplicons mapped to the genomic intervals;
f) comparing the plurality of features of amplicons in a first genomic interval with the plurality of features of amplicons in one or more different genomic intervals; and
g) wherein at least 100,000 amplicons are formed in the step of amplifying.

E32. The method of any one of embodiments E1-E31, comprising providing a value for aneuploidy, wherein the value is a function of the copy number of at least about 5, 10, 20, 30, 50, 100, 200, 500, or 1000 different genomic sequences disposed between the terminal repeated elements of a RE family.

E33. The method of any one of embodiments E1-E32, wherein the copy number is greater than 2 or is less than 2.

E34. The method of any one of embodiments E31-E33, wherein at least about 100,000 amplicons, about 150,000 amplicons, about 200,000 amplicons; about 250,000 amplicons; about 300,000 amplicons; about 350,000 amplicons; about 400,000 amplicons; about 450,000 amplicons; about 500,000 amplicons; about 550,000 amplicons; about 600,000 amplicons; about 650,000 amplicons; about 700,000 amplicons; about 750,000 amplicons; about 800,000 amplicons; about 850,000 amplicons; about 900,000 amplicons; about 950,000 amplicons; or about 1,000,000 amplicons are formed.

E35. The method of any one of embodiments E1-E34, comprising providing a value for aneuploidy, wherein the value is a function of:

(i) the copy number or length of a first genomic sequence disposed between the terminal repeated elements of a RE family, on a first segment of genomic DNA; and

(ii) the copy number or length of a second genomic sequence disposed between the terminal repeated elements of a (e.g., the same or a different) RE family, on a second segment of genomic DNA.

E36. The method of embodiment E35, wherein:

(i) the first segment of genomic DNA and the second segment of genomic DNA are on different arms of the same chromosome, e.g., the first segment is on the q arm and the second segment is on the p arm of the same chromosome; or the first segment is on the p arm and the second segment is on the q arm of the same chromosome;

(ii) the first segment of genomic DNA and the second segment of genomic DNA are on the same arm of the same chromosome, e.g., the first segment and the second segment are both on the p arm, or q arm of a chromosome; and/or

(iii) the first segment of genomic DNA and the second segment of genomic DNA are on different chromosomes, e.g., non-homologous chromosomes.

E37. The method of any one of embodiments E1-E36, comprising providing a value for aneuploidy, wherein the value is a function of:

the copy number or length of a third genomic sequence disposed between the terminal repeated elements of a RE family, on a third chromosome.

E38. The method of any one of embodiments E1-E37, comprising providing a value for aneuploidy, wherein the value is a function of:

the copy number or length of an N^{th} genomic sequence disposed between the terminal repeated elements of a RE family, on an N^{th} chromosome, wherein N is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23.

E39. The method of any one of embodiments E1-E38, comprising contacting subject genomic nucleic acid with a primer moiety which amplifies a sequence comprising a genomic sequence disposed between the terminal repeated elements of a RE family.

E40. The method of embodiment E39, wherein the primer moiety is complementary to a terminal element of the RE family.

E41. The method of embodiment E39 or E40, wherein the primer moiety comprises a pair of primers.

E42. The method of any one of embodiments E39-E41, wherein the primer moiety comprises a single primer, and e.g., is used with isothermal amplification.

E43. The method of any one of embodiments E1-E42, wherein, the number of biomarkers (e.g., number of driver gene mutations) detected is sufficient such that the sensitivity of detection of the cancer in the plurality of cancers with which each gene, *e.g.*, driver gene, is associated with, is not substantially increased by the detection of one or more additional genetic biomarkers.

E44. The method of any one of embodiments E1-E42, wherein detecting the genetic biomarker comprises providing, *e.g.*, by sequencing, the sequence (e.g., nucleotide sequence) of the genetic biomarker.

E45. The method of embodiment E44, wherein the number of genetic biomarker sequences provided is sufficient such that the sensitivity of detection of the cancer in the plurality of cancers with which each gene, *e.g.*, driver gene, is associated with is not substantially increased by the provision of one or more sequences of additional genetic biomarkers.

E46. The method of any one of embodiments E1-E42, wherein detecting the biomarker comprises providing the sequence (e.g., nucleotide sequence) of one or more subgenomic intervals comprising the genetic biomarker.

E47. The method of embodiment E46, wherein, the number of subgenomic interval sequences provided is sufficient such that the sensitivity of detection of the cancer in the plurality of cancers with which each gene, *e.g.*, driver gene, is associated with is not

substantially increased by the provision of one or more sequences (e.g., nucleotide sequences) of additional subgenomic intervals.

E48. The method of any one of embodiments E1-E42, wherein detecting the genetic biomarker comprises providing the sequence of an amplicon comprising the genetic biomarker.

E49. The method of embodiment E48, wherein, the number of amplicon sequences provided is sufficient such that the sensitivity of detection of the cancer in the plurality of cancers with which each gene, *e.g.*, driver gene, is associated with is not substantially increased by the provision of one or more sequences of additional amplicons.

E50. The method of embodiment E46, wherein the number of subgenomic interval sequences provided is sufficient such that the specificity of detection of the cancer in the plurality of cancers with which each gene, *e.g.*, driver gene, is associated with is not substantially decreased by the provision of one or more sequences of additional subgenomic intervals.

E51. The method of embodiment E48, wherein the number of amplicons provided is sufficient such that the specificity of detection of the cancer in the plurality of cancers with which each gene, *e.g.*, driver gene, of the plurality is associated with is not substantially decreased by the provision of one or more sequences of additional amplicons.

E52. The method of any of the preceding embodiments, wherein the plurality of cancers comprises 4, 5, 6, 7 or 8 cancers.

E53. The method of any of the preceding embodiments, wherein the plurality of cancers is chosen from solid tumors such as: mesothelioma (*e.g.*, malignant pleural mesothelioma), lung cancer (*e.g.*, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, or large cell lung cancer), pancreatic cancer (*e.g.*, pancreatic ductal adenocarcinoma), liver cancer (e.g., hepatocellular carcinoma, or cholangiocarcinoma), esophageal cancer (e.g., esophageal adenocarcinoma or squamous cell

carcinoma), head and neck cancer, ovarian cancer, colorectal cancer, bladder cancer, cervical cancer, uterine cancer (endometrial cancer), kidney cancer, breast cancer, prostate cancer, brain cancer (e.g., medulloblastoma, or glioblastoma), or sarcoma (e.g., Ewing sarcoma, osteosarcoma, rhabdomyosarcoma), or a combination thereof.

E54. The method of any of the preceding embodiments, wherein the plurality of cancers is chosen from liver cancer, ovarian cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, breast cancer, or prostate cancer, or a combination thereof.

E55. The method of any of the preceding embodiments, wherein one or more of the plurality of cancers is chosen from liver cancer, ovarian cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, or breast cancer.

E56. The method of any of the preceding embodiments, wherein one or more of the plurality of cancers is a hematological cancer.

E57. The method of any of the preceding embodiments, wherein no more than 60, 100, 150, 200, 300 or 400 subgenomic intervals or amplicons from the one or more genes, *e.g.*, one or more driver genes, *e.g.*, genes listed in Tables 60 and 61 of US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C,

IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2, are sequenced.

E58. The method of any of the preceding embodiments, wherein at least 30, 40, 50 or 60 subgenomic intervals or amplicons from the one or more genes, *e.g.*, one or more driver genes, *e.g.*, genes listed in Tables 60 and 61 of US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2, are sequenced.

E59. The method of any of the preceding embodiments, wherein at least 30 and not more than 400, at least 40 and not more than 300, at least 50 and no more than 200, at least 60 and no more than 150, or at least 60 and no more than 100, subgenomic intervals or amplicons from the one or more genes, *e.g.*, one or more driver genes, *e.g.*, one or more genes listed in Tables 60 and 61 of US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS,

MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2, are sequenced.

E60. The method of any of the preceding embodiments, wherein the number of subgenomic intervals or amplicons sequenced for a gene is no greater than 125, 150, 200, or 300 % of the lowest number that achieves plateau for sensitivity of detection of the cancer.

E61. The method of any of the preceding embodiments, wherein each subgenomic interval or amplicon of the genetic biomarker comprises 6-800bp, *e.g.*, 6-750bp, 6-700bp, 6-650bp, 6-600bp, 6-550bp, 6-500bp, 6-450bp, 6-400bp, 6-350bp, 6-300bp, 6-250bp, 6-200bp, 6-150bp, 6-100bp, 10-800bp, 15-800bp, 20-800bp, 25-800bp, 30-800bp, 35-800bp, 40-800bp, 45-800bp, 50-800bp, 55-800bp, 60-800bp, 65-800bp, 70-800bp, 75-800bp, 80-800bp, 85-800bp, 90-800bp, 95-800bp, 100-800bp, 200-800bp, 300-800bp, 400-800bp, 500-800bp, 600-800bp, 700-800bp, 10-700bp, 20-600bp, 30-500bp, 40-400bp, 50-300bp, 60-200bp, 61-150bp, 62-140bp, 63-130bp, 64-120bp, or 65-100bp, *e.g.*, 66-80bp.

E62. The method of any of the preceding embodiments, wherein each subgenomic interval or amplicon of the genetic biomarker comprises about 35, 40, 45, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 100, or 110 bp.

E63. The method of any of the preceding embodiments, wherein each subgenomic interval or amplicon of the genetic biomarker comprises no more than 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, or 800 bp.

E64. The method of any of the preceding embodiments, wherein each subgenomic interval or amplicon of the genetic biomarker comprises at least 6, 10, 15, 20, 25, 30, 35, 40, 45, or 50bp.

E65. The method of any of the preceding embodiments, wherein each subgenomic interval or amplicon of the genetic biomarker comprises at least 6pb and no more than 800bp, at least 10bp and no more than 700bp, at least 15bp and no more than 600bp, at least 20bp and no more than 600bp, at least 25bp and no more than 500bp, at least 30bp and no more than 400bp, at least 35bp and no more than 300bp, at least 40bp and no more than 200bp, at least 45bp and no more than 100bp, at least 50bp and no more than 95bp, or at least 55bp and no more than 90bp.

E66. The method of any of the preceding embodiments, wherein each subgenomic interval or amplicon of the genetic biomarker comprises 66-80bp.

E67. The method of any of the preceding embodiments, wherein the number of subgenomic intervals or amplicons of the genetic biomarker comprises no more than 2000, 2500, 3000, 3500, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, 15,000, or 20,000bp.

E68. The method of any of the preceding embodiments, wherein the number of subgenomic intervals or amplicons of the genetic biomarker comprises at least 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900 or 2000bp.

E69. The method of any of the preceding embodiments, wherein the number of subgenomic intervals or amplicons of the genetic biomarker comprises at least 200bp and no more than 20,000bp, at least 300bp and no more than 15,000bp, at least 400bp and no more than 10,000bp, at least 500bp and no more than 9000, at least 600bp and no more than 8000bp, at least 700bp and no more than 7000bp, at least 800bp and no more than 6000bp, at least 900bp and no more than 5000bp, at least 1000bp and no more than 4000bp, at least 1100bp and no more than 3500bp, at least 1200bp and no more than 3000bp, at least 1300bp and no more than 2500bp, or at least 1500bp and no more than 2000bp.

E70. The method of any of the preceding embodiments, wherein the number of subgenomic intervals or amplicons of the genetic biomarker comprises 200 + 15%, 300 + 15%, 400 + 15%, 500 + 15%, 600 + 15%, 700 + 15%, 800 + 15%, 900 + 15%, 1000 + 15%, 1100 + 15%, 1200 + 15%, 1300 + 15%, 1400 + 15%, 1500 + 15%, 1600 + 15%, 1700 + 15%, 1800 + 15%, 1900 + 15%, 2000 + 15%, 2500 + 15%, 3000 + 15%, 3500 + 15%, 4000 + 15%, 5000 + 15%, 6000 + 15%, 7000 + 15%, 8000 + 15%, 9000 + 15%, 10,000 + 15%, 15,000 + 15%, or 20,000bp + 15%, e.g., 2000bp + 15%.

E71. The method of any of the preceding embodiments, wherein the number of subgenomic intervals or amplicons of the genetic biomarker comprise 2000bp.

E72. The method of any of the preceding embodiments, wherein the average depth to which the number of subgenomic intervals or amplicons of the genetic biomarker is sequenced is at least 5× sequencing depth.

E73. The method of any of the preceding embodiments, wherein the average depth to which the number of subgenomic intervals or amplicons of the genetic biomarker is sequenced is no more than 500× sequencing depth.

E74. The method of any of the preceding embodiments, wherein the average depth to which the number of subgenomic intervals or amplicons of the genetic biomarker is sequenced is between 5× to 500× sequencing depth.

E75. The method of any of the preceding embodiments, wherein said detecting step comprises sequencing each subgenomic interval to a depth of at least 50,000 reads per base.

E76. The method of any of the preceding embodiments, wherein said detecting step comprises sequencing each subgenomic interval to a depth of no more than 150,000 reads per base.

E77. The method of any of the preceding embodiments, wherein said detecting step comprises sequencing each subgenomic interval to a depth of from 50,000 reads per base to 150,000 reads per base.

E78. The method of any of the preceding embodiments, wherein said detecting step comprises sequencing each subgenomic interval at a depth sufficient to detect a mutation in said region of interest at a frequency as low as 0.0005%.

E79. The method of any of the preceding embodiments, wherein no more than 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50, 55, 60,100, 200 or 300 bp, is sequenced for each biomarker, *e.g.*, each gene, *e.g.*, each driver gene, *e.g.*, each gene disclosed in Table 60 or 61 in US2019/0256924A1 e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2.

E80. The method of any of the preceding embodiments, wherein at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20bp, is sequenced in each biomarker, *e.g.*, each gene, *e.g.*, each driver gene, *e.g.*, each gene disclosed in Table 60 or 61 in US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1,

CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2.

E81. The method of any of the preceding embodiments, wherein at least 6 and no more than 300bp, at least 7 and no more than 200bp, at least 8bp and no more than 100bp, at least 9bp and no more than 60bp, at least 10bp and no more than 55bp, at least 11 bp and no more than 50bp, at least 12bp and no more than 45bp, at least 13bp and no more than 40bp, at least 14bp and no more than 35bp, at least 15bp and no more than 34bp, at least 14bp and no more than 33bp, at least 15bp and no more than 32bp, at least 16bp and no more than 31bp, at least 17bp and no more than 30bp, at least 18bp and no more than 29bp, at least 19bp and no more than 28bp, at least 20bp and no more than 27bp, is sequenced in each biomarker, *e.g.*, each gene, *e.g.*, each driver gene, *e.g.*, each gene disclosed in Table 60 or 61 in US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1,

SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2.

E82. The method of any of the preceding embodiments, wherein about 33bp is sequenced in each biomarker, *e.g.*, each gene, *e.g.*, each driver gene, *e.g.*, each gene disclosed in Table 60 or 61 in US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2.

E83. The method of any of the preceding embodiments, wherein detecting the biomarker comprises providing the sequence of the subgenomic interval or amplicon of no more than 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 40, 45, 50, 55, 60,100, 200 or 300 bp, in length and wherein the subgenomic interval or the amplicon comprises the biomarker, *e.g.*, a driver gene comprising a driver mutation.

E84. The method of any of the preceding embodiments, wherein detecting the biomarker comprises providing the sequence of the subgenomic interval or the amplicon of at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20bp, in length and wherein the subgenomic interval or the amplicon comprises the biomarker, *e.g.*, a driver gene comprising a driver mutation.

E85. The method of any of the preceding embodiments, wherein detecting the biomarker comprises providing the sequence of a subgenomic interval or amplicon of at least 6 and no more than 300bp, at least 7 and no more than 200bp, at least 8bp and no more than 100bp, at least 9bp and no more than 60bp, at least 10bp and no more than 55bp, at least 11 bp and no more than 50bp, at least 12bp and no more than 45bp, at least 13bp and no more than 40bp, at least 14bp and no more than 35bp, at least 15bp and no more than 34bp, at least 14bp and no more than 33bp, at least 15bp and no more than 32bp, at least 16bp and no more than 31bp, at least 17bp and no more than 30bp, at least 18bp and no more than 29bp, at least 19bp and no more than 28bp, at least 20bp and no more than 27bp, in length and wherein the subgenomic interval or amplicon comprises the biomarker, *e.g.*, driver gene comprising a driver mutation.

E86. The method of any of the preceding embodiments, wherein detecting the biomarker comprises providing the sequence of a subgenomic interval or amplicon of between 6bp and 300bp, 7bp and 200bp, or 8 and 100bp, 9bp and 60bp, 10bp and 50bp, 15bp and 40bp, 20bp and 35bp in length and wherein the subgenomic interval or amplicon comprises the biomarker, *e.g.*, driver gene comprising a driver mutation.

E87. The method of any of the preceding embodiments, wherein detecting the biomarker comprises providing the sequence of a subgenomic interval or amplicon of about 33bp in length and wherein the subgenomic interval or amplicon comprises the biomarker, *e.g.*, driver gene comprising a driver mutation.

E88. The method of any of the preceding embodiments, further comprising:

b) detecting the level of each of a plurality of, *e.g.*, at least four, protein biomarkers in a biological sample, wherein the level of each protein biomarker of the plurality is associated with the presence of a cancer of the plurality of cancers;

(optionally) (c) comparing the detected levels of each protein biomarker of the plurality of protein biomarkers to a reference level for the protein biomarker; and

d) identifying the presence of a cancer of the plurality of cancers in the subject when the presence of one or more genetic biomarkers and the level of one of the protein biomarkers of the plurality of protein biomarkers is detected.

E89. The method of any of the preceding embodiments, wherein:

(i) the subject has not yet been determined to have a cancer, *e.g.*, a cancer selected from the plurality of cancers,

(ii) the subject has not yet been determined to harbor a cancer cell, *e.g.*, a cancer cell selected from the plurality of cancers, or

(iii) the subject does not exhibit, or has not exhibited a symptom associated with a cancer, *e.g.*, a cancer selected from the plurality of cancers.

E90. The method of any of the preceding embodiments, wherein the subject:

(i) is a pediatric subject or a young adult; e.g., aged 6 months-21 years; or

(ii) is an adult, e.g., aged 18 years or older.

E91. The method of any of the preceding embodiments, wherein the sample comprises a tumor sample, *e.g.*, a biopsy sample (*e.g.*, a liquid biopsy sample (*e.g.*, a circulating tumor DNA sample, or a cell-free DNA sample) or a solid tumor biopsy sample); a blood sample (*e.g.*, a circulating tumor DNA sample, or a cell-free DNA sample), an apheresis sample, a urine sample, a cyst fluid sample (*e.g.*, a pancreatic cyst fluid sample), a Papanicolaou (Pap) sample, or a fixed tumor sample (*e.g.*, a formalin fixed sample or a paraffin embedded sample (FPPE)).

E92. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, genes comprises 1, 2, 3, or 4 genes from Tables 60 and 61 of US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2,

JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2.

E93. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, genes comprises 5, 6, 7, or 8 genes, chosen from Tables 60 and 61 of US2019/0256924A1, e.g., ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, or SKP2.

E94. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, genes is a gene selected from: NRAS, CTNNB1, PIK3CA, FBXW7, APC, EGFR, BRAF, CDKN2A, PTEN, FGFR2, HRAS, KRAS, AKT1, TP53, PPP2R1A, or GNAS.

E95. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, biomarkers (e.g., one or more genes) is chosen from KRAS, PIK3CA, HRAS, CDKN2A, TP53, AKT1, CTNNB1, APC, EGFR, GNAS, PPP2R1A, BRAF, FBXW7, PTEN, or FGFR2, or a combination thereof, and the cancer is chosen from: liver cancer, ovarian cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, breast cancer, or prostate cancer.

E96. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, biomarkers (e.g., one or more genes) is chosen from KRAS, PIK3CA, HRAS, CDKN2A, TP53, TERT, ERBB2, FGFR3, MET, MLL, or VHL, or a combination thereof, and the cancer is chosen from a bladder cancer or upper tract urothelial carcinoma (UTUC).

E97. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, biomarkers (e.g., one or more genes) is chosen from KRAS, PIK3CA, CDKN2A, TP53, CTNNB1, PPP2R1A, BRAF, PTEN, CSMD3, FAT3, BRCA, or ARID1A, or a combination thereof, and the cancer is an ovarian cancer or an endometrial cancer.

E98. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of, biomarkers (e.g., one or more genes) is chosen from KRAS, PIK3CA, CDKN2A, TP53, CTNNB1, GNAS, BRAF, NRAS, VHL, RNF43, or SMAD4, or a combination thereof, and the cancer is a pancreatic cancer, *e.g.*, a pancreatic ductal adenocarcinoma (PDAC).

E99. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of biomarkers, comprises 5, 6, 7, or 8 protein biomarkers.

E100. The method of any of the preceding embodiments, wherein the one or more, e.g., plurality of biomarkers, comprises a protein biomarker selected from: CA19-9, CEA, HGF, OPN, CA125, prolactin (PRL), TIMP-1, CA15-3, AFP or MPO.

E101. The method of any of the preceding embodiments, wherein detecting the presence of one or more genetic biomarkers comprises:

a. assigning a unique identifier (UID) to each of a plurality of template molecules present

in the sample;

b. amplifying each uniquely tagged template molecule to create UID-families; and

c. redundantly sequencing the amplification products.

E102. The method of any of the preceding embodiments, further comprising detecting the presence of aneuploidy in the sample, *e.g.*, detecting gain or loss in one or more chromosomes, *e.g.*, using the WALDO method as described in Example 6.

E103. The method of embodiment 102, wherein the method comprises: (i) estimating somatic mutation load; (ii) estimating carcinogen signature, and/or (iii) detecting microsatellite instability (MSI).

E104. The method of embodiment 102 or 103, wherein the method can be used to compare two samples, *e.g.*, two unrelated samples, to evaluate genetic similarities between the samples or to find somatic mutations within the samples, *e.g.*, within the LINE elements in the sample.

E105. The method of embodiment 102 or 103, wherein the method results in an increase in specificity and/or sensitivity of aneuploidy detection.

E106. The method of embodiment 102, wherein the presence of aneuploidy is detected on one or more chromosome arms.

E107. The method of any of the preceding embodiments, further comprising responsive to a value of: a genetic marker, a protein biomarker and/or aneuploidy status, assigning an origin or cancer type to the cancer.

E108. The method of any one of the preceding embodiments, wherein responsive to a value of: a genetic marker, a protein biomarker and/or aneuploidy status, the method comprises identifying the subject as having a cancer, or having a risk of developing a cancer.

E109. The method of embodiment E108, further comprising administering to the subject a therapeutic agent to treat the cancer, or selecting a therapeutic agent for treating the cancer in the subject.

E110. The method of embodiment E109, wherein the subject is administered the therapeutic agent in combination with one or more additional therapeutic agents.

E111. A reaction mixture comprising:

at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 detection reagents, wherein a detection reagent mediates a readout that is a value of the level or presence of:

(i) one or more genetic biomarkers referred to herein;

(ii) one or more protein biomarkers referred to herein; and/or

(iii) the copy number or length, e.g., aneuploidy, of a genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family) referred to herein.

E112. The reaction mixture of embodiment E111, comprising a plurality of detection reagents for (i).

E113. The reaction mixture of any of embodiments E111-E112, comprising a plurality of detection reagents for (ii).

E114. The reaction mixture of any of embodiments E111-E113, comprising a plurality of detection reagents for (iii).

E115. The reaction mixture of any of embodiments E111-E114, comprising a sample from a subject, e.g., a subject sample.

E116. A kit comprising:

(a) at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 detection reagents, wherein a detection reagent mediates a readout that is a value of the level or presence of:

(i) one or more genetic biomarkers referred to herein;

(ii) one or more protein biomarkers referred to herein; and/or

(iii) the copy number or length, e.g., aneuploidy, of a genomic sequence disposed between at least two terminal repeated elements of a repeated element family (RE Family) referred to herein; and

(b) instructions for using said kit.

E117. The reaction mixture of embodiment E116, comprising a plurality of detection reagents for (i).

E118. The reaction mixture of any of embodiments E116-E117, comprising a plurality of detection reagents for (ii).

E119. The reaction mixture of any of embodiments E116 to E118, comprising a plurality of detection reagents for (iii).

E120. The method of any one of embodiments E1-E110, wherein aneuploidy status is evaluated, e.g., determined, using a first primer and a second primer.

E121. The method of embodiment E120, wherein the first primer comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 96%, 98%, 99%, or 100% identical to SEQ ID NO: 1

E122. The method of embodiment E121, wherein the first primer comprises the sequence of SEQ ID NO: 1.

E123. The method of embodiment E120, wherein the second primer comprises a sequence that is at least 80%, 85%, 90%, 95%, 96%, 96%, 98%, 99%, or 100% identical to SEQ ID NO: 10.

E124. The method of embodiment E123, wherein the second primer comprises the sequence of SEQ ID NO: 10.

E125. The method of any one of embodiments E1-E110, or E120-E124, further comprising subjecting the subject to a radiologic scan, e.g., a PET-CT scan, of an organ or body region.

E126. The method of embodiment 125, wherein the radiologic scanning of an organ or body region characterizes the cancer.

E127. The method of embodiment 125, wherein the radiologic scanning of an organ or body region identifies the location of the cancer.

E128. The method of any one of embodiments E125-E127, wherein the radiologic scan is a PET-CT scan.

E129. The method of any one of embodiments E125-E128, wherein the radiologic scanning is performed after the subject is evaluated for the presence of each of a plurality of cancers.

E130. The method of any one of embodiments E1-E110, or E120-E129, comprising administering to the subject one or more therapeutic interventions (e.g., surgery, adjuvant chemotherapy, neoadjuvant chemotherapy, radiation therapy, immunotherapy, targeted therapy, and/or an immune checkpoint inhibitor).

E131. The method of any one of embodiments E1-E110, or E120-E130, wherein the evaluation comprises evaluating a sample from the subject at one time point or at different time points.

E132. The method of any one of embodiments E1-E110, or E120-E131, comprising evaluating one or more samples, e.g., multiple samples, obtained from the subject.

E133. The method of E132, wherein the one or more samples, e.g., multiple samples, are obtained yearly, e.g., within 1 year of one another.

E134. The method of any of embodiments E1-E110, or E120-E133, wherein the subject is evaluated simultaneously for the presence or absence of each of a plurality of cancers.

E135. The method of any of embodiments E1-E110, or E120-E134, wherein the subject is co-evaluated for the presence or absence of each of a plurality of cancers.

E136. The method of any of embodiments E1-E110, or E120-E135, comprising evaluating the presence of each of a plurality of cancers in a subject at one or more time points within a predetermined interval, e.g., at the same or substantially the same clinical stage of at least one of the cancers in the subject.

E137. The method of any of embodiments E1-E110, or E120-E136, comprising evaluating a sample, e.g., a single sample or multiple samples, obtained from the subject.

E138. The method of any of embodiments E1-E110, or E120-E137, wherein co-evaluation is performed on a single sample, aliquots of a single sample, or a plurality of samples taken, e.g., within 1, 5, 24 or 48 hours, of one another.

E139. The method of any embodiments E1-E110, or E120-E138, wherein the subject is asymptomatic for cancer.

E140. The method of any of embodiments E1-E110, or E120-E139, wherein the subject is asymptomatic for a cancer of the plurality.

E141. The method of any of embodiments E1-E110, or E120-E140, wherein the subject is not known or determined to harbor a cancer cell.

E142. The method of any of embodiments E1-E110, or E120-E141, wherein the subject has not been determined to have or diagnosed with a cancer.

E143. The method of any of embodiments E1-E110, or E120-E142, wherein the subject has an early stage cancer, e.g., Stage I or Stage II.

E144. The method of any of embodiments E1-E110, or E120-E143, wherein the subject is pre-metastatic.

E145. The method of any of embodiments E1-E110, or E120-E144, wherein the subject has no detectable metastasis.

E146. The method of any of embodiments E1-E110, or E120-E145, wherein the subject has not exhibited a symptom associated with a cancer.

E147. The method of any of embodiments E1-E110, or E120-E146, wherein the subject does not display one, two or more symptoms clinically associated with the cancer.

E148. The method of any of embodiments E1-E110, or E120-E147, wherein when the aneuploidy status is positive, the subject has an early stage cancer, e.g., Stage I or Stage II e.g., as provided in Table 3.

E149. The method of any of embodiments E1-E110, or E120-E147, wherein when the aneuploidy status is negative, the subject has an early stage cancer, e.g., Stage I or Stage II e.g., as provided in Table 3.

E150. A method of detecting aneuploidy in a sample comprising low input DNA.

E151. The method of any of embodiments E1-E110, or E120-E150, wherein the sample comprises about 0.01 picogram (pg) to 500 pg of DNA.

E152. The method of embodiment E151, wherein the sample comprises about 0.01-500pg, 0.05-400pg, 0.1-300pg, 0.5-200pg, 1-100pg, 10-90pg, or 20-50pg DNA.

E153. The method of embodiment E151, wherein the sample comprises at least 0.01 pg, at least .01 pg, at least 0.1 pg, at least 1 pg. at least 2 pg, at least 3 pg, at least 4 pg, at least 5 pg, at least 6 pg, at least 7 pg, at least 8 pg, at least 9 pg at least 10pg, at least 11 pg, at least 12 pg, at least 13 pg, at least 14 pg, at least 15 pg, at least 16 pg, at least 17 pg, at least 18 pg , at least 19 pg, at least 20 pg, at least 21 pg, at least 22 pg, at least 23 pg, at least 24 pg, at least 25 pg, at least 26 pg, at least 27 pg, at least 28 pg, at least 29 pg, at least 30 pg, at least 31 pg, at least 32 pg, at least 33 pg, at least 34 pg, at least 35 pg, at least 36 pg, at least 37 pg, at least 38 pg, at least 39 pg, at least 40 pg, at least 50 pg, at least 60 pg, at least 70 pg, at least 80 pg, at least 90 pg, at least 100pg, at least 150pg, at least 200 pg, at least 300 pg, at least 350 pg, at least 400 pg, at least 450 pg, or at least 500 pg DNA.

E154. A method of identifying or distinguishing a sample, e.g., using any of the methods disclosed herein.

E155. The method of embodiment E154, wherein a sample, e.g., a first sample, from a subject, e.g., a first subject, is distinguished from a second sample from a second subject.

E156. The method of embodiment E154, wherein a sample is identified as being from a subject based on a polymorphism (e.g., a plurality of polymorphisms, e.g., common polymorphisms).

E157. The method of embodiment E156, wherein a polymorphism, e.g., a common polymorphism, is present in a repetitive element, e.g., as described herein.

E158. The method of embodiment E154, wherein a method disclosed in Example 8 is used to identify and/or distinguish the sample.

E159. The method of any of embodiments E1-E110, or E120-E158, wherein the method is an in vitro method.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1A shows a distribution of amplicon size when using a single primer pair to amplify repetitive elements (see, e.g., Table 1 for a list of repetitive elements). The amplicon sizes shown in Figure 1A includes the number of bases in the primers.

Figure 1B shows a distribution of amplicon size when using a single primer pair to amplify repetitive elements (see, e.g., Table 1 for a list of repetitive elements). The amplicon sizes shown in Figure 1B do not include the number of bases in the primers.

Figure 1C shows a distribution of the number of amplicons observed in cell free DNA from 2231 plasma samples.

Figure 2A. Exemplary overview of an embodiment of a workflow described herein.

Figure 2B is an exemplary overview of an embodiment of the Repetitive Element AneupLoidy Sequencing System (RealSeqS).

Figure 3 shows aneuploidy sensitivity vs mutations (@99% specificity) in different cancer types. The percent of aneuploidy detected in each cancer type is depicted on the Y axis.

Figure 4 shows aneuploidy shows aneuploidy sensitivity compared to other cancer biomarkers. The percent of cancers detected (sensitivity) is depicted on the Y axis.

Figure 5 shows pseudocode to generate synthetics with multiple arm alterations.

Figure 6 shows estimation of the relationship between reads and DNA concentration.

Figure 7A shows a comparison of the sensitivity of cancer detection with different multi-analyte tests. Three different multi-analyte test evaluated sensitivity of detecting the eight indicated cancers. The three tests were: (1) aneuploidy status, somatic mutation analysis and protein biomarker evaluation; (2) aneuploidy status and somatic mutation analysis; and (3) aneuploidy status and protein biomarker evaluation.

Figure 7B shows the sensitivity of a test incorporating aneuploidy, mutations, and abnormally high levels of 8 proteins compared to a test comparing only aneuploidy + proteins or only mutations and proteins. All sensitivities were calculated at an aggregate of 99% specificity (i.e., only 1% of the plasma samples was positive for aneuploidy, mutations, or proteins in the test incorporating aneuploidy, mutations, and proteins using 10 iterations of 10 fold cross validation).

Figure 8 is a graph showing the true positive fraction (sensitivity) on the y-axis and the false positive fraction of cancer detection using the various tests. The tests include: (1) aneuploidy status; somatic mutation; and protein biomarker; (2) aneuploidy status and protein biomarker; (3) somatic mutation and protein biomarker; (4) aneuploidy status and somatic mutation; (5) aneuploidy status; and (6) somatic mutation. The true positive fraction (sensitivity) was calculated using a threshold at 99% specificity.

Figure 9 shows sensitivity of cancer detection for aneuploidy alone (@98% or 99% specificity) compared to sensitivity with aneuploidy and protein biomarkers (@95% specificity) in different stages of cancer.

Figure 10 shows aneuploidy (@99% specificity) in different stages of cancer.

Figure 11 shows aneuploidy (@99% specificity) in different cancer types.

Figure 12 shows sensitivity when aneuploidy (@99% specificity) is combined with detection of protein biomarkers.

Figure 13 shows pseudocode to generate in silico trisomy and monosomy samples used for the comparison of whole genome sequencing, FAST-SeqS and Real SeqS.

Figure 14 shows pseudocode to generate in silico simulated samples with multiple arm alterations that were used in the Genome Wide Aneuploidy SVM training set.

Figures 15A-15C show detection of Aneuploidy using Next Generation Sequencing Technologies. Sensitivities were calculated at 99% specificity. Error bars represent 95% confidence intervals. FIG. 15A Comparison of sensitivity for monosomies and trisomies across all 39 non-acrocentric chromosome arms at 5% cell fraction. FIG. 15B Comparison of

sensitivity for the 1.5 Mb DiGeorge deletion on 22q at 5% cell fraction. FIG. 15C Comparison of sensitivity for a 20 copy *ERBB2* focal amplification at 1% cell fraction.

Figures 16A-16B show examples of plasma samples with focal deletions or amplifications. FIG. 16A shows RealSeqS data on a plasma sample from a normal individual with a ~3 Mb deletion of chromosome 22, characteristic of DiGeorge Syndrome. Note that many patients with microdeletions at this locus have mild signs and symptoms and are clinically undetected. FIG. 16B shows RealSeqS data on a typical plasma sample from a normal individual, showing no deletion at the DiGeorge locus.

Figures 17A-17B show examples of plasma samples with focal deletions or amplifications. FIG. 17A shows RealSeqS data on a plasma sample from a patient with cancer showing a 2.5MB focal amplification that includes the *ERBB2* locus on chromosome 17q. FIG. 17B shows RealSeqS data on a typical plasma sample from a normal individual, showing no amplification at the *ERBB2* locus.

Figure 18 shows RealSeqS sensitivity for plasma samples with various amounts of tumor derived DNA. The amount of tumor DNA was estimated by the mutant allele frequency (MAF) of driver mutations present in the plasma sample.

Figures 19A-19B show detection of cancer in liquid biopsies from samples with non-metastatic cancers of eight different types. Sensitivities were calculated at 99% specificity during cross validation. Error bars represent 95% confidence intervals. FIG. 19A shows the comparison of aneuploidy status as assessed by RealSeqS to somatic mutations status with respect to tumor type. FIG. 19B shows the comparison of aneuploidy status as calculated by RealSeqS to somatic mutations status with respect to Cancer Stage.

DETAILED DESCRIPTION

Definitions

The term “driver gene mutation” or “driver mutation” as used herein, refers to a mutation that (i) occurs in a driver gene; and (ii) provides a growth advantage to the cell in which it occurs. A growth advantage for a cell can include:

a) an increase in the rate of cell division in a cell having a driver gene mutation, e.g., an increase in rate of cell division as compared to a reference cell, e.g., to an otherwise

similar cell, e.g., an otherwise similar cell adjacent to the cell, e.g., as compared to a cell of the same type not having the driver gene mutation;

b) an increase in the rate of clonal expansion in a cell having a driver gene mutation, e.g., an increase in rate of clonal expansion as compared to a reference cell, e.g., to an otherwise similar cell, e.g., an otherwise similar cell adjacent to the cell, e.g., as compared to a cell of the same type not having the driver mutation;

c) an increase in the number of cells that are progeny, e.g., a daughter cell, of the cell that has the driver gene mutation, e.g., an increase in number of progeny cells compared to the number of progeny cells expected if the cell did not have the driver gene mutation;

d) an increase in the ability to form tumors or promote tumor growth, e.g., tumor progression, e.g., as compared to a reference cell, e.g., to an otherwise similar cell not having the driver gene mutation; or

e) presence or appearance at a second or subsequent site or location in the subject.

In an embodiment, a driver gene mutation provides a 0.1-5%, e.g., a 0.1-4.5%, 0.1-4%, 0.1-3.5%, 0.1-3%, 0.1-2.5%, 0.1-2%, 0.1-1.5%, 0.1-1%, 0.1-0.5%, 0.5-5%, 1-5%, 1.5-5%, 2-5%, 2.5-5%, 3-5%, 3.5-5%, 4-5%, 4.5-5%, 0.5-4.5%, 1-4%, 1.5-3.5%, or 2-3%, growth advantage, e.g., increase in the difference between cell birth and cell death. In an embodiment, a driver gene mutation provides at least 0.1% 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, or 4.5%, e.g., about a 0.4 %, growth advantage, e.g., increase in the difference between cell birth and cell death. In an embodiment, a driver gene mutation, provides a proliferative capacity to the cell in which it occurs, e.g., allows for cell expansion, e.g., clonal expansion.

In some embodiments, the driver gene mutation can be causally linked to cancer progression.

In an embodiment, the driver gene mutation affects, e.g., alters the regulation, expression or function of, a protein coding gene. In an embodiment, a driver gene mutation affects, e.g., alters the function of, a noncoding region, e.g., non-protein coding region. In an embodiment, a driver gene mutation includes: a translocation, a deletion (e.g., a homozygous deletion), an insertion (e.g., an intragenic insertion), a small insertion and deletion (indels), a single base substitution (e.g., a synonymous mutation, non-synonymous mutation, nonsense mutation or a frameshift mutation), a copy number variation (CNV) (e.g., an amplification),

or a single nucleotide variation (SNV) (e.g., a single nucleotide polymorphism (SNP)). Exemplary driver mutations are disclosed in Tables 60 and 61 of US2019/0256924A1.

In some embodiments, the presence of a driver gene mutation in a cell can alter (e.g., increase or decrease) the expression of the gene product in that cell. In some embodiments, the presence of a driver gene mutation in a cell can alter the function of the gene product. In some cases, the presence of a driver gene mutation in a cell can provide that cell with a growth advantage. For example, the presence of a driver gene mutation in a cell can cause an increase the rate of proliferation (e.g., as compared to a reference cell). For example, the presence of a driver gene mutation in a cell can cause an increase in the rate of clonal expansion in a cell having a driver gene mutation (e.g., as compared to a reference cell). For example, the presence of a driver gene mutation in a cell can cause an increase in the number of progeny cells derived from the cell having the driver gene mutation (e.g., as compared to a reference cell). For example, the presence of a driver gene mutation in a cell can cause an increase in the ability of the cell to form a tumor (e.g., as compared to a reference cell). In some cases, a growth advantage can be measures as an increase in the difference between cytogenesis (e.g., the formation of new cells) and cell death. For example, the presence of a driver gene mutation in a cell can provide that cell with a growth advantage of at least about 0.1% (e.g., about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, or more). For example, the presence of a driver gene mutation in a cell can provide that cell with a growth advantage of about from 0.1% to about 5% (e.g., from about 0.1 to about 5%, from about 0.1 to about 4.5%, from about 0.1 to about 4%, from about 0.1 to about 3.5%, from about 0.1 to about 3%, from about 0.1 to about 2.5%, from about 0.1 to about 2%, from about 0.1 to about 1.5%, from about 0.1 to about 1%, from about 0.1 to about 0.5%, from about 0.5 to about 5%, from about 1 to about 5%, from about 1.5 to about 5%, from about 2 to about 5%, from about 2.5 to about 5%, from about 3 to about 5%, from about 3.5 to about 5%, from about 4 to about 5%, from about 4.5 to about 5%, from about 0.5 to about 4.5%, from about 1 to about 4%, from about 1.5 to about 3.5%, or from about 2 to about 3%).

In some cases, a driver gene can include more than one (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) driver gene mutations. In some cases, a driver gene including one or more driver gene mutations also can include one or more additional

mutations (e.g., passenger gene mutations (somatic mutations which are not a driver mutation)).

The term "driver gene" as used herein, refers to a gene which includes a driver gene mutation. In one embodiment, the driver gene is a gene in which one or more (e.g., one, two, three, four, five, six, seven, eight, nine, ten, or more) acquired mutations, e.g., driver gene mutations, can be causally linked to cancer progression. In an embodiment, a driver gene modulates one or more cellular processes including: cell fate determination, cell survival and genome maintenance. A driver gene can be associated with (e.g., can modulate) one or more signaling pathways. Examples of signaling pathways include, without limitation, a TGF-beta pathway, a MAPK pathway, a STAT pathway, a PI3K pathway, a RAS pathway, a cell cycle pathway, an apoptosis pathway, a NOTCH pathway, a Hedgehog (HH) pathway, an APC pathway, a chromatin modification pathway, a transcriptional regulation pathway, and a DNA damage control pathway. Examples of driver genes include, without limitation, ABL1, ACVR1B, AKT1, ALK, APC, AR, ARID1A, ARID1B, ARID2, ASXL1, ATM, ATRX, AXIN1, B2M, BAP1, BCL2, BCOR, BRAF, BRCA1, BRCA2, CARD11, CASP8, CBL, CDC73, CDH1, CDKN2A, CEBPA, CIC, CREBBP, CRLF2, CSF1R, CTNNB1, CYLD, DAXX, DNMT1, DNMT3A, EGFR, EP300, ERBB2, EZH2, FAM123B, FBXW7, FGFR2, FGFR3, FLT3, FOXL2, FUBP1, GATA1, GATA2, GATA3, GNA11, GNAQ, GNAS, H3F3A, HIST1H3B, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDM5C, KDM6A, KIT, KLF4, KRAS, MAP2K1, MAP3K1, MED12, MEN1, MET, MLH1, MLL2, MLL3, MPL, MSH2, MSH6, MYD88, NCOR1, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NPM1, NRAS, PAX5, PBRM1, PDGFRA, PHF6, PIK3CA, PIK3R1, PPP2R1A, PRDM1, PTCH1, PTEN, PTPN11, RB1, RET, RNF43, RUNX1, SETD2, SETBP1, SF3B1, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SOCS1, SOX9, SPOP, SRSF2, STAG2, STK11, TET2, TNFAIP3, TRAF7, TP53, TSC1, TSHR, U2AF1, VHL, WT1, CCND1, CDKN2C, IKZF1, LMO1, MAP2K4, MDM2, MDM4, MYC, MYCL1, MYCN, NCOA3, NKX2-1, and SKP2. Exemplary driver genes include oncogenes and tumor suppressors. In an embodiment, a driver gene has one or more driver gene mutations, e.g., as described herein. In an embodiment, a driver gene is a gene listed in Tables 60 or 61 in US2019/0256924A1. In an embodiment, a driver gene is a gene that modulates one or more cellular processes described in Tables 60 or 61 in US2019/0256924A1, e.g., cell fate determination, cell survival and genome maintenance. In an embodiment, a driver gene is a gene that modulates one or more

pathways described in Tables 60 or 61 in US2019/0256924A1. In an embodiment, a driver gene is a gene that modulates one or more signaling pathways described in Table 62 of US2019/0256924A1.

In an embodiment, a driver gene includes more than one driver mutation, and the first driver gene mutation, provides a selective growth advantage to the cell in which it occurs. In an embodiment, the subsequent mutation, e.g., second, third, fourth, fifth or later mutation, e.g., driver mutation in the driver gene, provides a proliferative capacity to the cell in which it occurs, e.g., allows for cell expansion, e.g., clonal expansion. In an embodiment, a driver gene has one or more passenger gene mutations, e.g., a somatic mutation that arises in the development of a cancer but which is not a driver mutation. In an embodiment, a driver gene can be present, e.g., expressed, in any cell type, e.g., a cell type derived from any one of the three germ cell layers: ectoderm, endoderm or mesoderm. In an embodiment, a driver gene is present, e.g., expressed, in a somatic cell. In an embodiment, a driver gene is present, e.g., expressed, in a germ cell. In an embodiment, a driver gene can be present in a large number of cancers, e.g., in more than 5% of cancers. In an embodiment, a driver gene can be present in a small number of cancer, e.g., in less than 5% of cancers. In an embodiment, a driver gene has a mutation pattern that is non-random and/or recurrent, i.e., the location at which a driver mutation occurs in the driver gene is the same in different cancer types. Exemplary recurrent driver gene mutations include mutations in the IDH1 gene at the substrate binding site, e.g., at codon 132, and mutations in the PIK3CA gene in the helical domain or the kinase domain, as depicted in Vogelstein et al (2013) Science 339: 1546-1558.

In an embodiment, a driver gene having a driver gene mutation is an oncogene. In an embodiment, an oncogene is a gene with an oncogene score of at least 20%, e.g., at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100%. In an embodiment, an oncogene score is defined as the number of mutations, e.g., clustered mutations (e.g., missense mutations at the same amino acid, or identical in-frame insertions or deletions) divided by the total number of mutations. In an embodiment, a driver gene having an amplification, e.g., as described herein, is an oncogene. In an embodiment, a driver gene having a driver gene mutation is a tumor suppressor gene (TSG). In an embodiment, a tumor suppressor gene is a gene with a tumor suppressor gene score of at least 20%, e.g., at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or 100%. In an embodiment, a tumor suppressor gene score is defined

as the number of inactivating mutations divided by the total number of mutations. In an embodiment, a driver gene having a deletion, e.g., as described herein, is a tumor suppressor gene.

The phrase "repeated element family" or "RE family" as used herein, refers to a family of repeat DNA elements (also known as repetitive DNA elements or repeating units or DNA repeats) which are present in the genome of an organism. A DNA repeat element can be interspersed throughout the genome of an organism or can be present in select chromosomes. An RE family can include one or more repeat DNA elements. Exemplary RE families in the human genome include: interspersed repeats (e.g., long interspersed nucleotide elements (LINE); short interspersed nucleotide elements (SINE)); and tandem repeats (e.g., microsatellites, mini-satellites, satellite DNA or multiple copy genes (e.g., ribosomal RNA)). In some embodiments, an RE family includes one or more repeat elements listed in Table 1, e.g., SINE.

"Acquire" or "acquiring" as the terms are used herein, refer to obtaining possession of a physical entity, or a value, *e.g.*, a numerical value, by "directly acquiring" or "indirectly acquiring" the physical entity or value. "Directly acquiring" as the term is used herein refers to performing a process (*e.g.*, performing a synthetic or analytical method) to obtain the physical entity or value. "Indirectly acquiring" as the term is used herein refers to receiving the physical entity or value from another party or source (*e.g.*, a third party laboratory that directly acquired the physical entity or value). Directly acquiring a physical entity includes performing a process that includes a physical change in a physical substance, *e.g.*, a starting material. Directly acquiring a value includes performing a process that includes a physical change in a sample or another substance, *e.g.*, performing an analytical process which includes a physical change in a substance, *e.g.*, a sample, analyte, or reagent (sometimes referred to herein as "physical analysis"), performing an analytical method, *e.g.*, a method which includes one or more of the following: separating or purifying a substance, *e.g.*, an analyte, or a fragment or other derivative thereof, from another substance; combining an analyte, or fragment or other derivative thereof, with another substance, *e.g.*, a buffer, solvent, or reactant; or changing the structure of an analyte, or a fragment or other derivative thereof.

"Biological sample," "sample," "patient sample," or "specimen" as the terms are used herein, each refer to a sample obtained from a subject or a patient. The source of the sample

can be a biopsy (e.g., a liquid biopsy), an aspirate; blood or any blood constituents; bodily fluids (e.g., cerebral spinal fluid, amniotic fluid, peritoneal fluid or interstitial fluid). The sample can comprise cells (e.g., any cell from a human body, e.g., normal cells and/or cancer cells) and/or cell-free DNA, e.g., circulating tumor DNA or circulating DNA from a normal cell. In an embodiment, the sample, e.g., the tumor sample, includes tissue or cells from a surgical margin. In another embodiment, the sample, e.g., tumor sample, includes one or more circulating tumor cells (CTC) (e.g., a CTC acquired from a blood sample).

As used herein, the term "sensitivity" refers to the ability of a method to detect or identify the presence of a disease in a subject. For example, when used in reference to any of the variety of methods described herein that can detect the presence of cancer in a subject, a high sensitivity means that the method correctly identifies the presence of cancer in the subject a large percentage of the time. For example, a method described herein that correctly detects the presence of cancer in a subject 95% of the time the method is performed is said to have a sensitivity of 95%. In some embodiments, a method described herein that can detect the presence of cancer in a subject provides a sensitivity of at least 70% (e.g., about 70%, about 72%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.5%, or about 100%). In some embodiments, methods provided herein that include detecting the presence of one or more members of two or more classes of biomarkers (e.g., genetic biomarkers and/or protein biomarkers) provide a higher sensitivity than methods that include detecting the presence of one or more members of only one class of biomarkers.

In some embodiments, sensitivity provides a measure of the ability of a method to detect a sequence variant in a heterogeneous population of sequences. A method has a sensitivity of S% for variants of F% if, given a sample in which the sequence variant is present as at least F% of the sequences in the sample, the method can detect the sequence at a confidence of C%, S% of the time. By way of example, a method has a sensitivity of 90% for variants of 5% if, given a sample in which the variant sequence is present as at least 5% of the sequences in the sample, the method can detect the sequence at a confidence of 99%, 9 out of 10 times (F=5%; C=99%; S=90%). Exemplary sensitivities include those of S=90%, 95%, 99%, 99.9% for sequence variants at F=0.5%, 1%, 5%, 10%, 20%, 50%, 100% at confidence levels of C= 90%, 95%, 99%, and 99.9%.

As discussed above, in embodiments, sensitivity is the ability of a test method to make an assignment of a first state identity to all first state samples, in other words, to find or identify all first state samples. (Sensitivity does not address the propensity of a method to mis-assign a first state sample as a second state sample). In an embodiment the first state is negativity, and sensitivity is the ability to identify all negative samples. In an embodiment the first state is positivity, and sensitivity is the ability to identify all positive samples.

As used herein, the term "specificity" refers to the ability of a method to detect the presence of a disease in a subject (e.g., the specificity of a method can be described as the ability of the method to identify the true positive over true negative in a subject and/or to distinguish a truly occurring sequence variant from a sequencing artifact or other closely related sequences). For example, when used in reference to any of the variety of methods described herein that can detect the presence of cancer in a subject, a high specificity means that the method correctly identifies the absence of cancer in the subject a large percentage of the time (e.g., the method does not incorrectly identify the presence of cancer in the subject a large percentage of the time). A method has a specificity of X % if, when applied to a sample set of NTotal sequences, in which XTrue sequences are truly variant and XNot true are not truly variant, the method can select at least X% of the not truly variant as not variant. For example, a method has a specificity of 90% if, when applied to a sample set of 1,000 sequences, in which 500 sequences are truly variant and 500 are not truly variant, the method selects 90 % of the 500 not truly variant sequences as not variant. For example, a method described herein that correctly detects the absence of cancer in a subject 95% of the time the method is performed is said to have a specificity of 95%. In some embodiments, a method described herein that can detect the absence of cancer in a subject provides a specificity of at least 80% (e.g., at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or higher). A method having high specificity results in minimal or no false positive results (e.g., as compared to other methods). False positive results can arise from any source. For example, in various methods described herein that correctly detect the absence of cancer and include sequencing a nucleic acid, false positives can result from errors introduced into the sequence of interest during sample preparation, sequencing errors, and/or inadvertent sequencing of closely related sequences such as pseudo-genes or members of a gene family. In some embodiments, methods provided herein that include detecting the presence of one or more members of two or more classes of biomarkers (e.g., genetic biomarkers and/or protein

biomarkers) provide a higher specificity than methods that include detecting the presence of one or more members of only one class of biomarkers.

As discussed above, in embodiments, specificity is the ability of a test method to make a true assignment of a first state identity to a sample. (Specificity does not address the ability of the method to find all true first state samples, that is sensitivity). In an embodiment the first state is negativity, and specificity is the ability to make true (as opposed to incorrect) assignments of negativity (and not mis-assign second state (e.g., positive) samples as first state (negative) sample). In an embodiment the first state is positivity, and specificity is the ability to make true (as opposed to incorrect) assignments of positivity (and not mis-assign second state (e.g., negative) samples as first state (positive) samples).

As used herein, the phrase "subgenomic interval" refers to a portion of a genomic sequence. A subgenomic interval can be any appropriate size (e.g., can include any appropriate number of nucleotides). In some embodiments, a subgenomic interval can include a single nucleotide (e.g., single nucleotide for which variants thereof are associated (positively or negatively) with a tumor phenotype). In some embodiments, a subgenomic interval can include more than one nucleotide. For example, a subgenomic interval can include at least about 2 (e.g., about 5, about 10, about 50, about 100, about 150, about 250, or about 300) nucleotides. In some cases, a subgenomic interval can include an entire gene. In some cases, a subgenomic interval can include a portion of gene (e.g., a coding region such as an exon, a non-coding region such as an intron, or a regulatory region such as a promoter, enhancer, 5' untranslated region (5' UTR), or 3' untranslated region (3' UTR)). In some cases, a subgenomic interval can include all or part of a naturally occurring (e.g., genomic) nucleotide sequence. For example, a subgenomic interval can correspond to a fragment of genomic DNA which can be subjected to a sequencing reaction. In some cases, a subgenomic interval can be a continuous nucleotide sequence from a genomic source. In some cases, a subgenomic interval can include nucleotide sequences that are not contiguous within the genome. For example, a subgenomic interval can include a nucleotide sequence that includes an exon-exon junction (e.g., in cDNA reverse transcribed from the subgenomic interval). In some cases, a subgenomic interval can include a mutation (e.g., a SNV, an SNP, a somatic mutation, a germ line mutation, a point mutation, a rearrangement, a deletion mutation (e.g., an in-frame deletion, an intragenic deletion, or a full gene deletion), an insertion mutation (e.g., an intragenic insertion), an inversion mutation (e.g., an intra-

chromosomal inversion), an inverted duplication mutation, a tandem duplication (e.g., an intrachromosomal tandem duplication), a translocation (e.g., a chromosomal translocation, or a non-reciprocal translocation), a change in gene copy number, or any combination thereof.

As used herein, the phrase "leukocyte parameter," refers to the sequence of a leukocyte nucleic acid, e.g., a chromosomal nucleic acid.

As used herein, the phrase "genomic event," refers to a sequence of a subgenomic interval that differs from the sequence of a reference sequence. A genomic event can be, e.g., a mutation, e.g., a point mutation or a rearrangement, e.g., a translocation.

Aneuploidy detection

This document provides methods and materials for identifying one or more chromosomal anomalies (e.g., aneuploidies) in a sample. In some embodiments, methods and materials described herein are used to identify one or more chromosomal anomalies (e.g., aneuploidies) in an embryo. In some embodiments, methods and materials described herein are used to identify one or more chromosomal anomalies (e.g., aneuploidies) in a mammal (e.g., a juvenile mammal or an adult mammal). For example, a mammal (e.g., a sample obtained from a mammal) can be assessed for the presence or absence of one or more chromosomal anomalies. In some cases, this document provides methods and materials for using amplicon-based sequencing data to identify a mammal as having a disease associated with one or more chromosomal anomalies (e.g., cancer). For example, methods and materials described herein can be applied to a sample obtained from a mammal to identify the mammal as having one or more chromosomal anomalies. For example, methods and materials described herein can be applied to a sample obtained from a mammal to identify the mammal as having a disease associated with one or more chromosomal anomalies (e.g., cancer). This document also provides methods and materials for identifying and/or treating a disease or disorder associated with one or more chromosomal anomalies (e.g., one or more chromosomal anomalies identified as described herein). In some cases, one or more chromosomal anomalies can be identified in DNA (e.g., genomic DNA) obtained from a sample obtained from a mammal. For example, a prenatal mammal (e.g., prenatal human)

can be identified as having a disease or disorder based, at least in part, on the presence of one or more chromosomal anomalies. In some embodiments, a mammalian embryo identified as having a disease or disease based, at least in part, on one or more chromosomal abnormalities can be assessed for the purposes of in vitro fertilization. In some embodiments, a mammal identified as having cancer based, at least in part, on the presence of one or more chromosomal anomalies can be treated with one or more cancer treatments. In some embodiments, a mammal can be identified as having congenital abnormalities based, at least in part, on the presence of one or more chromosomal abnormalities. In some embodiments, methods and materials provided herein are used to test an embryo (e.g., an embryo generated by in vitro fertilization) for chromosomal abnormalities prior to transfer to the uterus (e.g., a human uterus) for implantation.

Disclosed herein, *inter alia*, is a method of increasing the sensitivity of detecting one or more cancers, or a plurality of cancers, without altering the specificity of detecting said cancer or a plurality of cancers. In an embodiment, the sensitivity of detection of a cancer by evaluating (i) a genetic biomarker, e.g. a somatic mutation; (ii) a protein biomarker; and (iii) aneuploidy status, is higher, e.g., about 1.1, 1.2, 1.3, 1.4, 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 10 fold higher, than the sensitivity of detection of the cancer by evaluating (i) alone; (ii) alone; (iii) alone; (i) and (ii) only; (i) and (iii) only; or (ii) and (iii) only. The increase in sensitivity by a method comprising (i), (ii) and (iii) does not alter, e.g., reduce the specificity of detecting the cancer, or plurality of cancers. Exemplary increase in sensitivity of cancer detection using the method of the disclosure is demonstrated in Example 6 of this disclosure.

Any appropriate mammal can be assessed as described herein. A mammal can be a prenatal mammal (e.g., prenatal human). A mammal can be a mammal suspected of having a disease associated with one or more chromosomal anomalies (e.g., cancer or a congenital abnormality). In some cases, humans or other primates such as monkeys can be assessed for the presence of one or more chromosomal anomalies as described herein. In some cases, dogs, cats, horses, cows, pigs, sheep, mice, and rats can be assessed for the presence of one or more chromosomal anomalies as described herein. For example, a human can be assessed for the presence of one or more chromosomal anomalies as described herein.

Any appropriate sample from a mammal can be assessed as described herein (e.g., assessed for the presence of one or more chromosomal anomalies). A sample can include

genomic DNA. In some cases, a sample can include cell-free circulating DNA (e.g., cell-free circulating fetal DNA). In some cases, a sample can include circulating tumor DNA (ctDNA). Examples of samples that can contain DNA (e.g., ctDNA) include, without limitation, blood (e.g., whole blood, serum, or plasma), amnion, tissue, urine, cerebrospinal fluid, saliva, sputum, broncho-alveolar lavage, bile, lymphatic fluid, cyst fluid, stool, ascites, pap smears, cerebral spinal fluid, endo-cervical, endometrial, and fallopian samples. For example, a sample can be a plasma sample. For example, a sample can be a urine sample. For example, a sample can be a saliva sample. For example, a sample can be a cyst fluid sample. For example, a sample can be a sputum sample. In some cases, a sample can include a neoplastic cell fraction (e.g., a low neoplastic cell fraction).

In some embodiments, a sample can be processed to isolate and/or purify DNA from the sample. In some embodiments, DNA isolation and/or purification can include cell lysis (e.g., using detergents and/or surfactants). In some embodiments, further processing of DNA (e.g., an amplification reaction) is performed without purifying DNA from the cell lysis. In such cases, additional reagents are added to facilitate further processing including, without limitation, protease inhibitors. In some embodiments, DNA isolation and/or purification can include removing proteins (e.g., using a protease). In some cases, DNA isolation and/or purification can include removing RNA (e.g., using an RNase). In some embodiments, DNA isolation is performed using commercially available kits (for example, without limitation, Qiagen DNAeasy kit) or buffers known in the art (e.g., detergents in Tris-buffer).

In some embodiments, the amount DNA inputted ("input DNA") into the isolation and/or purification reaction may vary depending on a variety of factors including, without limitation, average length of DNA fragments, overall DNA quality, and/or type of DNA (e.g., gDNA, mitochondrial DNA, cfDNA). In some embodiments, any suitable amount of input DNA can be used in the methods described herein. In some embodiments, the amount of input DNA can be any amount from 1 picogram (pg) to 500 pg. In some embodiments, the amount of input DNA can be at least 0.01 pg, at least .01 pg, at least 0.1 pg or at least 1 pg. In some embodiments, the amount of input DNA can be at least 1 picogram (pg), at least 2 pg, at least 3 pg, at least 4 pg, at least 5 pg, at least 6 pg, at least 7 pg, at least 8 pg, at least 9 pg at least 10pg, at least 11 pg, at least 12 pg, at least 13 pg, at least 14 pg, at least 15 pg, at least 16 pg, at least 17 pg, at least 18 pg , at least 19 pg, at least 20 pg, at least 21 pg, at least 22 pg, at least 23 pg, at least 24 pg, at least 25 pg, at least 26 pg, at least 27 pg, at least 28 pg,

at least 29 pg, at least 30 pg, at least 31 pg, at least 32 pg, at least 33 pg, at least 34 pg, at least 35 pg, at least 36 pg, at least 37 pg, at least 38 pg, at least 39 pg or at least 40 pg. In some embodiments, the amount of input DNA is 3 pg.

In some embodiments, methods and materials for identifying one or more chromosomal anomalies (e.g., aneuploidies) as described herein can include amplification of a plurality of amplicons. In some embodiments, the plurality of amplicons is amplified from a plurality of chromosomal sequences in a DNA sample. In some embodiments, the plurality of amplicons can be amplified from any variety of repetitive elements (see e.g., Table 1 for a list of repetitive elements). In some embodiments, the plurality of amplicons is amplified from a plurality of short interspersed nucleotide elements (SINEs). In some embodiments, the plurality of amplicons is amplified from a plurality of long interspersed nucleotide elements (LINEs). Methods of amplifying a plurality of amplicons include, without limitation, the polymerase chain reaction (PCR) and isothermal amplification methods (e.g., rolling circle amplification or bridge amplification). In some embodiments, a second amplification step is performed. In some embodiments, the amplified DNA from a first amplification reaction is used as a template in a second amplification reaction. In some embodiments, the amplified DNA is purified before the second amplification reaction (e.g., PCR purification using methods known in the art).

In some embodiments, an amplification reaction includes using a single pair of primers comprising a first primer having or including SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 9. In some embodiments, an amplification reaction includes using a single pair of primers comprising a first primer having at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 96 %, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 9. In some embodiments, an amplification reaction includes using a single pair of primers comprising a second primer having or including SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19. In some embodiments, an amplification reaction includes using a single pair of primers comprising a second primer having at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 96 %, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 10, SEQ ID

NO: 11, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19.

In some embodiments, the first primer has a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95% at least 99%, or 100% identical) to CGACGTAAAACGACGGCCAGTNNNNNNNNNNNNNNNNGGTGAAACCCCGTCTCTACA (SEQ ID NO: 1). In some embodiments, the second primer has a sequence that is at least 80% identical (e.g., at least 85%, at least 90%, at least 95% at least 99%, or 100% identical) to CACACAGGAAACAGCTATGACCATGCCTCCTAAGTAGCTGGGACTACAG (SEQ ID NO: 10). In some embodiments, an amplification reaction includes using a single pair of primers comprising a first primer having SEQ ID NO. 1 and a second primer having SEQ ID NO. 10. In some embodiments, an amplification reaction includes using a single pair of primers comprising a first primer having at least 80% (e.g., at least 85%, a t least 90%, at least 95%, at least 96 %, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO. 1 and a second primer having at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 96 %, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO. 10.

In some embodiments, the first primer comprises from the 5' to 3' end: a universal primer sequence (UPS), a unique identifier DNA sequence (UID), and an amplification sequence. In some embodiments, the first primer comprises from the 5' to 3' end: a UPS sequence and an amplification sequence. In some embodiments, the first primer comprises from the 5' to 3' end: an amplification sequence. In such cases in which the first primer comprises at least an amplification sequence, any variety of library generation techniques known in the art can be used to generate a next generation sequencing library from the amplified amplicons.

In some embodiments, the universal primer sequence (UPS) facilitates the generation of a library of amplicons ready for next generation sequencing. For example, an amplicon generated during the amplification reaction using a first primer (SEQ ID NO. 1) and a second primer (SEQ ID NO. 10) is used as a template for a second amplification reaction. In such cases, a second set of primers designed to bind to the UPS includes the 5' grafting sequences necessary for hybridization to an Illumina flow cell.

In some embodiments, the UID comprises a sequence of 16-20 degenerate bases. In some embodiments, a degenerate sequence is a sequence in which some positions of a nucleotide sequence contain a number of possible bases. In some embodiments of any of the methods described herein, a degenerate sequence can be a degenerate nucleotide sequence comprising about or at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 nucleotides. In some embodiments, a nucleotide sequence contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 0, 10, 15, 20, 25, or more degenerate positions within the nucleotide sequence. In some embodiments, the degenerate sequence is used as a unique identifier DNA sequence (UID). In some embodiments, the degenerate sequence is used to improve the amplification of an amplicon. For example, a degenerate sequence may contain bases complementary to a chromosomal sequence being amplified. In such cases, the increased complementarity may increase a primers affinity for the chromosomal sequence. In some embodiments, the UID (e.g., degenerate bases) is designed to increase a primers affinity to a plurality of chromosomal sequences.

In some embodiments, an amplification reaction includes one or more pairs of primers (e.g., one or more pairs of primers selected from Table 2). In some embodiments, an amplification reaction includes at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 pairs of primers. In some embodiments, when an amplification reaction includes more than one pair or primers, at least one pair of primers includes a primer having SEQ ID NO: 1 as a first primer and a primer having SEQ ID NO: 10 as a second primer. In some embodiments, when an amplification reaction includes more than one pair of primers, at least one pair of primers includes a first primer with a sequence having at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 96 %, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 1 and a second primer with a sequence having at least 80% (e.g., at least 85%, at least 90%, at least 95%, at least 96 %, at least 97%, at least 98%, or at least 99%) sequence identity to SEQ ID NO: 10.

In some embodiments when an amplification reaction includes one or more pairs of primers, any variety of combinations of primers or pairs of primers can be selected from Table 2. For example, an amplification reaction containing 2 pairs of primers (e.g., 4 primers selected from Table 2) can include a first pair of primers (e.g., a first primer pair 1 from Table 2) that includes a first primer (e.g., a first primer having SEQ ID NO: 1) and a second primer (e.g., a second primer having SEQ ID NO: 10) and a second pair of primers (e.g., a second

primer pair 2 from Table 2) that includes a third primer (e.g., a third primer having SEQ ID NO: 2) and a fourth primer (e.g., a fourth primer having SEQ ID NO: 11). Combining any of the forward primers listed in Table 2 (e.g., a "FP" having SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 9) with any of the reverse primers listed in Table 2 (e.g., a "RP" having SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19) will generate amplicons from the repetitive elements as described herein (see e.g., Table 1 for a list of exemplary repetitive elements). For example, an amplification reaction containing 2 pairs of primers (e.g., 4 primers selected from Table 2) can include a first pair of primers (e.g., a first primer pair 1 from Table 2) that includes a first primer (e.g., a first primer having SEQ ID NO: 1) and a second primer (e.g., a second primer having SEQ ID NO: 10) and a second pair of primers (e.g., not listed as a primer pair in Table 2) that includes a third primer (e.g., a third primer having SEQ ID NO: 2) and a fourth primer (e.g., a fourth primer having SEQ ID NO: 12). In some embodiments, an amplification reaction includes one or more pairs of primers where a first primer is included in both pairs of primers. For example, an amplification reaction can include a first pair of primers (e.g., a first primer pair 1 from Table 2) that includes a first primer (e.g., a first primer having SEQ ID NO: 1) and a second primer (e.g., a second primer having SEQ ID NO: 10) and a second pair of primers that includes a third primer (e.g., a third primer having SEQ ID NO: 1) and a fourth primer (e.g., a fourth primer having SEQ ID NO: 11).

In some embodiments, a pair of primers are complementary to a plurality of chromosomal sequences. As used herein, the term "complementary" or "complementarity" refers to nucleic acid residues that are capable or participating in Watson-Crick type or analogous base pair interactions that is enough to support amplification. In some embodiments, an amplification sequence of a first primer is designed to amplify one or more chromosomal sequences. In some embodiments, the one or more chromosomal sequence include any of a variety of repetitive elements as described herein (see e.g., Table 1 for a list of exemplary repetitive elements). In some embodiments, the chromosomal sequences are SINEs. In some embodiments, the chromosomal sequences are LINEs. In some embodiments, the chromosomal sequences are a mixture of different types of repetitive elements (e.g., SINEs, LINEs and/or other exemplary repetitive elements list in Table 1). In

some embodiments when an amplification reaction includes two or more pairs of primers, each pair of primers amplifies a different type of repetitive element (see, e.g., Table 1 for a list of exemplary repetitive elements). For example, a first pair of primers can amplify SINEs, and a second pair of primers can amplify LINEs. Optionally, a third, fourth, fifth, etc. pair of primers can amplify a third, fourth, fifth, etc. type of repetitive element (see, e.g., Table 1 for a list of additional exemplary repetitive elements). In some embodiments when an amplification reaction includes two or more pairs of primers, each pair of primers generates amplicons from the same type of repetitive element (see, e.g., Table 1 for a list of exemplary repetitive elements). For example, a first pair of primers can amplify SINEs, and a second pair of primers amplify SINEs. Optionally, a third, fourth, fifth, etc. pair of primers can amplify SINEs. In some embodiments when an amplification reaction includes two or more primer pairs, each pair of primers generates amplicons from a mixture of different types of repetitive elements (see e.g., Table 1 for a list of exemplary repetitive elements).

Table 1: List of exemplary repetitive elements

ACRO1_Satellite	ALR/Alpha_Satellite	(A)n_Simple	A-rich_Low	Arthur1A_DNA
Arthur1B_DNA	Arthur1_DNA	AT_rich	BLACKJACK_DNA	(CAAAAA)n_Simple
(CAAAA)n_Simple	(CAA)n_Simple	(CAAT)n_Simple	(CA)n_Simple	(CATA)n_Simple
(CATATA)n_Simple	(CATTC)n_Satellite	(CCCCAG)n_Simple	Charlie13a_DNA	Charlie15a_DNA
Charlie16a_DNA	Charlie17a_DNA	Charlie19a_DNA	Charlie1a_DNA	Charlie1b_DNA
Charlie1_DNA	Charlie21a_DNA	Charlie22a_DNA	Charlie23a_DNA	Charlie25_DNA
Charlie2b_DNA	Charlie4a_DNA	Charlie4z_DNA	Charlie5_DNA	Charlie6_DNA
Charlie7a_DNA	Charlie7_DNA	Charlie8_DNA	Cheshire_DNA	(C)n_Simple
C-rich_Low	(CTA)n_Simple	CT-rich_Low	ERV3-16A3_I-int	ERVL-B4-int_LTR
ERVL-E-int_LTR	ERVL-int_LTR	Eulor11_DNA	Eulor1_DNA	HERV16-int_LTR
HERV30-int_LTR	HERV3-int_LTR	HERV4_I-int	HERVE_a-int	HERVFH19-int_LTR
HERVH48-int_LTR	HERVH-int_LTR	HERVI-int_LTR	HERVK14C-int_LTR	HERVK3-int_LTR
HERVK-int_LTR	HERVL66-int_LTR	HERVL74-int_LTR	HERVL-int_LTR	HERVP71A-int_LTR
HSAT4_Satellite	HSMAR1_DNA	HSMAR2_DNA	HUERS-P1-int_LTR	HUERS-P3-int_LTR
Kanga1a_DNA	Kanga1d_DNA	Kanga1_DNA	Looper_DNA	LOR1b_LTR
LOR1-int_LTR	LTR10F_LTR	LTR12C_LTR	LTR15_LTR	LTR16A1_LTR

LTR16A2_LTR	LTR16A_LTR	LTR16B1_LTR	LTR16B_LTR	LTR16C_LTR
LTR16E1_LTR	LTR19B_LTR	LTR1B_LTR	LTR1D_LTR	LTR24_LTR
LTR25-int_LTR	LTR25_LTR	LTR27B_LTR	LTR27_LTR	LTR28_LTR
LTR32_LTR	LTR33C_LTR	LTR33_LTR	LTR34_LTR	LTR35B_LTR
LTR37B_LTR	LTR3B_	LTR40c_LTR	LTR43_LTR	LTR45C_LTR
LTR48B_LTR	LTR48_LTR	LTR49-int_LTR	LTR49_LTR	LTR57-int_LTR
LTR5B_LTR	LTR5_Hs	LTR64_LTR	LTR66_LTR	LTR67B_LTR
LTR6A_LTR	LTR71B_LTR	LTR72_LTR	LTR77_LTR	LTR78B_LTR
LTR78_LTR	LTR79_LTR	LTR80B_LTR	LTR81A_LTR	LTR81B_LTR
LTR81C_LTR	LTR81_LTR	LTR82A_LTR	LTR84b_LTR	LTR85a_LTR
LTR85b_LTR	LTR86A2_LTR	LTR87_LTR	LTR8A_LTR	LTR8_LTR
LTR9_LTR	MADE1_DNA	MADE2_DNA	MamGypLTR1b_LTR	MamGypLTR1c_LTR
MamRep1161_DNA	MamRep1527_LTR	MamRep38_DNA	MamRep434_DNA	MamRep564_Unknown
MARNA_DNA	MER101-int_LTR	MER102a_DNA	MER102b_DNA	MER102c_DNA
MER103C_DNA	MER106A_DNA	MER110A_LTR	MER110-int_LTR	MER113B_DNA
MER113_DNA	MER115_DNA	MER11D_LTR	MER121_DNA	MER135_DNA
MER1A_DNA	MER1B_DNA	MER20B_DNA	MER20_DNA	MER21B_LTR
MER21-int_LTR	MER2_DNA	MER30_DNA	MER31A_LTR	MER31B_LTR
MER31-int_LTR	MER33_DNA	MER34A1_LTR	MER34A_LTR	MER34B-int_LTR
MER34B_LTR	MER34C_	MER34C2_LTR	MER34D_LTR	MER34_LTR
MER39_LTR	MER3_DNA	MER41A_LTR	MER41B_LTR	MER41-int_LTR
MER44A_DNA	MER44C_DNA	MER45R_DNA	MER46C_DNA	MER49_LTR
MER4A1_	MER4A_LTR	MER4B-int_LTR	MER4B_LTR	MER4C_LTR
MER4D1_LTR	MER4D_LTR	MER4E1_LTR	MER4-int_LTR	MER50_LTR
MER51C_LTR	MER52A_LTR	MER52-int_LTR	MER53_DNA	MER54A_LTR
MER57A-int_LTR	MER57B2_LTR	MER57E3_LTR	MER57F_LTR	MER57-int_LTR
MER58A_DNA	MER58B_DNA	MER58C_DNA	MER58D_DNA	MER5A1_DNA
MER5A_DNA	MER5B_DNA	MER5C_DNA	MER61B_LTR	MER61-int_LTR
MER63C_DNA	MER63D_DNA	MER65A_LTR	MER65-int_LTR	MER66B_LTR
MER66-int_LTR	MER67B_LTR	MER67C_LTR	MER67D_LTR	MER68-int_LTR
MER6_DNA	MER70A_LTR	MER70B_LTR	MER74A_LTR	MER74B_LTR
MER74C_LTR	MER81_DNA	MER82_DNA	MER83B-int_LTR	MER87_LTR
MER89-int_LTR	MER89_LTR	MER8_DNA	MER92B_LTR	MER94_DNA
MER96B_DNA	MLT1A0_LTR	MLT1A1_LTR	MLT1A-int_LTR	MLT1A_LTR
MLT1B-int_LTR	MLT1B_LTR	MLT1C_LTR	MLT1D_LTR	MLT1E1A_LTR
MLT1E2_LTR	MLT1E3-int_LTR	MLT1E3_LTR	MLT1F2_LTR	MLT1F-int_LTR
MLT1F_LTR	MLT1G1-int_LTR	MLT1G1_LTR	MLT1G3_LTR	MLT1G-int_LTR
MLT1G_LTR	MLT1G_LTR	MLT1H_LTR	MLT1I_LTR	MLT1J1-int_LTR
MLT1J1_LTR	MLT1J2_LTR	MLT1J_LTR	MLT1K_LTR	MLT1L_LTR
MLT1M_LTR	MLT1N2_LTR	MLT2B1_LTR	MLT2B4_LTR	MLT2D_LTR
MSTA-int_LTR	MSTA_LTR	MSTB1_LTR	MSTB-int_LTR	MSTD-int_LTR
ORSL-2b_DNA	PABL_A-int	PABL_B-int	PRIMA41-int_LTR	PRIMA4-

				int_LTR
Ricksha_b	Ricksha_c	Ricksha_DNA	SATR1_Satellite	SVA_B
SVA_C	(TAAAA)n_Simple	(TAAA)n_Simple	(TAA)n_Simple	(TAG)n_Simple
(TA)n_Simple	(TCCA)n_Simple	(TCTCTG)n_Simple	(TG)n_Simple	THE1A-int_LTR
THE1B-int_LTR	THE1B_LTR	THE1C-int_LTR	THE1C_LTR	THE1D_LTR
Tigger10_DNA	Tigger12c_DNA	Tigger12_DNA	Tigger13a_DNA	Tigger15a_DNA
Tigger16b_DNA	Tigger1a_Art	Tigger1_DNA	Tigger2a_DNA	Tigger2b_Pri
Tigger2_DNA	Tigger3a_DNA	Tigger3b_DNA	Tigger3_DNA	Tigger4a_DNA
Tigger4b_DNA	Tigger4_DNA	Tigger6a_DNA	Tigger7_DNA	Tigger8_DNA
Tigger9b_DNA	UCON23_DNA?	Zaphod2_DNA	Zaphod3_DNA	Zaphod_DNA

In some embodiments, one or both primers of a primer pair described herein include primer modifications. Examples of primer modifications include, without limitation, a spacer (e.g., C3 spacer, PC spacer, hexanediol, spacer 9, spacer 18, 1',2'-dideoxyribose (dspacer)), phosphorylation, phosphorothioate bond modifications, modified nucleic acids, attachment chemistry and/or linker modifications. Examples of modified nucleic acids include, without limitation, 2-Aminopurine, 2,6-Diaminopurine (2-Amino-dA), 5-Bromo dU, deoxyUridine, Inverted dT, Inverted Dideoxy-T, Dideoxy-C, 5-Methyl dC, deoxyInosine, Super T®, Super G®, Locked Nucleic Acids (LNA's), 5-Nitroindole, 2'-O-Methyl RNA Bases, Hydroxymethyl dC, Iso-dG, Iso-dC, Fluoro C, Fluoro U, Fluoro A, Fluoro G, 2-MethoxyEthoxy A, 2-MethoxyEthoxy MeC, 2-MethoxyEthoxy G, and/or 2-MethoxyEthoxy T. Examples of attachment chemistries and linker modifications include, without limitation, AcryditeTM, Adenylation, Azide (NHS Ester), Digoxigenin (NHS Ester), Cholesterol-TEG, I-Linker, Amino Modifiers (e.g., amino modifier C6, amino nodifier C12, amino modifier C6 dT, amino modifier, and/or Uni-LinkTM amino modifier), Alkynes (e.g., 5' Hexynyl and/or 5-Octadiynyl dU), Biotinylation (e.g., biotin, biotin (Azide), biotin dT, biotin-TEG, dual biotin, pC biotin, and/or desthiobiotin-TEG), and/or Thiol Modifications (e.g., thiol modifier C3 S-S, dithiol, and/or thiol modifier C6 S-S). In some embodiments, any primer as described herein includes synthetic nucleic acids.

In some embodiments, one or both primers of a primer pair described herein include primer modifications that enhance processing of amplified DNA. In some embodiments, any primer as described herein includes primer modifications that facilitate elimination of primers (e.g., elimination of primers following an amplification reaction). In some

embodiments, primer modifications are conveyed to a product of an amplification reaction (e.g., an amplification product contains modified bases). In such cases, the amplification product includes the modification and the inherent properties of the modification (e.g., the ability to select the amplification product containing the modification).

In some embodiments, methods for identifying one or more chromosomal anomalies as described herein include using amplicon-based sequencing reads. In some embodiments, a plurality of amplicons (e.g., amplicons obtained from a DNA sample) are sequenced. In some embodiments, each amplicon is sequenced at least 1, 2,3,4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more times. In some embodiments, each amplicon can be sequenced between about 1 and about 20 (e.g., between about 1 and about 15, between about 1 and about 12, between about 1 and about 10, between about 1 and about 8, between about 1 and about 5, between about 5 and about 20, between about 7 and about 20, between about 10 and about 20, between about 13 and about 20, between about 3 and about 18, between about 5 and about 16, or between about 8 and about 12) times. In some cases, amplicon-based sequencing reads can include continuous sequencing reads. In some cases, amplicons include short interspersed nucleotide elements (SINEs). In some cases, amplicon-based sequencing reads can include from about 100,000 to about 25 million (e.g., from about 100,000 to about 20 million, from about 100,000 to about 15 million, from about 100,000 to about 12 million, from about 100,000 to about 10 million, from about 100,000 to about 5 million, from about 100,000 to about 1 million, from about 100,000 to about 750,000, from about 100,000 to about 500,000, from about 100,000 to about 250,000, from about 250,000 to about 25 million, from about 500,000 to about 25 million, from about 750,000 to about 25 million, from about 1 million to about 25 million, from about 5 million to about 25 million, from about 10 million to about 25 million, from about 15 million to about 25 million, from about 200,000 to about 20 million, from about 250,000 to about 15 million, from about 500,000 to about 10 million, from about 750,000 to about 5 million, or from about 1 million to about 2 million) sequencing reads. For example, sequencing a plurality of amplicons can include assigning a unique identifier (UID) to each template molecule (e.g., to each amplicon), amplifying each uniquely tagged template molecule to create UID-families, and redundantly sequencing the amplification products. For example, sequencing a plurality of amplicons can include calculating a Z-score of a variant on said selected chromosome arm

using the equation $Z \sim \frac{\sum_{i=1}^{k} w_i Z_i}{\sqrt{\sum_{i=1}^{k} w_i^2}}$, where w_i is UID depth at a variant i, Z_i is the Z-score of variant i , and k is the number of variants observed on the chromosome arm. In some embodiments, methods of sequencing amplicons includes methods known in the art (see, e.g., US Pat. No. 2015/0051085; and Kinde et al. 2012 *PloS ONE* 7:e41162, which are herein incorporated by reference in their entireties). In some embodiments, amplicons are aligned to a reference genome (e.g., GRC37).

In some embodiments, a plurality of amplicons generated by methods described herein includes from about 10,000 to about 1,000,000 (e.g., from about 15,000 to about 1,000,000, from about 25,000 to about 1,000,000, from about 35,000 to about 1,000,000, from about 50,000 to about 1,000,000, from about 75,000 to about 1,000,000, from about 100,000 to about 1,000,000, from about 125,000 to about 1,000,000, from about 160,000 to about 1,000,000, from about 180,000 to about 1,000,000, from about 200,000 to about 1,000,000, from about 300,000 to about 1,000,000, from about 500,000 to about 1,000,000, from about 750,000 to about 1,000,000, from about 10,000 to about 800,000, from about 10,000 to about 500,000, from about 10,000 to about 250,000, from about 10,000 to about 150,000, from about 10,000 to about 100,000, from about 10,000 to about 75,000, from about 10,000 to about 50,000, from about 10,000 to about 40,000, from about 10,000 to about 30,000, or from about 10,000 to about 20,000) amplicons (e.g., unique amplicons). As one non-limiting example, a plurality of amplicons can include about 745,000 amplicons (e.g., 745,000 unique amplicons). Amplicons in a plurality of amplicons can include from about 50 to about 140 (e.g., from about 60 to about 140, from about 76 to about 140, from about 90 to about 140, from about 100 to about 140, from about 130 to about 140, from about 50 to about 130, from about 50 to about 120, from about 50 to about 110, from about 50 to about 100, from about 50 to about 90, from about 50 to about 80, from about 60 to about 130, from about 70 to about 125, from about 80 to about 120, or from about 90 to about 100) nucleotides. As one non-limiting example, an amplicon can include about 100 nucleotides.

In some embodiments, one or more amplicons in a plurality of amplicons generated by methods described herein can be greater than 1000 basepairs (bp) in length ("long amplicons"). In some embodiments, one or more long amplicons make up at least 4.0% of all amplicons within the total plurality of amplicons. In some embodiments, methods and

materials described herein can detect long amplicons when the long amplicons make up at least 4.0% of all the amplicons within the total plurality of amplicons. In some embodiments, methods and materials described herein can detect long amplicons when the long amplicons make up between 0.01% and 3.9% of all amplicons within the total plurality of amplicons.

In some embodiments, one or more amplicons with a length >1000bp originate from amplification of DNA from cells that do not contain a chromosomal abnormality. In some embodiments, cells that do not contain chromosomal abnormalities are considered contaminating cells. In some embodiments, cells that do not contain chromosomal abnormalities are used as control cells or samples. In some embodiments, contaminating cells can be any variety of cells that might be found in a plasma sample that may dilute amplification of the intended target. In some embodiments, contaminating cells are white blood cells (e.g., leukocyte, granulocyte, eosinophil, basophile, B-cell, T-cell or Natural Killer cell). For example, contaminating cells can be leukocytes.

In some embodiments, methods and materials for identifying one or more chromosomal anomalies as described herein include grouping sequencing reads (e.g., from a plurality of amplicons) into clusters (e.g., unique clusters) of genomic intervals. In some embodiments, a genomic interval is included in one or more clusters. In some embodiments, a genomic interval can belong to from about 100 to about 252 (e.g., from about 125 to about 252, from about 150 to about 252, from about 175 to about 252, from about 200 to about 252, from about 225 to about 252, from about 100 to about 250, from about 100 to about 225, from about 100 to about 200, from about 100 to about 175, from about 100 to about 150, from about 125 to about 225, from about 150 to about 200, or from about 160 to about 180) clusters. As one non-limiting example, a genomic interval can belong to about 176 clusters. In some embodiments, each cluster includes any appropriate number of genomic intervals. In some embodiments, each cluster includes the same number of genomic intervals. In some embodiments, different clusters include varying numbers of genomic clusters. As one non-limiting example, each cluster can include about 200 genomic intervals.

In some embodiments, genomic intervals are identified as having shared amplicon features. As used herein, the term “shared amplicon feature” refers to amplicons with one or more features that are similar. In some embodiments, a plurality of genomic intervals are grouped into a cluster based on one or more shared amplicon features of the sequencing reads

mapped to a genomic interval. In some embodiments, the shared amplicon feature is the number amplicons mapped to a genomic interval (e.g., sums of the distributions of the sequencing reads in each genomic interval). In some embodiments, the shared amplicon feature is the average length of the mapped amplicons.

In some embodiments, a cluster of genomic intervals includes from about 5000 to about 6000 (e.g., from about 5100 to about 6000, from about 5200 to about 6000, from about 5300 to about 6000, from about 5400 to about 6000, from about 5500 to about 6000, from about 5600 to about 6000, from about 5700 to about 6000, from about 5800 to about 6000, from about 5900 to about 6000, from about 5000 to about 5900, from about 5000 to about 5800, from about 5000 to about 5700, from about 5000 to about 5600, from about 5000 to about 5500, from about 5000 to about 5400, from about 5000 to about 5300, from about 5000 to about 5200, from about 5000 to about 5100, from about 5100 to about 5800, from about 5100 to about 5700, from about 5100 to about 5600, from about 5100 to about 5500, from about 5100 to about 5400, from about 5100 to about 5300, from about 5100 to about 5200, from about 5200 to about 5600, from about 5200 to about 5500, from about 5200 to about 5400, from about 5200 to about 5300, from about 5300 to about 5500, from about 5300 to about 5400, or from about 5400 to 5500 from about 5200 to about 5700, or from about 5300 to about 5500) genomic intervals. As one non-limiting example, a cluster of genomic intervals can include about 5344 genomic intervals. A genomic interval can be any appropriate length. For example, a genomic interval can be the length of an amplicon sequenced as described herein. For example, a genomic interval can be the length of a chromosome arm. In some cases, a genomic interval can include from about 100 to about 125,000,000 (e.g., from about 250 to about 125,000,000, from about 500 to about 125,000,000, from about 750 to about 125,000,000, from about 1,000 to about 125,000,000, from about 1,500 to about 125,000,000, from about 2,000 to about 125,000,000, from about 5,000 to about 125,000,000, from about 7,500 to about 125,000,000, from about 10,000 to about 125,000,000, from about 25,000 to about 125,000,000, from about 50,000 to about 125,000,000, from about 100,000 to about 125,000,000, from about 250,000 to about 125,000,000, from about 500,000 to about 125,000,000, from about 100 to about 1,000,000, from about 100 to about 750,000, from about 100 to about 500,000, from about 100 to about 250,000, from about 100 to about 100,000, from about 100 to about 50,000, from about 100 to about 25,000, from about 100 to about 10,000, from about 100 to about 5,000, from about

100 to about 2,500, from about 100 to about 1,000, from about 100 to about 750, from about 100 to about 500, from about 100 to about 250, from about 500 to about 1,000,000, from about 5000 to about 900,000, from about 50,000 to about 800,000, or from about 100,000 to about 750,000) nucleotides. As one non-limiting example, a genomic interval can include about 500,000 nucleotides. In some embodiments, clusters of genomic intervals are formed using any appropriate method known in the art. In some embodiments, clusters of genomic intervals are formed based on shared amplicon features of the genomic intervals (see, e.g., Douville et al. PNAS 201 115(8):1871-1876, which is herein incorporated by reference in its entirety).

In some embodiments, methods and materials described herein for identifying one or more chromosomal anomalies include assessing a genome (e.g., a genome of a mammal) for the presence or absence of one or more chromosomal anomalies (e.g., aneuploidies). The presence or absence of one or more chromosomal anomalies in the genome of a mammal can, for example, be determined by sequencing a plurality of amplicons obtained from a sample (e.g., a test sample) obtained from the mammal to obtain sequencing reads, and grouping the sequencing reads into clusters of genomic intervals. In some cases, read counts of genomic intervals can be compared to read counts of other genomic intervals within the same sample. In some cases where read counts of genomic intervals are compared to read counts of other genomic intervals within the same sample, a second (e.g., control or reference) sample is not assayed. In some cases, read counts of genomic intervals can be compared to read counts of genomic intervals in another sample. For example, when using methods and materials described herein to identify genetic relatedness, polymorphisms (e.g., somatic mutations), and/or microsatellite instability, genomic intervals can be compared to read counts of genomic intervals in a reference sample. A reference sample can be a synthetic sample. A reference sample can be from a database. In some cases where methods and materials described herein are used to identify anomalies (e.g., aneuploidies), a reference sample can be a normal sample obtained from the same cancer patient (e.g., a sample from the cancer patient that does not harbor cancer cells) or a normal sample from another source (e.g., a patient that does not have cancer). In some cases where method and materials described herein are used to identify anomalies (e.g., aneuploidies), a reference sample can be a normal sample obtained from the same patient (e.g., a sample from pre-natal human that contains only maternal cells).

In some embodiments, methods and materials described herein are used for detecting aneuploidy in a preimplantation embryo (e.g., an embryo generated via in vitro fertilization). In some embodiments, the presence or absence of one or more chromosomal anomalies in a preimplantation embryo is determined by sequencing a plurality of amplicons obtained from a sample taken from the preimplantation embryo (e.g., a test sample such, as without limitation, one or more cells obtained from a blastocyst) to obtain sequencing reads, and grouping the sequencing reads into clusters of genomic intervals. In some cases, read counts of genomic intervals can be compared to read counts of other genomic intervals within the same sample. In some cases where read counts of genomic intervals are compared to read counts of other genomic intervals within the same sample, a second (e.g., control or reference) sample is not assayed. In some cases, read counts of genomic intervals can be compared to read counts of genomic intervals in another sample (e.g., a reference sample). In some embodiments, a reference sample is a sample obtained from a reference mammal. In some embodiments, a reference sample is obtained from a database (e.g., the reference sample is an in silico sample having a known sequence and/or ploidy at the genomic position of interest). Exemplary aneuploidies that can be detected in preimplantation embryos include trisomies at chromosome 21 (e.g., resulting in Down's Syndrome), trisomies at chromosome 13, trisomies at chromosome 18, Turner Syndrome (e.g., women with only one X chromosome) and Klinefelter Syndrome (e.g., men with two or more X chromosomes).

In some embodiments, methods and materials described herein are used for detecting aneuploidy in a genome of mammal. For example, a plurality of amplicons obtained from a sample obtained from a mammal can be sequenced, the sequencing reads can be grouped into clusters of genomic intervals, the sums of the distributions of the sequencing reads in each genomic interval can be calculated, a Z-score of a chromosome arm can be calculated, and the presence or absence of an aneuploidy in the genome of the mammal can be identified. The distributions of the sequencing reads in each genomic interval can be summed. For example, sums of distributions of the sequencing reads in each genomic interval can be calculated using the equation $\sum_1^I R_i \sim N(\sum_1^I \mu_i, \sum_1^I \sigma_i^2)$, where R_i is the number of sequencing reads, I is the number of clusters on a chromosome arm, N is a Gaussian distribution with parameters μ_i and σ_i^2, μ_i is the mean number of sequencing reads in each genomic interval, and σ_i^2 is the variance of sequencing reads in each genomic interval. A Z-score of a chromosome

arm can be calculated using any appropriate technique. For example, a Z-score of a chromosome arm can be calculated using the quantile function 1-CDF($\sum_1^l \mu_i, \sum_1^l \sigma_i^2$). The presence of an aneuploidy in the genome of the mammal can be identified in the genome of the mammal when the Z-score is outside a predetermined significance threshold, and the absence of an aneuploidy in the genome of the mammal can be identified in the genome of the mammal when the Z-score is within a predetermined significance threshold. The predetermined threshold can correspond to the confidence in the test and the acceptable number of false positives. For example, a significance threshold can be ± 1.96, ± 3, or ± 5. In some embodiments, methods and materials described herein employ supervised machine learning. In some embodiments, supervised machine learning can detect small changes in one or more chromosome arms. For example, supervised machine learning can detect changes such as chromosome arm gains or losses that are often present in a disease or disorder associated with chromosomal anomalies, such as cancer or congenital anomalies. In some embodiments, supervised machine learning can detect changes such as chromosome arm gains or losses that are present in a preimplantation embryo (e.g., a preimplantation embryo generated by in vitro fertilization methods). In some cases, supervised machine learning can be used to classify samples according to aneuploidy status. For example, supervised machine learning can be employed to make genome-wide aneuploidy calls. In some cases, a support vector machine model can include obtaining an SVM score. An SVM score can be obtained using any appropriate technique. In some cases, an SVM score can be obtained as described elsewhere (see, e.g., Cortes 1995 *Machine learning* 20:273-297; and Meyer et al. 2015 *R package version*:1.6-3). At lower read depths, a sample will typically have a higher raw SVM score. Thus, in some cases, raw SVM probabilities can be corrected based on the read depth of a sample using the equation $\log\left(1-\frac{1}{r}\right) = Ax + B$, where r is the ratio of the SVM score at a particular read depth/minimum SVM score of a particular sample given sufficient read depth. A and B can be determined as described in Example 1. For example, A = -7.076*10^-7, x = the number of unique template molecules for the given sample, and B = -1.946*10^-1.

Also provided herein are new methods of normalization that reduce the amount of variability between samples. In some embodiments, a principal component analysis (PCA) can be used for normalization. In some embodiments, a PCA is performed on sequencing

data from the controls. For example, a PCA may reduce the number of 500 kb genomic intervals from n=5,344 to a more manageable number of dimensions. Using the PCA coordinates of the controls, a model can be generated that predicts whether a particular 500kb interval will be amplified more or less efficiently in future samples based on their PCA coordinates.

$$Correction\ Factor\ for\ 500kb\ Interval_i = \beta_{0i} + \beta_{1i} * PCA_1 + \beta_{2i} * PCA_2 + \beta_{3i} * PCA_3 + \beta_{4i} * PCA_4 + \beta_{5i} * PCA_5$$

For example, for each test sample, a sample can be projected into PCA space and the correction factor can be calculated for each 500kb interval as function of its PCA coordinates. After applying the correction factor to each 500 kb genomic interval, the test sample may be matched to one or more control samples based on the closest Euclidean distance of the 500 kb intervals.

In some embodiments, samples are excluded in order to ensure the quality of the data. In some embodiments, samples are excluded before, contemporaneously with, and/or after data analysis. In some embodiments, a list of factors can be applied to the data in order to exclude data that does not meet the criteria set forth in the list of factors. In some embodiments, the list of factors may be any reasonable number of factors. For example, a list of five factors can be used to exclude samples. Any combination of factors can be used to determine that a sample should be excluded. In some embodiments, samples with fewer than 2.5M reads may be excluded. In some embodiments, samples with sufficient evidence of contamination may be excluded. For example, a sample may be considered contaminated if the sample has at least 10 significant allelic imbalanced chromosome arms (z score >= 2.5) and fewer than ten significant chromosome arms gains or losses (z >= 2.5 or z<= -2.5). In some embodiments, allelic imbalance can be determined from SNPs, while gains or losses can be assessed through WALDO. In some embodiments, when examining the quality of the plasma samples, samples may be excluded in which more than 8.5% of the amplicons were larger than 94 bps (50 base pairs between the forward and reverse primers). Without wishing to be bound by theory, such samples may be contaminated with leukocyte DNA. In some embodiments, samples outside the dynamic range of the assay, as defined by the equation below, may be excluded.

$$QC\ Dynamic\ Range\ Metric = \sum_{i}^{2q,3q,4q,5q,6q,8q,13q} \frac{Reads\ on\ chr_i}{\sum_{j=1}^{38} Reads\ on\ chr_j}$$

For example, the distribution of this metric has long tails. The values of >0.2450 and 0.2320 may be selected as a dynamic range that could evaluate cutoffs. In some embodiments, plasma samples with known aneuploidy in the leukocytes of the same patients may be excluded. For example, such patients may have Clonal Hematopoiesis of Indeterminate Potential (CHIP) or congenital disorders.

In some embodiments, provided herein are methods to detect copy number variants (CNVs) of indeterminate length. In some embodiments, provided herein are methods to detect copy number variation of near-fixed length. In some embodiments, detecting copy number variation include calculating the values of one or more variables. In some embodiments, using a log ratio of the observed test sample and WALDO predicted values from every 500 kb interval across each chromosomal arm, a circular binary segmentation algorithm can be applied to determine copy number variants throughout each chromosome arm. For example, copy number variant ≤ 5Mb in size can be flagged. In some embodiments, the flagged CNVs can be removed before, contemporaneously with, and/or after the analysis. In some embodiments, small CNVs may be used to assess microdeletions or microamplifications. For example, microdelections or microamplifications occur in DiGeorge Syndrome (chromosome 22q11.2 or in breast cancers (chromosome 17q12).

In some embodiments, provided herein are methods of using synthetic aneuploid samples. In some embodiments, synthetic aneuploidy samples can be created by adding (or subtracting) reads from several chromosome arms to the reads from these normal DNA samples. For example, reads can be added or subtracted from 1, 10, 15, or 20 chromosome arms to each sample. The additions and subtractions can be designed to represent neoplastic cell fractions ranging from 0.5% to 1.5% and resulted in synthetic samples containing exactly ten million reads. The reads from each chromosome arm can be added or subtracted uniformly. In some embodiments, provided herein are methods of generating synthetic aneuploid samples using exemplary pseudocode (Figure 5). In some embodiments, a person of ordinary skill in the art will be able to generate a synthetic sample by applying known coding languages and techniques to the exemplary pseudocode shown in Figure 5.

Examples of chromosomal anomalies that can be detected using methods and materials described herein include, without limitation, numerical disorders, structural abnormalities, allelic imbalances, and microsatellite instabilities. A chromosomal anomaly can include a numerical disorder. For example, a chromosomal anomaly can include an aneuploidy (e.g., an abnormal number of chromosomes). In some cases, an aneuploidy can include an entire chromosome. In some cases, an aneuploidy can include part of a chromosome (e.g., a chromosome arm gain or a chromosome arm loss). Examples of aneuploidies include, without limitation, monosomy, trisomy, tetrasomy, and pentasomy. A chromosomal anomaly can include a structural abnormality. Examples of structural abnormalities include, without limitation, deletions, duplications, translocations (e.g., reciprocal translocations and Robertsonian translocations), inversions, insertions, rings, and isochromosomes. Chromosomal anomalies can occur on any chromosome pair (e.g., chromosome 1, chromosome 2, chromosome 3, chromosome 4, chromosome 5, chromosome 6, chromosome 7, chromosome 8, chromosome 9, chromosome 10, chromosome 11, chromosome 12, chromosome 13, chromosome 14, chromosome 15, chromosome 16, chromosome 17, chromosome 18, chromosome 19, chromosome 20, chromosome 21, chromosome 22, and/or one of the sex chromosomes (e.g., an X chromosome or a Y chromosome). For example, aneuploidy can occur, without limitation, in chromosome 13 (e.g., trisomy 13), chromosome 16 (e.g., trisomy 16), chromosome 18 (e.g., trisomy 18), chromosome 21 (e.g., trisomy 21), and/or the sex chromosomes (e.g., X chromosome monosomy; sex chromosome trisomy such as XXX, XXY, and XYY; sex chromosome tetrasomy such as XXXX and XXYY; and sex chromosome pentasomy such as XXXXX, XXXXY, and XYYYY). For example, structural abnormalities can occur, without limitation, in chromosome 4 (e.g., partial deletion of the short arm of chromosome 4), chromosome 11 (e.g., a terminal 11q deletion), chromosome 13 (e.g., Robertsonian translocation at chromosome 13), chromosome 14 (e.g., Robertsonian translocation at chromosome 14), chromosome 15 (e.g., Robertsonian translocation at chromosome 15), chromosome 17 (e.g., duplication of the gene encoding peripheral myelin protein 22), chromosome 21 (e.g., Robertsonian translocation at chromosome 21), and chromosome 22 (e.g., Robertsonian translocation at chromosome 22).

In some embodiments, methods and materials as described herein are used for identifying and/or treating a disease associated with one or more chromosomal anomalies

(e.g., one or more chromosomal anomalies identified as described herein, such as, without limitation, an aneuploidy). In some cases, a DNA sample (e.g., a genomic DNA sample) obtained from a mammal can be assessed for the presence or absence of one or more chromosomal anomalies. For example, a mammal (e.g., a human) can be identified as having a disease based, at least in part, on the presence of one or more chromosomal anomalies can be treated with one or more cancer treatments. In some embodiments, a mammal identified as having cancer based, at least in part, on the presence of one or more chromosomal anomalies is treated with one or more cancer treatments. In some embodiments, a mammal (e.g., a prenatal human) can be identified as having a disease or disorder based, at least in part, on the presence of one or more chromosomal anomalies. In some embodiments, an embryo (e.g., an embryo generated by in vitro fertilization) can be identified as being unsuitable for to transfer to the uterus (e.g., a human uterus) for implantation based, at least in part, on the presence of one or more chromosomal anomalies. In some embodiments, an embryo (e.g., an embryo generated by in vitro fertilization) can be identified as being suitable for to transfer to the uterus (e.g., a human uterus) for implantation based, at least in part, on the absence of one or more chromosomal anomalies.

In some embodiments, a mammal identified as having a disease or disorder associated with one or more chromosomal anomalies as described herein (e.g., based at least in part on the presence of one or more chromosomal anomalies, such as, without limitation, an aneuploidy) can have the disease or disorder diagnosis confirmed using any appropriate method. Examples of methods that can be used to confirm the presence of one or more chromosomal anomalies include, without limitation, karyotyping, fluorescence in situ hybridization (FISH), quantitative PCR of short tandem repeats, quantitative fluorescence PCR (QF-PCR), quantitative PCR dosage analysis, quantitative mass spectrometry of SNPs, comparative genomic hybridization (CGH), whole genome sequencing, and exome sequencing.

Multi-analyte test for cancer detection

In some embodiments, detection of aneuploidy is used to identify a mammal as having cancer (e.g., any of the exemplary cancers described herein). In some embodiments, detection of one or more genetic biomarkers is used to confirm or identify a mammal as having cancer (e.g., any of the exemplary cancers described herein). In some embodiments,

an elevated level of one or more peptide biomarkers is used to confirm or identify a mammal as having cancer (e.g., any of the exemplary cancers described herein). In some embodiments, a mammal identified as having cancer as described herein (e.g., based on detection of aneuploidy, and/or at least in part on the presence or absence of one or more genetic biomarkers (e.g., mutations) and/or an elevated level of one or more protein biomarkers (e.g., peptides)) can have the cancer diagnosis confirmed using any appropriate method. Examples of methods that can be used to diagnose or confirm diagnosis of a cancer include, without limitation, physical examinations (e.g., pelvic examination), imaging tests (e.g., ultrasound or CT scans), cytology, and tissue tests (e.g., biopsy).

In some embodiments, methods for identifying one or more chromosomal anomalies (e.g., aneuploidy) provided herein are used to identify a mammal as having a distinct stage of cancer. In some embodiments, a cancer can be a Stage I cancer. In some embodiments, a cancer can be a Stage II cancer. In some embodiments, a cancer can be a Stage III cancer. In some embodiments, a cancer can be a Stage IV cancer. In some embodiments, methods for identifying one or more chromosomal anomalies (e.g., aneuploidy) provided herein are used to identify a mammal as having a stage of cancer that conventional methods of detecting cancer cannot reliably detect. For example, methods for identifying one or more chromosomal anomalies (e.g., aneuploidy) provided herein can be used to identify a mammal as having a Stage I cancer that conventional methods of detecting cancer cannot reliably detect. In some embodiments, methods provided herein for identifying: 1) one or more chromosomal anomalies (e.g., aneuploidy), and 2) one or more genetic biomarkers (e.g., any of the genetic biomarkers provided herein) are used to identify a mammal as having a stage of cancer that conventional methods of detecting cancer cannot reliably detect. In some embodiments, methods provided herein for identifying: 1) one or more chromosomal anomalies (e.g., aneuploidy), and 2) one or more protein biomarkers (e.g., any of the protein biomarkers provided herein) are used to identify a mammal as having a stage of cancer that conventional methods of detecting cancer cannot reliably detect. Non-limiting examples of cancers that be identified as described herein (e.g., based on detection of aneuploidy, and/or at least in part on the presence or absence of one or more genetic biomarkers (e.g., mutations) and/or an elevated level of one or more protein biomarkers (e.g., peptides)) include, liver cancer, ovarian cancer, esophageal cancer, stomach cancer, pancreatic cancer, colorectal cancer, lung cancer, breast cancer, and prostate cancer.

In some embodiments, the subject in which the presence of one or more chromosomal anomalies (e.g., aneuploidies) is detected may be selected for further diagnostic testing. In some embodiments, methods provided herein can be used to select a subject for further diagnostic testing at a time period prior to the time period when conventional techniques are capable of diagnosing the subject with an early-stage cancer. For example, methods provided herein for selecting a subject for further diagnostic testing can be used when a subject has not been diagnosed with cancer by conventional methods and/or when a subject is not known to harbor a cancer. In some embodiments, a subject selected for further diagnostic testing can be administered a diagnostic test (e.g., any of the diagnostic tests described herein) at an increased frequency compared to a subject that has not been selected for further diagnostic testing. For example, a subject selected for further diagnostic testing can be administered a diagnostic test at a frequency of twice daily, daily, bi-weekly, weekly, bi-monthly, monthly, quarterly, semi-annually, annually, or any at frequency therein. In some embodiments, a subject selected for further diagnostic testing can be administered one or more additional diagnostic tests compared to a subject that has not been selected for further diagnostic testing. For example, a subject selected for further diagnostic testing can be administered two diagnostic tests or more, whereas a subject that has not been selected for further diagnostic testing is administered only a single diagnostic test (or no diagnostic tests). In some embodiments, the diagnostic testing method can determine the presence of the same type of cancer as the originally detected cancer. Additionally or alternatively, the diagnostic testing method can determine the presence of a different type of cancer from the originally detected cancer.

In some embodiments, the diagnostic testing method is a scan. In some embodiments, the scan is a bone scan, a computed tomography (CT), a CT angiography (CTA), an esophagram (a Barium swallow), a Barium enema, a gallium scan, a magnetic resonance imaging (MRI), a mammography, a monoclonal antibody scan (e.g., ProstaScint® scan for prostate cancer, OncoScint® scan for ovarian cancer, and CEA-Scan® for colon cancer), a multigated acquisition (MUGA) scan, a PET scan, a PET/CT scan, a thyroid scan, an ultrasound (e.g., a breast ultrasound, an endobronchial ultrasound, an endoscopic ultrasound, a transvaginal ultrasound), an X-ray, a DEXA scan.

In some embodiments, the diagnostic testing method is a physical examination, such as, without limitation, an anoscopy, a biopsy, a bronchoscopy (e.g., an autofluorescence

bronchoscopy, a white-light bronchoscopy, a navigational bronchoscopy), a digital breast tomosynthesis, a digital rectal exam, an endoscopy, including but not limited to a capsule endoscopy, virtual endoscopy, an arthroscopy, a bronchoscopy, a colonoscopy, a colposcopy, a cystoscopy, an esophagoscopy, a gastroscopy, a laparoscopy, a laryngoscopy, a neuroendoscopy, a proctoscopy, a sigmoidoscopy, a skin cancer exam, a thoracoscopy, an endoscopic retrograde cholangiopancreatography (ERCP), an ensophagogastroduodenoscopy, a pelvic exam.

In some embodiments, the diagnostic testing method is a biopsy (e.g., a bone marrow aspiration, a tissue biopsy). In some embodiments, the biopsy is performed by fine needle aspiration or by surgical excision. In some embodiments, the diagnostic testing method(s) further include obtaining a biological sample (e.g., a tissue sample, a urine sample, a blood sample, a check swab, a saliva sample, a mucosal sample (e.g., sputum, bronchial secretion), a nipple aspirate, a secretion or an excretion). In some embodiments, the diagnostic testing method(s) include determining exosomal proteins (e.g., an exosomal surface protein (e.g., CD24, CD147, PCA-3)) (Soung et al. (2017) Cancers 9(1):pii:E8). In some embodiments, the diagnostic testing method is an oncotype DX® test (Baehner (2016) Ecancermedicalscience 10:675).

In some embodiments, the diagnostic testing method is a test, such as without limitation, an alpha-fetoprotein blood test, a bone marrow test, a fecal occult blood test, a human papillomavirus test, low-dose helical computed tomography, a lumbar puncture, a prostate specific antigen (PSA) test, a pap smear, or a tumor marker test.

In some embodiments, the diagnostic testing method includes determining the level of a known protein biomarker (e.g., CA-125 or prostate specific antigen (PSA)). For example, a high amount of CA-125 can be found in subject's blood, which subject has ovarian cancer, endometrial cancer, fallopian tube cancer, pancreatic cancer, stomach cancer, esophageal cancer, colon cancer, liver cancer, breast cancer, or lung cancer. The term "biomarker" as used herein refers to "a biological molecule found in blood, other bodily fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease", e.g., as defined by the National Cancer Institute. (see, e.g., the URL www.cancer.gov/publications/dictionaries/cancer-terms?CdrID=45618). A biomarker can include a genetic biomarker such as, without limitation, a nucleic acid (e.g., a DNA molecule, a RNA molecule (e.g., a microRNA, a long non-coding RNA (lncRNA) or other non-coding

RNA) A biomarker can include a protein biomarker such as, without limitation, a peptide, a protein, or a fragment thereof.

In some embodiments, the biomarker is FLT3, NPM1, CEBPA, PRAM1, ALK, BRAF, KRAS, EGFR, Kit, NRAS, JAK2, KRAS, HPV virus, ERBB2, BCR-ABL, BRCA1, BRCA2, CEA, AFP, and/or LDH. See e.g., Easton et al. (1995) Am. J. Hum. Genet. 56: 265-271, Hall et al. (1990) Science 250: 1684-1689, Lin et al. (2008) Ann. Intern. Med. 149: 192-199, Allegra et al. (2009) (2009) J. Clin. Oncol. 27: 2091-2096, Paik et al. (2004) N. Engl. J. Med. 351: 2817-2826, Bang et al. (2010) Lancet 376: 687-697, Piccart-Gebhart et al. (2005) N. Engl. J. Med. 353: 1659-1672, Romond et al. (2005) N. Engl. J. Med. 353: 1673-1684, Locker et al. (2006) J. Clin. Oncol. 24: 5313-5327, Giligan et al. (2010) J. Clin. Oncol. 28: 3388-3404, Harris et al. (2007) J. Clin. Oncol. 25: 5287-5312; Henry and Hayes (2012) Mol. Oncol. 6: 140-146. In some embodiments, the biomarker is a biomarker for detection of breast cancer in a subject, such as, without limitation, MUC-1, CEA, p53, urokinase plasminogen activator, BRCA1, BRCA2, and/or HER2 (Gam (2012) World J. Exp. Med. 2(5): 86-91). In some embodiments, the biomarker is a biomarker for detection of lung cancer in a subject, such as, without limitation, KRAS, EGFR, ALK, MET, and/or ROS1 (Mao (2002) Oncogene 21: 6960-6969; Korpanty et al. (2014) Front Oncol. 4: 204). In some embodiments, the biomarker is a biomarker for detection of ovarian cancer in a subject, such as, without limitation, HPV, CA-125, HE4, CEA, VCAM-1, KLK6/7, GST1, PRSS8, FOLR1, ALDH1 (Nolen and Lokshin (2012) Future Oncol. 8(1): 55-71; Sarojini et al. (2012) J. Oncol. 2012:709049). In some embodiments, the biomarker is a biomarker for detection of colorectal cancer in a subject, such as, without limitation, MLH1, MSH2, MSH6, PMS2, KRAS, and BRAF (Gonzalez-Pons and Cruz-Correa (2015) Biomed. Res. Int. 2015: 149014; Alvarez-Chaver et al. (2014) World J. Gastroenterol. 20(14): 3804-3824). In some embodiments, the diagnostic testing method determines the presence and/or expression level of a nucleic acid (e.g., microRNA (Sethi et al. (2011) J. Carcinog. Mutag. S1-005), RNA, a SNP (Hosein et al. (2013) Lab. Invest doi: 10.1038/labinvest.2013.54; Falzoi et al. (2010) Pharmacogenomics 11: 559-571), methylation status (Castelo-Branco et al. (2013) Lancet Oncol 14: 534-542), a hotspot cancer mutation (Yousem et al. (2013) Chest 143: 1679-1684)). Non-limiting examples of methods of detecting a nucleic acid in a sample include: PCR, RT-PCR, sequencing (e.g., next generation sequencing methods, deep sequencing), a DNA microarray, a microRNA microarray, a SNP microarray, fluorescent in situ

hybridization (FISH), restriction fragment length polymorphism (RFLP), gel electrophoresis, Northern blot analysis, Southern blot analysis, chromogenic in situ hybridization (CISH), chromatin immunoprecipitation (ChIP), SNP genotyping, and DNA methylation assay. See, e.g., Meldrum et al. (2011) Clin. Biochem. Rev. 32(4): 177-195; Sidranksy (1997) Science 278(5340): 1054-9.

In some embodiments, the diagnostic testing method includes determining the presence of a protein biomarker in a sample (e.g., a plasma biomarker (Mirus et al. (2015) Clin. Cancer Res. 21(7): 1764-1771)). Non-limiting examples of methods of determining the presence of a protein biomarker include: western blot analysis, immunohistochemistry (IHC), immunofluorescence, mass spectrometry (MS) (e.g., matrix assisted laser desorption/ionization (MALDI)-MS, surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF)-MS), enzyme-linked immunosorbent assay (ELISA), flow cytometry, proximity assay (e.g., VeraTag proximity assay (Shi et al. (2009) Diagnostic molecular pathology: the American journal of surgical pathology, part B: 18: 11-21, Huang et al. (2010) AM. J. Clin. Pathol. 134: 303-11)), a protein microarray (e.g., an antibody microarray (Ingvarsson et al. (2008) Proteomics 8: 2211-9, Woodbury et al. (2002) J. Proteome Res. 1: 233-237), an IHC-based microarray (Stromberg et al. (2007) Proteomics 7: 2142-50), a microarray ELISA (Schroder et al. (2010) Mol. Cell. Proteomics 9: 1271-80). In some embodiments, the method of determining the presence of a protein biomarker is a functional assay. In some embodiments, the functional assay is a kinase assay (Ghosh et al. (2010) Biosensors & Bioelectronics 26: 424-31, Mizutani et al. (2010) Clin. Cancer Res. 16: 3964-75, Lee et al. (2012) Biomed. Microdevices 14: 247-57), a protease assay (Lowe et al. (2012) ACS nano. 6: 851-7, Fujiwara et al. (2006) Breast cancer 13: 272-8, Darragh et al. (2010) Cancer Res 70: 1505-12). See, e.g., Powers and Palecek (2015) J. Heathc Eng. 3(4): 503-534, for a review of protein analytical assays for diagnosing cancer patients.

In some embodiments, any appropriate disease or condition associated with one or more chromosomal anomalies as described herein (e.g., based at least in part on the presence of one or more chromosomal anomalies, such as, without limitation, an aneuploidy) is identified as described herein. In some embodiments, the disease is cancer. Examples of cancers that can be associated with one or more chromosomal anomalies include, without limitation, lung cancer (e.g., small cell lung carcinoma or non-small cell lung carcinoma), papillary thyroid cancer, medullary thyroid cancer, differentiated thyroid cancer, recurrent

thyroid cancer, refractory differentiated thyroid cancer, lung adenocarcinoma, bronchioles lung cell carcinoma, multiple endocrine neoplasia type 2A or 2B (MEN2A or MEN2B, respectively), pheochromocytoma, parathyroid hyperplasia, breast cancer, colorectal cancer (e.g., metastatic colorectal cancer), papillary renal cell carcinoma, ganglioneuromatosis of the gastroenteric mucosa, inflammatory myofibroblastic tumor, or cervical cancer, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), cancer in adolescents, adrenal cancer, adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, atypical teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain stem glioma, brain tumor, breast cancer, bronchial tumor, Burkitt lymphoma, carcinoid tumor, unknown primary carcinoma, cardiac tumors, cervical cancer, childhood cancers, chordoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloproliferative neoplasms, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, bile duct cancer, ductal carcinoma in situ, embryonal tumors, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing sarcoma, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer, fallopian tube cancer, fibrous histiocytoma of bone, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumors (GIST), germ cell tumor, gestational trophoblastic disease, glioma, hairy cell tumor, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular cancer, histiocytosis, Hodgkin's lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumors, pancreatic neuroendocrine tumors, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leukemia, lip and oral cavity cancer, liver cancer, lung cancer, lymphoma, macroglobulinemia, malignant fibrous histiocytoma of bone, osteocarcinoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer, midline tract carcinoma, mouth cancer, multiple endocrine neoplasia syndromes, multiple myeloma, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative neoplasms, myelogenous leukemia, myeloid leukemia, multiple myeloma, myeloproliferative neoplasms, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin's lymphoma, non-small cell lung cancer, oral cancer, oral cavity cancer, lip cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, hepatobiliary cancer, upper urinary tract cancer, papillomatosis, paraganglioma, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromosytoma,

pituitary cancer, plasma cell neoplasm, pleuropulmonary blastoma, pregnancy and breast cancer, primary central nervous system lymphoma, primary peritoneal cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach cancer, T-cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, unknown primary carcinoma, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom Macroglobulinemia, Wilms' tumor, 1p36 deletion syndrome, 1q21.1 deletion syndrome, 2q37 deletion syndrome, Wolf-Hirschhorn syndrome, Cri du chat, 5q deletion syndrome, Williams syndrome, Monosomy 8p, Monosomy 8q, Alfi's syndrome, Kleefstra syndrome, Monosomy 10p, Monosomy 10q, Jacobsen syndrome, Patau syndrome, Angelman syndrome, Prader–Willi syndrome, Miller-Dieker syndrome, Smith-Magenis syndrome, Edwards syndrome, Down syndrome, DiGeorge syndrome, Phelan-McDermid syndrome, 22q11.2 distal deletion syndrome, Cat eye syndrome, XYY syndrome, Triple X syndrome, Klinefelter syndrome, Wolf-Hirschhorn syndrome, Jacobsen syndrome, Charcot-Marie-Tooth disease type 1A, and Lynch Syndrome.

Once identified as having a disease associated with one or more chromosomal anomalies as described herein (e.g., based at least in part on the presence of one or more chromosomal anomalies, such as, without limitation, an aneuploidy), a mammal (e.g., a human) can be treated accordingly. For example, when a mammal is identified as having a cancer associated with one or more chromosomal anomalies as described herein, the mammal can be treated with one or more cancer treatments. The one or more cancer treatments can include any appropriate cancer treatments. A cancer treatment can include surgery. A cancer treatment can include radiation therapy. A cancer treatment can include administration of a pharmacotherapy such chemotherapy, hormone therapy, targeted therapy, and/or cytotoxic therapy. Examples of cancer treatments include, without limitation, platinum compounds (such as cisplatin or carboplatin), taxanes (such as paclitaxel or docetaxel), albumin bound paclitaxel (nab-paclitaxel), altretamine, capecitabine, cyclophosphamide, etoposide (vp-16), gemcitabine, ifosfamide, irinotecan (cpt-11), liposomal doxorubicin, melphalan, pemetrexed, topotecan, vinorelbine, luteinizing-hormone-releasing hormone (LHRH) agonists (such as goserelin and leuprolide), anti-estrogen therapy (such as tamoxifen), aromatase inhibitors

(such as letrozole, anastrozole, and exemestane), angiogenesis inhibitors (such as bevacizumab), poly(ADP)-ribose polymerase (PARP) inhibitors (such as olaparib, rucaparib, and niraparib), external beam radiation therapy, brachytherapy, radioactive phosphorus, and any combinations thereof.

Multi-analyte test to increase sensitivity of detection

In some embodiments, methods provided herein to detect aneuploidy (e.g., using the analysis of chromosomal sequences (see e.g., Table 1 for an exemplary list of repetitive elements that can be analyzed)) increase sensitivity of cancer detection compared to cancer detection using the presence of one or more genetic biomarkers as indicators of cancer. In some embodiments, methods provided herein to detect aneuploidy (e.g., using the analysis of chromosomal sequences (see e.g., Table 1 for an exemplary list of repetitive elements that can be analyzed)) increase sensitivity of cancer detection compared to cancer detection using the presence of one or more protein biomarkers as indicators of cancer.

In some embodiments, methods provided herein to detect aneuploidy (e.g., using the analysis of chromosomal sequences (see e.g., Table 1 for an exemplary list of repetitive elements that can be analyzed)) are combined with one or more methods to detect the presence of one or more genetic biomarkers (e.g., mutations). In some embodiments, the combination of aneuploidy detection with genetic biomarker detection increases the specificity and/or sensitivity of detecting cancer. In some embodiments, methods provided herein to detect aneuploidy (e.g., using the analysis of chromosomal sequences (see e.g., Table 1 for an exemplary list of repetitive elements that can be analyzed)) are combined with one or more methods to detect the presence of one or more members of a panel of protein biomarkers (e.g., peptides). In some embodiments, the combination of aneuploidy detection with protein biomarker detection increases the specificity and/or sensitivity of detecting cancer. In some embodiments, methods provided herein to detect aneuploidy (e.g., using the analysis of chromosomal sequences (see e.g., Table 1 for an exemplary list of repetitive elements that can be analyzed)) are combined with methods to detect the presence of one or more genetic biomarkers (e.g., mutations) and/or methods to detect the presence of one or more members of a panel of protein biomarkers (e.g., peptide). In some embodiments, the combination of aneuploidy detection with genetic and/or protein biomarker detection increases the specificity and/or sensitivity of detecting cancer.

In some embodiments, methods provided herein to detect aneuploidy are combined with methods to detect the presence of one or more genetic biomarkers (e.g., mutations) in one or more genes selected from the group consisting of: NRAS, PTEN, FGFR2, KRAS, POLE, AKT1, TP53, RNF43, PPP2R1A, MAPK1, CTNNB1, PIK3CA, FBXW7, PIK3R1, APC, EGFR, BRAF. In some embodiments, methods provided herein to detect aneuploidy are combined with methods to detect the presence of one or more genetic biomarkers (e.g., mutations) in one or more genes selected from the group consisting of: PTEN, TP53, PIK3CA, PIK3R1, CTNNB1, KRAS, FGFR2, POLE, APC, FBXW7, RNF43, and PPP2R1A. In some embodiments, an assay includes detection of genetic biomarkers (e.g., mutations) in one or more of any of the genes disclosed herein including, without limitation, CDKN2A, FGF2, GNAS, ABL1, EVI1, MYC, APC, IL2, TNFAIP3, ABL2, EWSR1, MYCL1, ARHGEF12, JAK2, TP53, AKT1, FEV, MYCN, ATM, MAP2K4, TSC1, AKT2, FGFR1, NCOA4, BCL11B, MDM4, TSC2, ATF1, FGFR1OP, NFKB2, BLM, MEN1, VHL, BCL11A, FGFR2, NRAS, BMPR1A, MLH1, WRN, BCL2, FUS, NTRK1, BRCA1, MSH2, WT1, BCL3, GOLGA5, NUP214, BRCA2, NF1, BCL6, GOPC, PAX8, CARS, NF2, BCR, HMGA1, PDGFB, CBFA2T3, NOTCH1, BRAF, HMGA2, PIK3CA, CDH1, NPM1, CARD11, HRAS, PIM1, CDH11, NR4A3, CBLB, IRF4, PLAG1, CDK6, NUP98, CBLC, JUN, PPARG, SMAD4, PALB2, CCND1, KIT, PTPN11, CEBPA, PML, CCND2, KRAS, RAF1, CHEK2, PTEN, CCND3, LCK, REL, CREB1, RB1, CDX2, LMO2, RET, CREBBP, RUNX1, CTNNB1, MAF, ROS1, CYLD, SDHB, DDB2, MAFB, SMO, DDX5, SDHD, DDIT3, MAML2, SS18, EXT1, SMARCA4, DDX6, MDM2, TCL1A, EXT2, SMARCB1, DEK, MET, TET2, FBXW7, SOCS1, EGFR, MITF, TFG, FH, STK11, ELK4, MLL, TLX1, FLT3, SUFU, ERBB2, MPL, TPR, FOXP1, SUZ12, ETV4, MYB, USP6, GPC3, SYK, ETV6, IDH1, and/or TCF3. In some embodiments, combining the detection of aneuploidy with the detection of one or more genetic biomarkers (e.g., mutations) increases the specificity and/or sensitivity of detecting cancer.

In some embodiments, detection of a genetic biomarker (e.g., one or more genetic biomarkers) includes any of the variety of methods described in U.S. Patent No. 7,700,286, which is hereby incorporated by reference in its entirety. Any of the variety of methods of messenger RNA ("mRNA") isolation known in the art may be used to isolate RNA from a sample (e.g., Qiagen RNeasy Kit). Any of the variety of methods of genomic DNA ("gDNA") isolation known in the art may be used to isolate gDNA from the sample (e.g.,

Qiagen DNeasy Kit). In some embodiments, detection of a genetic biomarker includes a cancer detection assay. In some embodiments, the amount of gDNA and/or mRNA in a sample are measured for any of the genetic biomarkers disclosed herein. Changes in the amount of gDNA and/or mRNA may indicate cancer. For example, when measuring gDNA, gene amplification (e.g., increased copy number of chromosomal sequences (e.g., coding regions of genes or non-coding DNA (see e.g., Table 1 for an exemplary list of repetitive elements that can be measured)) may indicate cancer. For example, when measuring mRNA, increases in the amount of RNA (e.g., increased expression of a genetic biomarker) may indicate cancer. In some cases, changes in DNA and RNA may correlate.

In some embodiments, methods provided herein to detect aneuploidy can be combined with methods to detect the presence of one or more protein biomarkers (e.g., peptides) in one or more proteins selected from the group consisting of: AFP, CA19-9, CEA, HGF, OPN, CA-125, CA15-3, MPO, prolactin (PRL) and/or TIMP-1 to determine the presence of cancer (e.g., ovarian or endometrial). In some embodiments, a protein biomarker can be any appropriate peptide biomarker. In some embodiments, a peptide biomarker can be a peptide biomarker associated with cancer. For example, a peptide biomarker can be a peptide having elevated levels in a cancer (e.g., as compared to a reference level of the peptide).

Exemplary and non-limiting threshold levels for certain protein biomarkers include: CA19-9 (>92 U/ml), CEA (>7,507 pg/ml), CA125 (>577 U/ml), AFP (>21,321 pg/ml), Prolactin (>145,345 pg/ml), HGF (>899 pg/ml), OPN (>157,772 pg/ml), TIMP-1 (>176,989 pg/ml), Follistatin (>1,970 pg/ml), and CA15-3 (>98 U/ml). In some embodiments, threshold levels for protein biomarkers can be higher (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or higher) than the exemplary threshold levels described herein. In some embodiments, threshold levels for protein biomarkers can be lower (e.g., about 10%, about 20%, about 30%, about 40%, about 50%, or lower) than the exemplary threshold levels described herein.

In some embodiments, a threshold level of CA19-9 can be at least about 92 U/mL (e.g., about 92 U/mL). In some embodiments, a threshold level of CA19-9 can be 92 U/mL. In some embodiments, a threshold level of CEA can be at least about 7,507 pg/ml (e.g., about 7,507 pg/ml). In some embodiments, a threshold level of CEA can be 7.5 ng/mL. In some embodiments, a threshold level of HGF can be at least about 899 pg/ml (e.g., about 899

pg/ml). In some embodiments, a threshold level of HGF can be 0.92 ng/mL. In some embodiments, a threshold level of OPN can be at least about 157,772 pg/ml (e.g., about 157,772 pg/ml). In some embodiments, a threshold level of OPN can be 158 ng/mL. In some embodiments, a threshold level of CA125 can be at least about 577 U/ml (e.g., about 577 U/ml). In some embodiments, a threshold level of CA125 can be 577 U/mL. In some embodiments, a threshold level of AFP can be at least about 21,321 pg/ml (e.g., about 21,321 pg/ml). In some embodiments, a threshold level of AFP can be 21,321 pg/ml. In some embodiments, a threshold level of prolactin can be at least about 145,345 pg/ml (e.g., about 145,345 pg/ml). In some embodiments, a threshold level of prolactin can be 145,345 pg/ml. In some embodiments, a threshold level of TIMP-1 can be at least about 176,989 pg/ml (e.g., about 176,989 pg/ml). In some embodiments, a threshold level of TIMP-1 can be 176,989 pg/ml. In some embodiments, a threshold level of follistatin can be at least about 1,970 pg/ml (e.g., about 1,970 pg/ml). In some embodiments, a threshold level of CA15-3 can be at least about 98 U/ml (e.g., about 98 U/ml). In some embodiments, a threshold level of CA15-3 can be 98 U/ml. In some embodiments, a threshold level of CA19-9, CEA, and/or OPN can be 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% or more greater than the threshold levels listed above (e.g., greater than a threshold level of 92 U/mL for CA-19-9, 7,507 pg/ml for CEA, 899 pg/ml for HGF, 157,772 pg/ml for OPN, 577 U/ml for CA125, 21,321 pg/ml for AFP, 145,345 pg/ml for prolactin, 176,989 pg/ml for TIMP-1, 1,970 pg/ml for follistatin, and/or 98 U/ml for CA15-3).

In some embodiments, a threshold level of protein biomarker can be greater than the levels that are typically tested for diagnostic or clinical purposes. For example, the threshold level of CA19-9 can be greater than about 37 U/ml (e.g., greater than about 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or more U/mL). Additionally or alternatively, the threshold level of CEA can be greater than about 2.5 ug/L (e.g., greater than about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 or more ug/L). Additionally or alternatively, the threshold level of CA125 can be greater than about 35 U/mL (e.g., greater than about 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 or more U/mL). Additionally or alternatively, the threshold level of AFP can be greater than about 21 ng/mL (e.g., greater than about 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400 or more ng/L). Additionally or alternatively, the threshold level of TIMP-1 can be greater than

about 2300 ng/mL (e.g., greater than about 2,500, 3,000, 4,000, 5,000 , 6,000, 7,000, 8,000, 9,000, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000 or more ng/L). Additionally or alternatively, the threshold level of follistatin can be greater than about 2 ug/mL (e.g., greater than about 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 or more ug/L). Additionally or alternatively, the threshold level of CA15-3 can be greater than about 30 U/mL (e.g., greater than about 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or more U/mL). In some embodiments, detecting one or more protein biomarkers at threshold levels that are higher than are typically tested for during traditional diagnostic or clinical assays can improve the sensitivity of cancer detection.

Examples of peptide biomarkers include, without limitation, AFP, Angiopoietin-2, AXL, CA125, CA 15-3, CA19-9, CD44, CEA, CYFRA 21-1, DKK1, Endoglin, FGF2, Follistatin, Galectin-3, G-CSF, GDF15, HE4, HGF, IL-6, IL-8, Kallikrein-6, Leptin, LRG-1, Mesothelin, Midkine, Myeloperoxidase, NSE, OPG, OPN, PAR, Prolactin, sEGFR, sFas, SHBG, sHER2/sEGFR2/sErbB2, sPECAM-1, TGFa, Thrombospondin-2, TIMP-1, TIMP-2, and Vitronectin. For example, a peptide biomarker can include one or more of OPN, IL-6, CEA, CA125, HGF, Myeloperoxidase, CA19-9, Midkine and/or TIMP-1. In some embodiments, combining the detection of aneuploidy with the detection of one or more protein biomarkers (e.g., peptides) increases the specificity and/or sensitivity of detecting cancer.

In some embodiments, the presence of a genetic and/or protein biomarker may be detected in any of a variety of biological samples isolated or obtained from a subject (e.g., a human subject) including, but not limited to blood, plasma, serum, urine, cerebrospinal fluid, saliva, sputum, broncho-alveolar lavage, bile, lymphatic fluid, cyst fluid, stool, ascites, and combinations thereof. Any protein biomarker known in the art may be detected when a threshold value is obtained above which normal, healthy human subjects do not fall, but human subjects with cancer do fall. Any appropriate method can be used to detect the level of one or more protein biomarkers as described herein. In some embodiments, the level of one or more protein biomarkers is compared to a predetermined threshold. In some embodiments, the predetermined threshold is a general or global threshold. In some embodiments, the predetermined threshold is a threshold that is relevant to a particular protein biomarker. In some embodiments, the level of the one or more protein biomarkers is compared to an absolute amount of a reference protein biomarker. In some embodiments, the

level of the one or more protein biomarkers is relative to an amount of a reference protein biomarker. In some embodiments, the level of the one or more protein biomarkers is an elevated level. In some embodiments, the level of the one or more protein biomarkers is above a predetermined threshold. In some embodiments, the level of the one or more protein biomarkers is within a predetermined threshold range. In some embodiments, the level of the one or more protein biomarkers is or approximates a predetermined threshold. In some embodiments, the level of the one or more protein biomarkers is below a predetermined threshold. In some embodiments, the level of the one or more protein biomarkers from a biological sample is lower than a particular threshold. In some embodiments, the level of the one or more protein biomarkers from a biological sample is depressed compared to a predetermined threshold.

In some embodiments, methods and materials described herein can be used for detecting one or more polymorphisms (e.g., somatic mutations) in a genome of a mammal. For example, a plurality of amplicons obtained from a sample obtained from a first mammal (e.g., a test mammal or a mammal suspected of harboring one or more polymorphisms) can be sequenced, a plurality of amplicons obtained from a sample obtained from a second mammal (e.g., a reference mammal) can be sequenced, variant sequencing reads from the sample obtained from the first mammal can be grouped into clusters of genomic intervals, reference sequencing reads from the sample obtained from the second mammal can be grouped into clusters of genomic intervals, a chromosome arm having a sum of the variant sequencing reads and the reference sequencing reads on both alleles that is greater than about 3 (e.g., greater than about 4, greater than about 5, greater than about 6, greater than about 7, greater than about 8, greater than about 9, greater than about 10, greater than about 12, greater than about 15, greater than about 18, greater than about 20, greater than about 22, greater than about 25, or greater than about 30) can be selected, a variant-allele frequency (VAF) of the selected chromosome arm can be determined, and the presence or absence of one or more polymorphisms on the selected chromosome arm can be identified. A VAF of the selected chromosome arm can be determined using any appropriate technique. For example, a VAF of the selected chromosome arm can be the number of variant sequencing reads / total number of sequencing reads. The presence of one or more polymorphisms in the genome of the mammal can be identified in the genome of the mammal when the VAF is between about 0.2 and about 0.8 (e.g., between about 0.3 and about 0.8, between about 0.4

and about 0.8, between about 0.5 and about 0.8, between about 0.6 and about 0.8, between about 0.2 and about 0.7, between about 0.2 and about 0.6, between about 0.2 and about 0.5, or between about 0.2 and about 0.4), and the absence of one or more polymorphisms in the genome of the mammal can be identified in the genome of the mammal when the VAF is within a predetermined significance threshold. For example, without limitation, the presence of one or more polymorphisms in the genome of the mammal can be identified in the genome of the mammal when the VAF is between about 0.4 and 0.6.

In some embodiments, methods and materials described herein can be used for sample identification. The repetitive elements amplified by the methods described herein include common polymorphisms that can be used to establish or refute sample identify among samples (e.g., plasma, tumor, and blood). For example, the genotype at each polymorphic location can be identified and compared across samples. Overall similarities between samples at polymorphic locations can be used to determine sample identity.

In some cases the diseases associated with one or more chromosomal anomalies as described herein (e.g., based at least in part on the presence of one or more chromosomal anomalies, such as, without limitation, an aneuploidy) are also associated with increased mutation rates (e.g., increased mutation rates can be associated with stage of disease) when compared to a control (e.g., non-disease sample). In such cases, the materials and methods described herein can be used to (a) identify the presence of one or more chromosomal anomalies (e.g., aneuploidy) and (b) identify the stage (e.g., cancer stages I, II, III, and IV) of the disease based on a determination of the mutation rate (e.g., number of mutations) compared to a control.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Detection of aneuploidy in patients with cancer

This example describes a novel adaptation of amplicon-based aneuploidy detection. An approach called WALDO for Within-Sample-AneupLoidy-DetectiOn, which employs supervised machine learning to detect changes in chromosome arms, improved aneuploidy detection sensitivity compared to previous methods. It is shown here that using WALDO to

analyze amplicons of short interspersed nucleotide elements (SINEs) from a DNA sample increases sensitivity of aneuploidy detection. In addition, the ~1,000,000 SINE amplicons with an average length of about 100bp reduce the input requirement for cell free DNA input while also increasing sensitivity of detection.

Materials and methods

Primers

To generate a list of candidate primers, the frequency of all possible 6-mers (4^6 = 4096) within the RepeatMasker track of hg19 were calculated. Next, the frequency of all possible 4-mers (4^4 = 256) within 75 bp upstream or downstream from the 6-mers were calculated. Joining the 6-mers with the 4-mers generated 2,097,152 candidate pairs. These pairs were selected for further assessment based on the number of unique genomic loci expected from their PCR-mediated amplification, the average size between the 6-mer and its corresponding 4-mers, and the distribution of these sizes, aiming for a unimodal distribution. This filtering criteria generated 16 potential k-mer pairs, leading to the design of 16 primer pairs that incorporated these k-mer pairs at their 3-ends. A k-mer is understood in the art to refer to a subsequence of length k which is contained within a sequence.

In total, 16 primers were initially designed and tested (Table 2). One primer (SEQ ID NO: 1) consistently had fewer primer dimers and was selected for use in testing a cohort. A primer pair having SEQ ID NO: 1 as one of the primers uniquely amplified 745,184 amplicons, which amplicons had an average amplicon size of ~88bp (Figure 1A). The amplicons sizes shown in Figure 1A include 45 bp of primers. For example, when not including the primers, the amplicons have an average size of ~43 base pairs (Figure 1B).

Table 2.

Primer Pair	SEQ ID NO:	Primer Name	Sequence
1	1	FP1_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNGGTGAAACCCCGTCTCTACA
2	2	FP2_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNGGTGAAACCCCGTCTCTAC
3	3	FP3_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNGGTGAAACCCCGTCTCTACT
4	4	FP4_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNCATGCCTGTAGTCCCAGCTACT

5	5	FP5_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNATAGTGAAACCCCATCTCTACAAAA
6	6	FP6_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNGGTGAAACCCCATCTCTACAA
7	7	FP7_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNATAGTGAAACCCCATCTCTACAAA
8	8	FP8_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNGAGGTGGGAGGATTGCTT
9	9	FP9_n16	cgacgtaaaacgacggccagtNNNNNNNNNNNNNNNNACCAGCCTGGGCAACATA
1	10	RP1_z4	cacacaggaaacagctatgaccatgCCTCCTAAGTAGCTGGGACTACAG
2	11	RP2_z4	cacacaggaaacagctatgaccatgCCTCCTAAGTAGCTGGGACTACAG
3	12	RP3_z4	cacacaggaaacagctatgaccatgCCTCCTAAGTAGCTGGGACTACAG
4	14	RP4_z4	cacacaggaaacagctatgaccatgTGCAGTGGCACGATCATAGCTCACTGCAGCCTTGA
5	15	RP5_z4	cacacaggaaacagctatgaccatgCTCCCGAGTAGCTGGGACT
6	16	RP6_z4	cacacaggaaacagctatgaccatgCTCCCGAGTAGCTGGGACTAC
7	17	RP7_z4	cacacaggaaacagctatgaccatgCCCGAGTAGCTGGGACTACA
8	18	RP8_z4	cacacaggaaacagctatgaccatgAGGCTGGAGTGCAGTGG
9	19	RP9_z4	cacacaggaaacagctatgaccatgCCACCATGCCTGGCTAA

Sequencing library preparation

The first primer having SEQ ID NO: 1 included from the 5' to 3' end: a universal primer sequence (UPS), a unique identifier DNA sequence (UID), and an amplification sequence. Polymerase chain reaction (PCR) was performed in 25 uL reactions containing 7.25 uL of water, 0.125 uL of each primer, 12.5 uL of NEBNext Ultra II Q5 Master Mix (New England Biolabs cat # M0544S), and 5 uL of DNA. The cycling conditions were: one cycle of 98°C for 120 s, then 15 cycles of 98°C for 10 s, 57°C for 120 s, and 72°C for 120 s. For experiments with plasma, the amount of DNA in 5 uL was 0.14 ng. A second round of PCR was then performed to add dual indexes (barcodes) to each PCR prior to sequencing. The forward and reverse primers used for the second round of PCR are listed in Table 2. The initial amplification primers were not removed and the amplification product from the first reaction was diluted 1:20. The dilution was used directly for a second round of amplification using primers that annealed to the UPS site introduced by the first round primers and that additionally contained the 5' grafting sequences necessary for hybridization to the Illumina flow cell.

FIndexes (e.g., sequences used to differentiate between samples) were introduced to each sample using the second reverse primer to later allow multiplexed sequencing. The second round of PCR was performed in 25 uL reactions containing 7.25 uL of water, 0.125 uL of each primer, 12.5 uL of NEBNext Ultra II Q5 Master Mix (New England Biolabs cat # M0544S), and 5 uL of DNA containing 5% of the PCR product from the first round. The cycling conditions were: one cycle of 98°C for 120 s, then 15 cycles of 98°C for 10 s, 65°C for 15 s, and 72°C for 120 s. Amplification products were run on agarose gels to check for amplification. Amplification products were purified with AMPure XP beads at 1.2X and were quantified by spectrophotometry, real time PCR, an Agilent 2100 Bioanalyzer or an automated electrophoresis using an Aiglent TapeStation. All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, Iowa).

Sequencing and sequencing analysis

Bowtie2 was used to align reads of the amplicons generated with each of the 7 primer pairs to the human reference genome assembly GRC37 (Langmead et al. 2012). With primer pair 1 (the primer having SEQ ID NO: 1 and the primer having SEQ ID NO: 10), an average of 51.1% of the total reads could be uniquely aligned and the average amplicon size was 88bp (Figure 1A). The amplicons sizes shown in Figure 1A include 45 bp of primers. For example, when not including the primers, the amplicons have an average size of ~43 base pairs (Figure 1B). Primer pair 1 was theoretically able to amplify up to 745,184 repetitive elements that can be uniquely aligned, but the average sample contained an average of 350,000 repetitive elements, see Figure 1C. Without wishing to be bound by theory, there were several potential reasons for the discrepancy between the potential number and the actual observed number of amplicons in plasma samples. (1) Polymorphisms within the sequences may have caused misalignment and result in "missing amplicons." (2) Polymorphisms within the primers may not have amplified. (3) Each amplicon may have had a different PCR efficiency with low efficiency amplicons outcompeted during PCR. (4) Smaller DNA fragments may have been preferentially amplified and long amplicons (>100 bp) may not have been amplified. (5) Long amplicons may have been absent in cell free DNA due to the small sizes of the DNA fragments in cell free DNA. (6) The amount of sequencing used for these samples may not have been high enough to observe every amplicon especially those with low PCR efficiencies. (7) Finally, some repetitive elements

may not have been present in every individual. Within the amplicons generated by the primer pair of SEQ ID NO: 1 and SEQ ID NO: 10, 52,762 polymorphisms were identified. The average number of heterozygous sites in the test cohort of 1348 normal plasmas and 883 plasmas from cancer patient individuals was 2,200. These sites could be used to measure allelic imbalance, genetically identify samples, and determine whether samples had been accidentally mixed together. Using the same SNPs, synthetic experiments were used to estimate that sample mixing could be detected when the amount of sample one DNA was >4% of the amount of sample two DNA in a given mixture.

Statistical analysis

Read-depth–based analytical methods have been widely applied to whole-genome sequencing (WGS) protocols. Under the assumption that reads are uniformly and independently distributed, regions of normal copy number are expected to follow a Poisson or normal distribution (Zhao et al 2013 and Pirooznia et al 2015). Amplicon-based protocols achieve high coverage depth at relatively low cost, and they are an attractive alternative to WGS, but aligned reads from amplicon sequencing such as those resulting from the above described assay have properties different from those resulting from WGS and WES. Because these reads are limited to a relatively small number of discrete loci, they are discontinuous. The reads are also not randomly distributed, which makes it difficult to use the statistical models of read depth coverage designed for WGS and WES. The Within-Sample AneupLoidy DetectiOn (WALDO), is an algorithm specifically designed for amplicon-based aneuploidy detection (see, e.g., Douville et al. PNAS 201 115(8):1871-1876). WALDO was applied to sequencing reads that mapped to the above described genomic loci (e.g., SINE). The genome-wide aneuploidy score was used to identify whether a sample had the presence of aneuploidy.

Statistical principles underlying WALDO

Unlike most conventional approaches for assessing copy number changes, WALDO does not compare normalized read counts from each chromosome arm in a test sample to the fraction of reads in each chromosome arm in other samples. Such conventional comparisons are subject to batch effects and other artifacts associated with variables that are difficult to control. To evaluate whole genome sequencing data, aneuploidy was detected by comparing

the read counts within 5344 genomic intervals each containing 500-kb of sequence. The read counts within the 500-kb genomic intervals within a sample were only compared to the read counts of other genomic intervals within the same sample - hence the "Within-Sample" designation in WALDO. The previously described WALDO protocol was tailored in this Example, which resulted in several analytical changes (see Figure 2). The modifications included a new normalization step, a new way to call small copy number changes of indeterminate length, and an improved way to detect genome-wide aneuploidy, as described below. These analytical improvements coupled with the increased genomic density of amplicons achieved with the SEQ ID NO: 1 and SEQ ID NO: 10 primer pair enabled greater sensitivity as well as the detection of focal amplifications and deletions less than 1 Mb in size.

In euploid samples, the number reads within each 500-kb genomic interval should track with the number of reads in certain other genomic regions. Genomic intervals that track together do so because the amplicons within them amplify to similar extents. Here, such genomic regions that track together are called "clusters". It is possible identify clusters from sequencing data on euploid samples. In a test sample, it is determined whether the number of reads in each genomic interval in each pre-defined cluster is within the expected bound of the other clusters from that same sample. If the reads within a genomic interval are outside the statistically expected bound, and there are many such outsiders on the same chromosome arm, then that chromosome arm is classified as aneuploid. The statistical basis of this test is described elsewhere (e.g., Douville et al. PNAS 201 115(8):1871-1876). In brief, while the number of reads is not randomly distributed across the genome, the distribution of scaled reads within each cluster is approximately Normal. A convenient property of Normal distributions is that the sum of multiple Normal distributions is also a Normal distribution. It is thus possible to compute the theoretical mean and variance of the summed reads on each chromosome arm simply by summing the means and variances of all the clusters represented on that chromosome arm.

WALDO also employs several other innovations that make it applicable to the analysis of PCR-generated amplicons from clinical samples. One of these innovations is controlling amplification bias stemming from the strong dependence of the data on the size of the initial template. Another is the use of a machine learning algorithm (e.g., a Support

Vector Machine (SVM)) to enable the detection of aneuploidy in samples containing low neoplastic fractions.

Normalization

The improved WALDO methods described in this Example include a new method of normalization that reduced the amount of variability between samples. In this normalization, a principal component analysis (PCA) was first performed on sequencing data from the controls. PCA reduced the number of 500 kb genomic intervals from n=5,344 to a more manageable number of dimensions. Using the PCA coordinates of the controls, a modeled was created to predict whether a particular 500kb interval will be amplified more or less efficiently in future samples based on their PCA coordinates.

$$Correction\,Factor\,for\,500kb\,Interval_i = \beta_{0i} + \beta_{1i} * PCA_1 + \beta_{2i} * PCA_2 + \beta_{3i} * PCA_3 + \beta_{4i} * PCA_4 + \beta_{5i} * PCA_5$$

For each test sample, the sample was projected into PCA space and the correction factor was calculated for each 500kb interval as function of its PCA coordinates. After applying the correction factor to each 500 kb genomic interval, the test sample was matched to 7 control samples based on the closest Euclidean distance of the 500 kb intervals.

Generation of synthetic aneuploidy samples.

Data was selected from 84 presumably euploid plasma samples, each containing at least 10 million reads, and each derived from the DNA of normal WBCs. Synthetic aneuploid samples were created by adding (or subtracting) reads from several chromosome arms to the reads from these normal DNA samples. The reads were added or subtracted from 1, 10, 15, or 20 chromosome arms to each sample. The additions and subtractions were designed to represent neoplastic cell fractions ranging from 0.5% to 1.5% and resulted in synthetic samples containing exactly ten million reads. The reads from each chromosome arm were added or subtracted uniformly. For example, when modeling five chromosome arms that were lost, each was lost to the identical degree and we did not incorporate tumor heterogeneity into the model. Furthermore, synthetic samples were not created containing more than three of any chromosome arm; e.g. 4 copies of chromosome 3p. This simplified approach did not comprehensively cover all biologically plausible aneuploidy events.

However, limiting the possible combinations of altered arms made sample generation computationally tractable, and the resulting support vector machine worked well in practice. The synthetically generated samples in which reads from only a single chromosome arm were added or subtracted enabled us to estimate the performance of WALDO when only a single chromosome arm of interest was gained or lost. The pseudocode to generate synthetic samples is shown in Figure 5.

Determination of genome wide aneuploidy

A two-class support vector machine (SVM) was trained to discriminate between euploid samples and aneuploid samples. The training set contained a negative class of 1348 presumably euploid plasma samples from normal individuals containing at least 2.5M reads and 635 aneuploid samples. The aneuploid class contained a mixture of synthetic and actual aneuploid samples. SVM training was done with the e1071 package in R, using radial basis kernel and default parameters. Each sample had 39 Z-score features, representing chromosome arm gains and losses. During training, the positive class was randomly sampled so that the positive class was 10% the size of the negative class. The positive class was randomly sampled at a ratio of two real samples to one synthetic sample. Ten iterations of this procedure were performed. The final genome wide aneuploidy score was the average of the raw svm score across the 10 iterations.

Results

The performance of this assay was assessed on a cohort of 1348 euploid plasma samples and 883 plasma samples from cancer patients (Table 3). The samples from cancer patients included Breast, Colorectum, Esophagus, Liver, Lung, Ovary, Pancreas, and Stomach cancers (Figure 3). Using a cutoff of that resulted in 99% specificity defined in our cohort of 1348 euploid samples, it was found that 49% plasmas from cancer samples had aneuploidy.

Sample exclusion criteria

To ensure that all samples included in the results section of paper were of high quality, several exclusion criteria were developed. First, samples with fewer than 2.5M reads were excluded. Second, samples with sufficient evidence of contamination were excluded.

To be labeled as contaminated, the sample had to have at least 10 significant allelic imbalanced chromosome arms (z score >= 2.5) and fewer than ten significant chromosome arms gains or losses (z >= 2.5 or z<= -2.5). Allelic imbalance is determined from SNPs, while gains or losses were assessed through WALDO. As determined through mixing experiments, a relatively large number of allelic imbalanced chromosome arms in the absence of a large number of gains or losses indicated contamination of the sample with DNA from another individual. Third, in plasma analyses, samples in which more than 8.5% of the amplicons were larger than 94 bps (50 base pairs between the forward and reverse primers) were excluded. Such samples were likely to be contaminated with leukocyte DNA. Fourth, samples outside the dynamic range of the assay, as defined by the equation below, were excluded.

$$QC\ Dynamic\ Range\ Metric = \sum_{i}^{2q,3q,4q,5q,6q,8q,13q} \frac{Reads\ on\ chr_i}{\sum_{j=1}^{38} Reads\ on\ chr_j}$$

The distribution of this metric has long tails. The values of >0.2450 and 0.2320 were selected as a dynamic range that we could evaluate cutoffs. Fifth, plasma samples with known aneuploidy in the leukocytes of the same patients; such patients were assumed to have Clonal Hematopoiesis of Indeterminate Potential (CHIP) or congenital disorders.

Detection of cancer using a multi-analyte test

Whether aneuploidy could be integrated as an additional biomarker into the published framework, as well as the predictive ability of a logistic regression model with aneuploidy and protein markers against the original logistic regression model that uses somatic mutations and protein markers, was compared.

Here, 1348 plasma samples from healthy people and 883 cancer patients were analyzed. Of the 1348 healthy samples, only 248 overlapped with the original study. All 883 cancer samples were included in the original study. The sample demographic information was provided in Table 3.

Using the original 812 healthy samples (Cohen et al.) and the 883 cancer samples, a logistic regression model was trained and then used to assess performance using ten rounds

of tenfold cross validation. A full list of samples and their biomarker values was provided in Table 3. Because 564 of the original healthy samples were not analyzed for aneuploidy, the list of scores from the 1348 normal samples was randomly sampled and assigned each missing sample an aneuploidy value. Ten rounds of analysis were performed and each new round, the collection of 1348 normal scores again randomly sampled to assign the 564 samples a new score.

To account for variations in the lower limits of detection across different experiments, the 90th percentile feature value was used in the healthy training samples. Any feature value below this threshold and set all values to the 90th percentile threshold. This transformation was done for all training and testing samples. This procedure was done for aneuploidy scores, somatic mutation scores, and protein concentrations. The 90th percentile thresholds and final feature coefficients from the logistic regression model were listed in Table 4.

Table 4. Logistic regression coefficients and thresholds.

	90th Percentile Values	Coefficients
Intercept		-11.8552
Aneuploidy	0.116389196	8.014704
Omega	1.145773698	2.343129
AFP	2866.68	9.26E-06
CA.125	6.9024	0.085206
CA19.9	22.6652	0.019665
CEA	2063.1449	0.00037
HGF	264.6446	0.004534
OPN	53651.1756	2.19E-05
Prolactin	21304.0703	4.84E-05
TIMP.1	85363.9233	1.18E-05

Comparison of aneuploidy sensitivity detection with other cancer biomarkers

The aneuploidy results were benchmarked against a driver gene mutation panel and collection of 7 proteins markers (AFP, CA-125, CA15-3, CA19-9, CEA, HGF, OPN, TIMP1) that were recently published as key biomarkers for cancer detection in plasma samples (Figure 4) (Cohen et. al 2018, *Science* 359(6378): 926-930). Aneuploidy outperformed all protein markers. Aneuploidy was also able to detect 42% of the samples that were missed by mutations and 34% of the samples that were missed by the mutation panel as well as the proteins. Due to the high specificity of this aneuploidy assay and the utility of each additional cancer biomarker, it will be understood that these components can be combined into a multi-analyte test for cancer detection.

Example 2: Detection of aneuploidy with low input DNA from trisomy 21 samples

Reliably detecting aneuploidy in only a few picograms (pg) of DNA is necessary for preimplantation diagnostics as well as forensic applications. In preimplantation diagnosis, a few cells picked from a blastocyst are used to assess copy number variations. For example, preimplantation diagnosis includes identifying a mammals as having aneuploidy related to Down Syndrome. To test the limit of detection with respect to input DNA for the methods featured in this disclosure, samples with aneuploidy associated with trisomy 21 were

analyzed at input DNA concentrations ranging from 3-225 pg. The relationship of reads to DNA was based on negative controls (water wells with no DNA) and the known concentration of the euploid control (Figure 6). Trisomy 21 aneuploidy was detected in every sample tested, even those with 3 pg of input DNA, representing half of a diploid cell. No chromosome arms other than chromosome 21 were found to be aneuploid in the Trisomy 21 samples. No chromosome arms, including chromosome 21, were found to be aneuploid in the euploid controls used in these experiments.

Example 3: Detection of aneuploidy with low input DNA from biobank samples

Samples from biobanks with low input DNA were assessed for either aneuploidy or identification purposes. The methods as described herein were applied to 793 plasma DNA samples, which had been stored in PCR plates for as long as 10 years. For each of the wells in the PCR plates, all of the DNA volume had been used for other experiments. Five microliters of water was added to the dried (empty) wells and then subjected to the methods as described herein. In 728 samples, more than 2.5 million aligned reads were sequenced, which is a number sufficient to reliably assess aneuploidy. In 768 of these samples, more than 1 million aligned reads were sequenced, a number sufficient to confirm the identity of the plasma DNA to other samples from the same donor.

Example 4: Detection of leukocyte DNA contamination in plasma sample

Plasma cfDNA is often contaminated with DNA that has leaked out of leukocytes, either through phlebotomy or preparation of plasma. This contaminating leukocyte DNA can reduce the sensitivity of aneuploidy testing from plasma samples because leukocytes are not derived from either fetal cells (in NIPT) or cancer cells (in liquid biopsies). Leukocyte genomic DNA (gDNA) has an average fragment size of >1000 bp while cell-free plasma DNA has an average size of <160 bp. Given that small fragments are amplified more efficiently during a PCR reaction, detection of contaminating leukocyte gDNA is difficult because the shorter cfDNA is preferentially amplified. Application of the methods described herein enabled the detection of contaminating leukocyte gDNA by virtue of the amplicons generated with primers SEQ ID NO: 1 and SEQ ID NO: 10. Using these methods, 1241

amplicons were identified that are typically present in gDNA but not cfDNA. Sequencing reads of these amplicons thereby indicated leukocyte contamination in plasma samples. Through mixing of leukocyte DNA with cell-free plasma DNA and using the methods described herein, samples containing >4% of leukocyte DNA could be detected, as shown in Table 5.

Table 5. Prediction of gDNA contamination in plasma.

	Total Reads	Reads that map to 1241 amplicons	Fraction of Reads that map to 1241 amplicons	Ratio of Fraction of reads to cfDNA
gDNA control	1420121	1302	0.000916823	13.6900667
euploid cell free DNA	9810368	657	6.697E-05	1
54% gDNA	16666542	31138	0.001868294	27.8974908
37% gDNA	13990980	19264	0.001376887	20.5597705
26% gDNA	10760112	10907	0.001013651	15.135907
19% gDNA	10976478	8769	0.00079889	11.929081
10% gDNA	9408703	3415	0.000362962	5.41977026
5.5% gDNA	9904155	2058	0.000207792	3.10275776
5.0% gDNA	9083013	1987	0.00021876	3.2665391
4.5% gDNA	8470920	1790	0.000211311	3.15531251
4% gDNA	8852813	2336	0.000263871	3.9401384

Example 5: Copy number analysis of indeterminate length

Copy number variants of indeterminate length were detected. First, the log ratio of the observed test sample and WALDO predicted values from every 500 kb interval across each chromosomal arm were calculated. Using the log ratio, a circular binary segmentation algorithm was applied to find copy number variants throughout each chromosome arm. Any copy number variant ≤ 5Mb in size was flagged. Before calculating the statistical significance across each chromosome arm, these flagged CNVs were removed. In general, small CNVs can be used to assess microdeletions or microamplifications, such as those occurring in DiGeorge Syndrome (chromosome 22q11.2 or in breast cancers (chromosome 17q12).

Example 6: Sensitivity of cancer detection with multi-analyte tests

This Example describes the sensitivity of cancer detection with different multi-analyte tests.

Three different multi-analyte tests were used to evaluate the sensitivity of detecting eight cancers: breast, ovary, liver, lung, pancreas, esophagus, stomach, and colorectum, in the plasma sample from patients. The three tests were: (1) a three component test using aneuploidy status, somatic mutation analysis and protein biomarker evaluation; (2) a two component test using aneuploidy status and somatic mutation analysis; and (3) a two component test using aneuploidy status and protein biomarker evaluation. The eight protein biomarkers tested and somatic mutations tested were as described in Cohen et al., *Science* **359**, pp. 926-930, the entire contents of which are hereby incorporated by reference.

As shown in **FIGs. 7A-7B**, the median sensitivity of detection of ovary, liver, lung, pancreas, esophagus, stomach, and colorectum cancer with the three component multi-analyte test was 80%, with a range of sensitivity of detection of 77% to 97%. The sensitivity of detection of breast cancer with the three component multi-analyte test was 38%. The sensitivities were calculated using a threshold at 99% specificity.

FIG. 8 further demonstrates true positive fraction (measure of sensitivity) of cancer detection using the following tests: (1) aneuploidy status; somatic mutation; and protein biomarker; (2) aneuploidy status and protein biomarker; (3) somatic mutation and protein biomarker; (4) aneuploidy status and somatic mutation; (5) aneuploidy status; and (6) somatic mutation. The specificity of detection was maintained at 99%.

As shown in **FIG. 8**, the three component multi-analyte test (aneuploidy status, somatic mutation analysis and protein biomarker evaluation) detected cancer at a sensitivity of 73% and with a specificity of 99%. The true positive fraction (a measure of sensitivity) was highest with the three component multi-analyte test as compared to the other tests.

As shown in Figure 9, a multi-analyte test (aneuploidy status and protein biomarker evaluation) detected cancer at a greater sensitivity than aneuploidy alone when looking at samples based cancer stage.

Thus, the data disclosed in this Example shows that the three component multi-analyte test with aneuploidy status, somatic mutation analysis and protein biomarker

evaluation can increase the sensitivity of detecting cancer while maintaining a high specificity of cancer detection.

Example 7: Determining somatic/germline status

The materials and methods described herein can be used to identify somatic mutations within the sequences of repetitive elements amplified from a sample (e.g., a tumor sample or a non-tumor sample (i.e., a normal sample)). For example, when two samples, a non-tumor sample and a tumor sample, are available from the same patient, mutations that are in one sample but not the other can be discerned. For each sample, the number of somatic mutations can be counted and the spectrum of single base substitutions (SBS) (e.g., A->T, A->C, etc.) determined. When the samples are also analyzed by exomic sequencing, a correlation between the number of SBSs in the repetitive elements amplified herein and the number of SBS in the exomes can be determined. Thus, the materials and methods as described herein can be used identify somatic mutations within a sample.

Example 8: Sample identification

The materials and methods described herein can be used to identify and/or distinguish samples (e.g., distinguish between a sample from one subject from a sample from a second subject). In such cases, samples are identified based on the common polymorphisms present in the repetitive elements amplified by materials and methods described herein. Samples are then distinguished from other samples by comparing the sequence at common polymorphisms between samples. Determining the genotype of each polymorphism for each of the amplicons assigns a genotype to the sample. Genotypes can be compared across samples in order to identify samples (e.g., distinguish tumor sample from non-tumor sample or a sample from one subject from a sample from a different subject). Samples can be considered to be from different samples if concordance (e.g., percent similarity between the genoytpes) was < 0.99 and at least 5,000 amplicons had adequate coverage.

Example 9: Detecting aneuploidy in different stages and different types of cancer

A set of experiments was performed to assess detection of aneuploidy in different stages and different types of cancer. In these experiments, plasma from subjects having

different stages of breast, colorectum, esophagus, liver, lung, ovary, pancreas and stomach cancers were isolated according to the methods described herein. Figure 10 shows aneuploidy (at 99% specificity) for Stage I (n=109), Stage II (n=276), and Stage III (173). Figure 11 shows aneuploidy (at 99% specificity) for the same cancers in Figure 7 displayed by cancer type (Figure 11) rather than cancer stage (Figure 10).

Using the Real Seq method, aneuploidy was detected more commonly than mutations in plasma samples from cancer patients. Aneuploidy was detected more commonly than mutations in plasma samples from cancer patients (49% and 34% of 883 samples, respectively; P < 10-20, one sided binomial test, **Fig. 19A**). With respect to tissue type, aneuploidy was detected more commonly than mutations in samples from patients with cancers of the esophagus, colorectum, pancreas, lung, stomach, and breast, (all P-values <0.01), less commonly in ovary (P=0.048), and equally commonly in liver cancer (**FIG. 19A**). With respect to stage, aneuploidy was detected more commonly than mutations in all stages especially stages I and II (**FIG. 19B**, P-values <10-9).

Example 10: Detecting cancer in samples using aneuploidy and protein biomarkers

A set of experiments was performed to assess sensitivity of cancer detection when combining aneuploidy detection with protein biomarker detection as described herein. In these experiments, plasma from the same cohort as in example 8 (e.g., different stages of breast, colorectum, esophagus, liver, lung, ovary, pancreas and stomach cancer) were assayed for aneuploidy and protein biomarkers. Figure 12 shows sensitivity of detection in the different stages of cancer (Stage I (n=109), Stage II (n=276), and Stage III (n=173).

Example 11: Comparison of Real Seq to Other Next Generation Sequencing Technologies

A set of experiments was performed to assess performance of Real Seq compared to other next generation sequencing technologies.

In the most common form of NIPT, detection of a gain or loss of a chromosome (e.g., chromosome 21 in Down Syndrome) is the goal. Whole genome sequencing (WGS), FAST-SeqS, and RealSeqS were used to assess performance on samples for DNA admixtures typically encountered in non-invasive prenatal testing (NIPT), i.e., when the fraction of fetal

DNA was 5%. For this purpose, actual data obtained with the three methods was used, but then a defined number of reads from various chromosome regions from the same samples were added to simulate what would happen if there was aneuploidy in these regions. The pseudocode used to generate these *in silico* simulated samples is described **FIG.13** and **FIG. 14**. The performance was calculated using a frequently used z-score that compares the observed fraction of reads on a particular chromosome arm to the average fraction of reads from a normal panel divided by the standard deviation in the normal panel. The results in total reads needed for all three approaches is reported, assuming single-end 100 bp reads and accounting for differences in alignment rates and filtering criteria typically used.

As shown in **FIG. 15A**, RealSeqS consistently achieved higher sensitivity at lower amounts of sequencing. For example, RealSeqS had 99% sensitivity (at 99% specificity) for monosomies and trisomies at a 5% cell fraction, while WGS and FAST-SeqS had 94% and 81% sensitivity, respectively (**FIG. 15A**).

Another important aspect of assays for copy number variation is the detection of relatively small regions which are deleted or amplified. For example, the DiGeorge Syndrome deletions are often as small as 1.5 Mb. For data simulating a 5% deletion-containing cell fraction, RealSeqS had 75.0% sensitivity for the 1.5 Mb DiGeorge deletion (at 99% Specificity) while WGS and FAST-SeqS had 19.0% and 29.0% sensitivity, respectively (**FIG. 15B**; and **FIGs. 16A-16B**).

The detection of amplifications, such as those on *ERBB2* in breast cancer, are important for deciding whether patients should be treated with trastuzumab or other targeted therapies. Following the same protocol as described above in this Example, *in silico* simulated samples with focal amplifications of the ~42 Kb ERBB2 gene (20 copies) were generated for WGS, FAST-SeqS, and RealSeqS. RealSeqS detected amplifications in the *in silico* simulated samples with significantly less sequencing compared to WGS or Fast-SeqS. For a 1% cell fraction, RealSeqS had a 91.0% sensitivity while WGS had 50.0% (**FIG. 15C;** and **FIGs. 17A-17B**).

This data shows that the Real SEQ technique can detect small regions that are amplified or deleted and the method has a higher sensitivity at lower amounts of sequencing.

Example 12: Detection of aneuploidy in samples with small concentration of tumor-derived DNA

A set of experiments was performed to assess detection of aneuploidy using the Real SEQ method in samples with varying concentrations of tumor-derived DNA. In assessing 302 samples in which the mutant allele fraction had been determined by the analysis of mutations that were present in the plasma (Cohen et al., *Science* 359; 926-930), aneuploidy was detected in 92% of 65 samples that had mutant allele fraction ≥ 2%, 71% of 65 samples with mutant allele fractions of 0.5% to 2%, and in 49% of 172 samples with mutant allele frequencies ranging from 0.01% to 0.5% (**FIG. 18**). The differences in aneuploidy among these three classes of samples was significant (P<10-3, one sided binomial test).

The data shows that the Real Seq method can detect aneuploidy, e.g., even at low concentrations of tumor DNA. Therefore, the sensitivity of detecting aneuploidy is related to the concentration of circulating tumor DNA in the sample.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7666.9_faster1		110962	INDI 250 PL	Colorectum	II	1001795	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7561.9_faster1		10772	INDI 260 PL	Stomach	I	1004248	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7649.7_faster1		110913	INDI 548 PL	Breast	II	1053642	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7678.11_faster1		111047	INDI 927 PL	Breast	II	1091466	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7560.11_faster1		108985	INDI 534 PL	Stomach	III	1112490	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7537.3_faster1		110697	PAPA 1350	Ovary	II	1118254	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.10_faster1		110925	INDI 354 PL	Colorectum	III	1196533	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7613.11_faster1		109125	INDI 854 PL	Colorectum	II	1213691	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7563.8_faster1		109084	INDI 762 PL	Esophagus	II	1217677	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7537.11_faster1		110621	PANC 675 P	Pancreas	II	1228946	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
7591.11_faster1		109110	INDI 818 PL	Ovary	III	1320220	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7586.5_faster1		109014	INDI 622 PL	Liver	III	1336412	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive
7561.4_faster1		109075	INDI 743 PL	Stomach	II	1388535	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7589.8_faster1		109143	INDI 896 PL	Lung	I	1412655	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7586.6_faster1		109020	INDI 636 PL	Liver	III	1470803	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7671.10_faster1		110996	INDI 767 PL	Colorectum	II	1492197	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7560.10_faster1		108981	INDI 526 PL	Esophagus	III	1514882	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7740.10_faster1		111066	CRC 478 PL	Colorectum	III	1559066	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7671.12_faster1		110998	INDI 769 PL	Colorectum	II	1562172	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7013.8_faster1		109015	INDI 623 PL	Breast	III	1597394	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7645.6_faster1		110872	INDI 593 PL	Breast	II	1598322	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7014.12_faster1		110796	INDI 244 PL	Colorectum	III	1615175	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7009.10_faster1		10888	INDI 445 PL	Breast	II	1629340	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7541.9_faster1		110631	PANCA 100	Pancreas	II	1666957	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7665.5_faster1		110951	INDI 679 PL	Colorectum	II	1693864	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7541.11_faster1		110633	PANCA 100	Pancreas	II	1733623	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
7567.8_faster1		108991	INDI 545 PL	Lung	I	1734591	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7542.10_faster1		110642	PANCA 102	Pancreas	II	1773560	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7678.10_faster1		111046	INDI 926 PL	Breast	II	1778136	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7673.12_faster1		111018	INDI 816 PL	Colorectum	II	1780759	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7666.4_faster1		110968	INDI 903 PL	Breast	I	1795451	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7562.10_faster1		109108	INDI 814 PL	Stomach	I	1843118	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7537.9_faster1		110619	PANC 673 P	Pancreas	II	1851442	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7594.3_faster1		10859	INDI 389 PL	Lung	III	1900877	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6837.6_faster1		109118	INDI 844 PL	Stomach	I	1953864	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7613.10_faster1		109122	INDI 849 PL	Colorectum	II	1998995	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7589.12_faster1		10856	INDI 384 PL	Pancreas	II	2067166	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7537.12_faster1		110622	PANC 676 P	Pancreas	II	2084372	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Negative
7591.12_faster1		109129	INDI 862 PL	Pancreas	II	2093931	Negative	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7012.7_faster1	Yes	109090	INDI 778 PL	Liver	II	2117457	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7667.9_faster1		110975	INDI 286 PL	Colorectum	I	2160308	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7548.9_faster1		10811	INDI 324 PL	Lung	I	2184419	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7665.12_faster1		110957	INDI 898 PL	Breast	I	2253404	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7671.3_faster1		110991	INDI 750 PL	Colorectum	III	2288848	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7536.3_faster1		110687	PAPA 1335	Ovary	III	2325428	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6857.4_faster1		109067	INDI 729 PL	Colorectum	II	2350770	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7676.8_faster1		111019	INDI 793 PL	Breast	II	2366123	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7561.7_faster1		109072	INDI 737 PL	Stomach	I	2456741	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7590.9_faster1		109070	INDI 735 PL	Pancreas	II	2464857	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7591.8_faster1		109068	INDI 731 PL	Pancreas	II	2481059	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7643.9_faster1		110852	INDI 610 PL	Colorectum	II	2503195	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7613.6_faster1		10758	INDI 230 PL	Colorectum	I	2539061	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7666.5_faster1		110966	INDI 897 PL	Breast	III	2577755	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7666.8_faster1		110961	INDI 248 PL	Colorectum	II	2641351	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7560.12_faster1		109141	INDI 892 PL	Stomach	I	2671691	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7590.4_faster1		108984	INDI 533 PL	Pancreas	II	2692393	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7639.4_faster1		110820	INDI 431 PL	Colorectum	II	2783016	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7637.3_faster1		110799	INDI 295 PL	Colorectum	II	2793496	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7611.9_faster1		10724	INDI 192 PL	Colorectum	II	2812098	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7612.3_faster1		10728	INDI 197 PL	Colorectum	II	2816342	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7645.12_faster1		110878	INDI 606 PL	Breast	II	2818021	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7646.5_faster1		110881	INDI 627 PL	Breast	II	2833357	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7546.10_faster1		10572	INDI 022 PL	Lung	III	2834538	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7613.7_faster1		10760	INDI 233 PL	Colorectum	I	2871111	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7543.11_faster1		110655	PANCA 105	Pancreas	II	2959475	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7676.11_faster1		111022	INDI 819 PL	Breast	III	2969142	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7563.6_faster1		109079	INDI 753 PL	Esophagus	III	2987451	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
6851.10_faster1		109077	INDI 751 PL	Colorectum	III	2991204	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7613.5_faster1		10757	INDI 229 PL	Colorectum	II	3055625	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7672.10_faster1		111001	INDI 725 PL	Colorectum	II	3073272	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7673.10_faster1		111016	INDI 809 PL	Colorectum	III	3105813	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7544.3_faster1		110657	PANCA 105	Pancreas	II	3128340	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7646.6_faster1		110882	INDI 629 PL	Breast	I	3214436	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7041.3_faster1		109053	INDI 701 PL	Colorectum	II	3276672	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6851.12_faster1		109092	INDI 781 PL	Colorectum	II	3295690	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6837.11_faster1		10739	INDI 210 PL	Stomach	III	3317316	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7590.8_faster1		109069	INDI 734 PL	Pancreas	II	3318380	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7638.11_faster1		110817	INDI 427 PL	Colorectum	II	3323134	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative
7561.5_faster1		109106	INDI 803 PL	Esophagus	II	3348473	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7567.10_faster1		108994	INDI 549 PL	Lung	III	3353566	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7678.7_faster1		111043	INDI 922 PL	Breast	II	3355196	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7740.7_faster1		111063	CRC 475 PL	Colorectum	I	3360401	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7590.3_faster1		10858	INDI 388 PL	Pancreas	II	3361282	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7589.3_faster1		109045	INDI 691 PL	Lung	I	3394538	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7542.12_faster1		110646	PANCA 103	Pancreas	II	3397925	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7678.4_faster1		111040	INDI 919 PL	Breast	II	3424684	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7534.12_faster1		110667	PAP 944 PL	Ovary	III	3428688	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7642.12_faster1		110848	INDI 599 PL	Colorectum	II	3480043	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7611.6_faster1		10711	INDI 178 PL	Colorectum	III	3487293	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7541.12_faster1		110634	PANCA 101	Pancreas	I	3488313	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7585.5_faster1		10722	INDI 190 PL	Liver	III	3507555	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative
7639.6_faster1		110822	INDI 434 PL	Colorectum	II	3557770	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7592_combined.7_faste	Yes	10818	INDI 335 PL	Lung	I	3560831	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7677.4_faster1		111030	INDI 830 PL	Breast	II	3584227	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7566.8_faster1		10730	INDI 199 PL	Stomach	II	3594027	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7739.8_faster1		111054	CRC 462 PL	Colorectum	I	3597935	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7584.8_faster1		10701	INDI 167 PL	Stomach	II	3615650	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7670.10_faster1		110986	INDI 658 PL	Colorectum	I	3655451	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7549.6_faster1		108964	INDI 495 PL	Lung	I	3683648	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7535.5_faster1		110677	PAP 957 PL	Ovary	III	3701875	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7740.6_faster1		111062	CRC 474 PL	Colorectum	III	3704739	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

WO 2020/236625 PCT/US2020/033209

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7542.9_faster1		110641	PANCA 102	Pancreas	II	3743768	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
7589.10_faster1		10786	INDI 278 PL	Ovary	III	3747939	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7640.7_faster1		110833	INDI 477 PL	Colorectum	III	3762894	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6857.9_faster1	Yes	10855	INDI 383 PL	Lung	III	3790321	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7667.10_faster1		110976	INDI 290 PL	Colorectum	I	3795297	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7649.4_faster1		110910	INDI 512 PL	Breast	II	3801892	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7640.8_faster1		110834	INDI 483 PL	Colorectum	II	3821378	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7643.6_faster1		110853	INDI 611 PL	Colorectum	II	3824328	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7543.4_faster1		110648	PANCA 103	Pancreas	II	3835324	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7589.6_faster1		10624	INDI 075 PL	Ovary	III	3846965	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7638.12_faster1		110818	INDI 428 PL	Colorectum	III	3869695	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative
7563.11_faster1		10803	INDI 313 PL	Esophagus	II	3887013	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
6839_redo.8_faster1		109001	INDI 574 PL	Colorectum	III	3890070	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7643.10_faster1		110856	INDI 617 PL	Colorectum	III	3901698	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7547.6_faster1		10759	INDI 231 PL	Lung	III	3915987	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7664.12_faster1		110948	INDI 551 PL	Colorectum	II	3925074	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7677.10_faster1		111036	INDI 842 PL	Breast	I	3973438	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7591.4_faster1		109051	INDI 699 PL	Pancreas	II	3993186	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7671.11_faster1		110997	INDI 768 PL	Colorectum	II	4013502	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7591.5_faster1		109055	INDI 703 PL	Lung	I	4019055	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7672.12_faster1		111008	INDI 782 PL	Colorectum	III	4055831	Positive	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7586.7_faster1		109024	INDI 645 PL	Liver	III	4073694	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7562.4_faster1		10761	INDI 236 PL	Stomach	II	4149785	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7566.9_faster1		10733	INDI 203 PL	Stomach	II	4178571	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7664.10_faster1		110946	INDI 528 PL	Colorectum	II	4210687	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7676.4_faster1		111025	INDI 825 PL	Colorectum	III	4269747	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7547.11_faster1		10775	INDI 264 PL	Lung	III	4275481	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
7638.5_faster1		110811	INDI 314 PL	Colorectum	I	4287508	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7664.9_faster1		110945	INDI 527 PL	Colorectum	III	4309014	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7603.12_faster1		10652	INDI 108 PL	Colorectum	II	4311628	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6835.3_faster1		110675	PAP 955 PL	Ovary	III	4325563	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7677.3_faster1		111029	INDI 823 PL	Breast	II	4334923	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7013.6_faster1		109011	INDI 609 PL	Colorectum	II	4345601	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7305.10_faster1		109137	INDI 880 PL	Breast	II	4389478	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7678.12_faster1		111048	INDI 929 PL	Stomach	II	4398098	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7011.7_faster1		10717	INDI 185 PL	Stomach	II	4409233	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive
7610.10_faster1		10698	INDI 163 PL	Colorectum	III	4431295	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7637.7_faster1		110803	INDI 302 PL	Colorectum	I	4442068	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7589.7_faster1		109140	INDI 888 PL	Pancreas	II	4482143	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7013.12_faster1		10825	INDI 343 PL	Colorectum	III	4512396	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7536.6_faster1		110690	PAPA 1342	Ovary	III	4547749	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7560.7_faster1		10807	INDI 318 PL	Stomach	III	4556278	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
7585.10_faster1		10889	INDI 446 PL	Esophagus	II	4580159	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
7640.12_faster1		110838	INDI 564 PL	Colorectum	I	4604216	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7543.3_faster1		110647	PANCA 103	Pancreas	II	4606920	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7042.3_faster1		109113	INDI 827 PL	Colorectum	III	4629206	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7645.8_faster1		110874	INDI 597 PL	Breast	II	4629350	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6858.5_faster1		10543	CRC 494 PL	Colorectum	II	4631558	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7663.11_faster1		110937	INDI 484 PL	Colorectum	II	4635397	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7610.12_faster1		10700	INDI 165 PL	Colorectum	I	4671536	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7672.5_faster1		111005	INDI 774 PL	Colorectum	I	4676699	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7549.11_faster1		108973	INDI 513 PL	Lung	I	4706497	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.12_faster1		110927	INDI 374 PL	Colorectum	III	4752071	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7666.11_faster1		110964	INDI 253 PL	Colorectum	I	4756889	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7542.4_faster1		110636	PANCA 101	Pancreas	II	4841695	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7677.7_faster1		111033	INDI 833 PL	Breast	I	4842387	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7677.8_faster1		111034	INDI 839 PL	Breast	II	4846046	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7672.3_faster1		111003	INDI 772 PL	Colorectum	II	4846546	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7593.8_faster1		10847	INDI 371 PL	Lung	III	4871802	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7560.8_faster1		10808	INDI 319 PL	Stomach	I	4915897	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7535.8_faster1		110678	PAP 959 PL	Ovary	III	4940585	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7562.6_faster1		10765	INDI 241 PL	Stomach	III	4948571	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.3_faster1		110928	INDI 889 PL	Colorectum	II	4974578	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7640.6_faster1		110832	INDI 472 PL	Colorectum	II	4977178	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7546.6_faster1		10566	INDI 013 PL	Lung	III	4982928	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7010.8_faster1		10567	INDI 014 PL	Lung	II	4993575	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7041.11_faster1		109085	INDI 764 PL	Colorectum	II	5040751	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7542.7_faster1		110639	PANCA 101	Pancreas	III	5064729	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative

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Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7676.10_faster1		111021	INDI 795 PL	Breast	II	5082422	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7010.11_faster1		108980	INDI 524 PL	Lung	III	5095014	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7593.10_faster1		10849	INDI 373 PL	Lung	I	5110714	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7601.12_faster1		10585	INDI 036 PL	Colorectum	I	5136788	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7611.7_faster1		10712	INDI 179 PL	Colorectum	III	5145980	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7603.5_faster1		10641	INDI 094 PL	Colorectum	I	5157994	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7672.7_faster1		111007	INDI 779 PL	Colorectum	II	5168461	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7563.10_faster1		109089	INDI 776 PL	Esophagus	II	5205936	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7536.10_faster1		110694	PAPA 1347	Ovary	I	5206646	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7664.11_faster1		110947	INDI 544 PL	Colorectum	III	5295602	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7534.8_faster1		110673	PAP 951 PL	Ovary	III	5341189	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6836.4_faster1		10579	INDI 029 PL	Colorectum	II	5358468	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7603.10_faster1		10648	INDI 102 PL	Colorectum	III	5389348	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7542.8_faster1		110640	PANCA 101	Pancreas	I	5459643	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7676.3_faster1		111023	INDI 821 PL	Colorectum	III	5507106	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7609.5_faster1		10662	INDI 122 PL	Colorectum	II	5524030	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
6838_redo.4_faster1		109120	INDI 847 PL	Liver	III	5531516	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7584.9_faster1		10702	INDI 168 PL	Stomach	II	5532836	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Positive	Negative
7612.5_faster1		10736	INDI 207 PL	Colorectum	II	5541362	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7541.4_faster1		110624	PANC 679 P	Pancreas	II	5689544	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7613.9_faster1		109121	INDI 848 PL	Colorectum	II	5696303	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7606.6_faster1	Yes	10634	INDI 085 PL	Stomach	I	5707150	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7602.3_faster1		10586	INDI 037 PL	Colorectum	I	5727492	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7042.7_faster1	Yes	10827	INDI 346 PL	Lung	I	5738285	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7041.12_faster1		109091	INDI 780 PL	Colorectum	II	5750070	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7642.5_faster1		110841	INDI 573 PL	Colorectum	I	5761449	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7672.4_faster1		111004	INDI 773 PL	Colorectum	II	5842790	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7535.6_faster1		110669	PAP 946 PL	Ovary	III	5847253	Negative	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7662.6_faster1		110921	INDI 325 PL	Colorectum	III	5847693	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7673.8_faster1		111009	INDI 708 PL	Colorectum	III	5876494	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7534.3_faster1		110663	PAP 938 PL	Ovary	III	5879145	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7613.8_faster1		109119	INDI 846 PL	Colorectum	II	5880716	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7759.5_faster1	Yes	10657	INDI 113 PL	Colorectum	II	5880730	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative
7739.6_faster1		111052	CRC 459 PL	Colorectum	II	5886699	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

WO 2020/236625 PCT/US2020/033209

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7637.5_faster1		110801	INDI 298 PL	Colorectum	I	5905840	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7603.6_faster1		10643	INDI 096 PL	Colorectum	I	5947450	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7537.5_faster1		110700	PAPA 1356	Ovary	II	5951081	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative
6857.5_faster1		109112	INDI 826 PL	Colorectum	II	5985530	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7678.9_faster1		111045	INDI 924 PL	Breast	III	6013647	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7541.7_faster1		110629	PANCA 100	Pancreas	II	6099173	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7667.4_faster1		110970	INDI 259 PL	Colorectum	II	6123288	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7672.8_faster1		110999	INDI 718 PL	Colorectum	II	6127303	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7535.12_faster1		110686	PAPA 1334	Ovary	III	6128628	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7665.9_faster1		110956	INDI 732 PL	Colorectum	III	6144208	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7306.11_faster1	Yes	10810	INDI 323 PL	Breast	I	6160359	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7536.4_faster1		110688	PAPA 1336	Ovary	I	6166344	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7742.8_faster1		111084	CRC 508 PL	Colorectum	I	6174674	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7546.5_faster1		10564	INDI 011 PL	Lung	I	6198380	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7663.7_faster1		110933	INDI 386 PL	Colorectum	III	6229120	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7671.5_faster1		110990	INDI 747 PL	Colorectum	III	6268955	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
7662.11_faster1		110926	INDI 356 PL	Colorectum	I	6313478	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7566.6_faster1		10679	INDI 140 PL	Stomach	III	6315974	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7013.5_faster1		109010	INDI 602 PL	Colorectum	II	6319245	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7010.9_faster1		10790	INDI 285 PL	Lung	II	6332234	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7642.8_faster1		110844	INDI 584 PL	Colorectum	II	6358208	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7759.4_faster1	Yes	10655	INDI 111 PL	Stomach	II	6406233	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7544.7_faster1		110661	PANCA 106	Pancreas	II	6418433	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7546.7_faster1		10568	INDI 015 PL	Lung	I	6421654	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6836.5_faster1		10584	INDI 035 PL	Colorectum	II	6441354	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7673.7_faster1		111014	INDI 806 PL	Colorectum	II	6483291	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7642.9_faster1		110845	INDI 587 PL	Colorectum	II	6510398	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7562.7_faster1		109102	INDI 799 PL	Stomach	II	6515486	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7612.11_faster1		10749	INDI 221 PL	Colorectum	III	6591134	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7670.12_faster1		110988	INDI 749 PL	Colorectum	III	6600357	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6835.9_faster1		110643	PANCA 102	Pancreas	III	6600637	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7590.10_faster1		109071	INDI 736 PL	Lung	III	6627333	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7548.4_faster1		10781	INDI 273 PL	Lung	I	6643421	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7742.9_faster1		111085	CRC 509 PL	Colorectum	I	6656386	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7009.5_faster1		10613	INDI 064 PL	Breast	II	6670500	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7673.11_faster1		111017	INDI 815 PL	Colorectum	II	6700399	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7611.5_faster1		10708	INDI 175 PL	Colorectum	III	6720895	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
6838_redo.11_faster1		10823	INDI 341 PL	Liver	II	6731601	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Positive
7565.10_faster1		10672	INDI 132 PL	Esophagus	III	6748979	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7593.3_faster1		10834	INDI 357 PL	Lung	I	6771463	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7673.6_faster1		111013	INDI 805 PL	Colorectum	II	6780095	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7011.9_faster1		10729	INDI 198 PL	Stomach	III	6793614	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7603.7_faster1		10645	INDI 098 PL	Colorectum	II	6797379	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7642.10_faster1		110846	INDI 594 PL	Colorectum	III	6797686	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6851.3_faster1		10766	INDI 245 PL	Colorectum	III	6799700	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7534.6_faster1		110681	PAP 974 PL	Ovary	I	6896369	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7586.4_faster1		109006	INDI 586 PL	Liver	III	6916059	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7663.3_faster1		110929	INDI 376 PL	Colorectum	I	6954053	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7544.5_faster1		110659	PANCA 105	Pancreas	II	7045546	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
6851.4_faster1		10770	INDI 255 PL	Colorectum	II	7093072	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7307.12_faster1		109035	INDI 672 PL	Breast	II	7145901	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.3_faster1		110969	INDI 256 PL	Colorectum	III	7157328	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7757.10_faster1	Yes	10608	INDI 059 PL	Breast	II	7168762	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7536.12_faster1		110696	PAPA 1349	Ovary	III	7185446	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Positive
6836.11_faster1		10573	INDI 023 PL	Lung	II	7249605	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7535.7_faster1		110670	PAP 947 PL	Ovary	III	7250255	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7640.3_faster1		110829	INDI 463 PL	Colorectum	II	7284271	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7541.5_faster1		110625	PANC 680 P	Pancreas	II	7288598	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7609.12_faster1		10686	INDI 148 PL	Colorectum	II	7304513	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative
7640.5_faster1		110831	INDI 468 PL	Colorectum	II	7330421	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7014.3_faster1		10826	INDI 344 PL	Colorectum	II	7374671	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7543.8_faster1		110652	PANCA 104	Pancreas	II	7374965	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7639.11_faster1		110827	INDI 452 PL	Colorectum	II	7402986	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
6837.4_faster1		10878	INDI 415 PL	Lung	II	7421121	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7665.8_faster1		110954	INDI 685 PL	Colorectum	III	7485730	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7759.7_faster1	Yes	10660	INDI 120 PL	Stomach	III	7527786	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7535.11_faster1		110685	PAPA 1333	Ovary	III	7562225	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative
7639.12_faster1		110828	INDI 456 PL	Colorectum	II	7585556	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7671.6_faster1		110992	INDI 758 PL	Colorectum	II	7592504	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
7664.6_faster1		110942	INDI 521 PL	Colorectum	II	7619123	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7739.10_faster1		111056	CRC 464 PL	Colorectum	III	7661499	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.4_faster1		109061	INDI 715 PL	Breast	III	7674698	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7645.11_faster1		110877	INDI 604 PL	Breast	II	7683175	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7541.8_faster1		110630	PANCA 100	Pancreas	II	7733461	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7758.5_faster1	Yes	10631	INDI 082 PL	Stomach	II	7735285	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7541.6_faster1		110626	PANC 757 F	Pancreas	II	7765953	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
6835.7_faster1		110698	PAPA 1354	Ovary	I	7771957	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7593.6_faster1		10841	INDI 364 PL	Lung	I	7821491	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7676.12_faster1		111024	INDI 822 PL	Colorectum	II	7842252	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.6_faster1		110972	INDI 271 PL	Colorectum	III	7853461	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7643.8_faster1		110855	INDI 615 PL	Colorectum	II	7866361	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7544.8_faster1		10556	INDI 001 PL	Lung	II	7887098	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7548.8_faster1		10795	INDI 292 PL	Lung	III	7923986	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7670.6_faster1		110982	INDI 650 PL	Colorectum	III	7954809	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7601.7_faster1		108954	INDI 480 PL	Breast	III	7955468	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7606.8_faster1	Yes	10764	INDI 239 PL	Lung	III	7977349	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7014.11_faster1		10623	INDI 074 PL	Pancreas	II	7990226	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7670.9_faster1		110985	INDI 655 PL	Colorectum	II	8062805	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7664.4_faster1		110940	INDI 508 PL	Colorectum	III	8148475	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7759.3_faster1	Yes	10654	INDI 110 PL	Stomach	I	8193900	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6836.8_faster1		10562	INDI 009 PL	Lung	II	8217852	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7564.4_faster1		10718	INDI 186 PL	Stomach	II	8224625	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Positive
6850.10_faster1		108996	INDI 555 PL	Colorectum	II	8231841	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7307.9_faster1		108962	INDI 493 PL	Breast	II	8263363	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7014.4_faster1		10842	INDI 365 PL	Colorectum	II	8272863	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7676.5_faster1		111026	INDI 829 PL	Colorectum	III	8309300	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7673.3_faster1		111010	INDI 783 PL	Colorectum	III	8375574	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7639.9_faster1		110825	INDI 438 PL	Colorectum	II	8414491	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.8_faster1		109095	INDI 787 PL	Breast	II	8446437	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6851.5_faster1		10796	INDI 293 PL	Colorectum	II	8469925	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7756.3_faster1	Yes	10536	CRC 470 PL	Colorectum	II	8479440	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7637.8_faster1		110804	INDI 303 PL	Colorectum	I	8479921	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7643.7_faster1		110854	INDI 612 PL	Colorectum	II	8511230	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6835.11_faster1		10619	INDI 070 PL	Breast	III	8512193	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7304.12_faster1	Yes	109027	INDI 659 PL	Breast	II	8533292	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.7_faster1		109065	INDI 722 PL	Breast	I	8569420	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7542.11_faster1		110644	PANCA 102	Pancreas	III	8585600	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6836.12_faster1		10748	INDI 220 PL	Lung	I	8603684	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7547.12_faster1		10777	INDI 266 PL	Lung	I	8648492	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7590.11_faster1		109050	INDI 698 PL	Ovary	III	8685561	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7612.9_faster1		10745	INDI 216 PL	Colorectum	III	8704765	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7663.6_faster1		110932	INDI 385 PL	Colorectum	I	8710642	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7546.4_faster1		10563	INDI 010 PL	Lung	II	8717278	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7607.8_faster1	Yes	108934	INDI 454 PL	Breast	III	8718537	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7308.10_faster1		109097	INDI 790 PL	Breast	II	8724316	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7547.5_faster1		10755	INDI 227 PL	Lung	II	8748209	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7677.12_faster1		111037	INDI 915 PL	Breast	II	8772028	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7534.9_faster1		110665	PAP 940 PL	Ovary	III	8792693	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7561.6_faster1		108974	INDI 514 PL	Stomach	I	8794282	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7012.8_faster1		10630	INDI 081 PL	Colorectum	II	8817504	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7306.10_faster1	Yes	10756	INDI 228 PL	Breast	II	8829767	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.7_faster1		110922	INDI 328 PL	Colorectum	I	8840984	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7610.4_faster1		10689	INDI 152 PL	Colorectum	I	8956285	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7548.12_faster1		10877	INDI 414 PL	Lung	II	8958632	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7592_combined.6_faste	Yes	10870	INDI 405 PL	Lung	I	8959025	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7638.6_faster1		110812	INDI 418 PL	Colorectum	I	8981237	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7594.10_faster1		109147	INDI 909 PL	Esophagus	II	9046563	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7041.10_faster1		109078	INDI 752 PL	Colorectum	III	9074211	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7609.11_faster1		10684	INDI 146 PL	Colorectum	II	9111916	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6857.8_faster1		10789	INDI 284 PL	Colorectum	II	9113264	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7543.9_faster1		110653	PANCA 104	Pancreas	II	9121697	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6838_redo.10_faster1		10885	INDI 441 PL	Esophagus	II	9134834	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative
7739.3_faster1		111049	CRC 455 PL	Colorectum	I	9143292	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6858.8_faster1		10546	CRC 502 PL	Colorectum	I	9149440	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7303.4_faster1	Yes	10616	INDI 067 PL	Breast	III	9228387	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7043.10_faster1		10548	CRC 506 PL	Colorectum	III	9248101	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7548.6_faster1		10792	INDI 288 PL	Lung	III	9282231	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7305.11_faster1		109138	INDI 882 PL	Breast	II	9338220	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7308.3_faster1		109060	INDI 713 PL	Breast	II	9357095	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7757.7_faster1	Yes	10591	INDI 043 PL	Colorectum	II	9366800	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7611.10_faster1		10725	INDI 194 PL	Colorectum	II	9392094	Negative	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7608.4_faster1	Yes	109040	INDI 684 PL	Liver	III	9409297	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7757.12_faster1	Yes	10614	INDI 065 PL	Breast	II	9414696	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7606.9_faster1	Yes	10767	INDI 246 PL	Lung	II	9434379	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7603.8_faster1		10646	INDI 100 PL	Colorectum	III	9451781	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7608.10_faster1	Yes	109116	INDI 841 PL	Colorectum	III	9466736	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7041.4_faster1		109057	INDI 709 PL	Colorectum	II	9517584	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7592_combined.12_fas	Yes	10833	INDI 355 PL	Lung	I	9526010	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7640.10_faster1		110836	INDI 560 PL	Colorectum	I	9530486	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7601.6_faster1		108953	INDI 479 PL	Breast	III	9541909	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7606.10_faster1	Yes	10774	INDI 262 PL	Breast	III	9555061	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7305.3_faster1	Yes	109030	INDI 664 PL	Breast	I	9557747	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7303.10_faster1		10887	INDI 443 PL	Breast	II	9563313	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7042.9_faster1	Yes	10865	INDI 395 PL	Lung	I	9572740	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7592_combined.5_faste	Yes	10816	INDI 333 PL	Lung	II	9598766	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7536.8_faster1		110692	PAPA 1345	Ovary	III	9607992	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7543.7_faster1		110651	PANCA 104	Pancreas	II	9631283	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7671.8_faster1		110994	INDI 763 PL	Colorectum	I	9670355	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7646.10_faster1		110886	INDI 641 PL	Breast	III	9693253	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7589.5_faster1		109047	INDI 694 PL	Lung	I	9721620	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7642.4_faster1		110840	INDI 572 PL	Colorectum	II	9722277	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7306.8_faster1		10604	INDI 056 PL	Breast	II	9734458	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7563.12_faster1		10715	INDI 183 PL	Stomach	I	9735365	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7672.11_faster1		111002	INDI 726 PL	Colorectum	I	9752953	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7760.10_faster1	Yes	10695	INDI 160 PL	Colorectum	II	9782990	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7663.8_faster1		110934	INDI 396 PL	Colorectum	I	9785678	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7011.3_faster1		109009	INDI 595 PL	Esophagus	II	9794909	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7303.3_faster1	Yes	10612	INDI 063 PL	Breast	III	9856414	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7637.11_faster1		110807	INDI 306 PL	Colorectum	III	9948291	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive
7665.7_faster1		110953	INDI 683 PL	Colorectum	II	9971674	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

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Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7606.11_faster1	Yes	10788	INDI 283 PL	Lung	I	9981617	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7676.6_faster1		111027	INDI 835 PL	Colorectum	III	10028345	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6857.10_faster1	Yes	10864	INDI 394 PL	Lung	II	10043000	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7646.12_faster1		110888	INDI 656 PL	Breast	III	10052211	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7644.4_faster1		110865	INDI 628 PL	Colorectum	II	10122473	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7012.6_faster1		109021	INDI 639 PL	Liver	II	10166996	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Negative	Positive
7759.12_faster1	Yes	10671	INDI 131 PL	Stomach	II	10187523	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7758.8_faster1	Yes	10636	INDI 087 PL	Stomach	I	10207839	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7642.3_faster1		110839	INDI 570 PL	Colorectum	II	10224108	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7757.8_faster1	Yes	10595	INDI 047 PL	Colorectum	II	10273757	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7637.4_faster1		110800	INDI 296 PL	Colorectum	II	10302905	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7607.7_faster1	Yes	10854	INDI 382 PL	Breast	II	10327454	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6837.12_faster1		10650	INDI 104 PL	Stomach	III	10346980	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
6851.6_faster1		108975	INDI 516 PL	Pancreas	II	10382498	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7041.8_faster1		109016	INDI 624 PL	Colorectum	II	10523846	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6838_redo.8_faster1		10744	INDI 215 PL	Liver	II	10635260	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7666.12_faster1		110965	INDI 258 PL	Colorectum	I	10661884	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7590.5_faster1		108987	INDI 537 PL	Ovary	I	10688024	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7756.12_faster1	Yes	10577	INDI 027 PL	Colorectum	II	10705925	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7608.9_faster1	Yes	109066	INDI 723 PL	Ovary	III	10787127	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7670.3_faster1		110979	INDI 644 PL	Colorectum	I	10792862	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7644.3_faster1		110864	INDI 621 PL	Colorectum	II	10808736	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.11_faster1		110978	INDI 742 PL	Breast	II	10866829	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7544.10_faster1		10558	INDI 003 PL	Lung	III	10894563	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
7663.12_faster1		110938	INDI 501 PL	Colorectum	III	10896229	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7593.4_faster1		10835	INDI 358 PL	Lung	II	10897648	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7304.10_faster1	Yes	109003	INDI 579 PL	Breast	I	10914894	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7638.9_faster1		110815	INDI 422 PL	Colorectum	II	10943979	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
7740.5_faster1		111061	CRC 473 PL	Colorectum	II	10946083	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7608.7_faster1	Yes	109052	INDI 700 PL	Lung	III	10952423	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
6857.6_faster1		109115	INDI 838 PL	Colorectum	III	10963339	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7307.11_faster1		108990	INDI 540 PL	Breast	II	10967901	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7758.9_faster1	Yes	10637	INDI 089 PL	Colorectum	II	10985306	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7759.9_faster1	Yes	10668	INDI 128 PL	Colorectum	III	11005383	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>2132 1)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >15777 2	TIMP-1 (pg/ml) + > 176989	Mut+
7537.10_faster1		110620	PANC 674 F	Pancreas	II	11007356	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative
7739.9_faster1		111055	CRC 463 PL	Colorectum	I	11037586	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7646.9_faster1		110885	INDI 638 PL	Breast	I	11134045	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7609.10_faster1		10683	INDI 145 PL	Colorectum	II	11146634	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7756.11_faster1	Yes	10576	INDI 026 PL	Lung	I	11148157	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7607.12_faster1	Yes	108971	INDI 507 PL	Lung	II	11155220	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7758.4_faster1	Yes	10625	INDI 076 PL	Esophagus	II	11178927	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6850.8_faster1		108982	INDI 529 PL	Colorectum	I	11205319	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7756.5_faster1	Yes	10554	CRC 531 PL	Colorectum	III	11205988	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7759.8_faster1	Yes	10667	INDI 127 PL	Colorectum	II	11226932	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7547.10_faster1		10771	INDI 257 PL	Lung	III	11239146	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7758.6_faster1	Yes	10633	INDI 084 PL	Stomach	II	11265793	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6858.10_faster1		10551	CRC 521 PL	Colorectum	III	11273801	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7541.10_faster1		110632	PANCA 100	Pancreas	II	11278288	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.4_faster1		110919	INDI 321 PL	Colorectum	III	11281006	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7561.3_faster1		108993	INDI 547 PL	Stomach	III	11284513	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7607.10_faster1	Yes	108959	INDI 490 PL	Breast	III	11318226	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7306.12_faster1	Yes	10815	INDI 332 PL	Breast	I	11332438	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7304.11_faster1	Yes	109013	INDI 619 PL	Breast	II	11334967	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6850.12_faster1		109038	INDI 678 PL	Colorectum	III	11356102	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7590.6_faster1		109041	INDI 686 PL	Pancreas	II	11430231	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7306.6_faster1		10607	INDI 058 PL	Breast	I	11455317	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7643.12_faster1		110858	INDI 620 PL	Colorectum	II	11474235	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7665.10_faster1		110955	INDI 692 PL	Colorectum	III	11477099	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7760.3_faster1	Yes	10673	INDI 133 PL	Stomach	I	11499086	Negative	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7756.9_faster1	Yes	10574	INDI 024 PL	Lung	II	11518949	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7304.5_faster1	Yes	108949	INDI 474 PL	Breast	II	11532501	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7534.7_faster1		110664	PAP 939 PL	Ovary	III	11546417	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
6837.8_faster1		10632	INDI 083 PL	Stomach	II	11547580	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7304.3_faster1	Yes	108939	INDI 460 PL	Breast	III	11621552	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7014.10_faster1		109043	INDI 688 PL	Colorectum	II	11688516	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7643.5_faster1		110851	INDI 608 PL	Colorectum	II	11689087	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7607.4_faster1	Yes	10688	INDI 151 PL	Stomach	II	11728749	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive
7593.7_faster1		10846	INDI 369 PL	Lung	I	11728834	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7303.8_faster1	Yes	10798	INDI 300 PL	Breast	III	11740790	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7756.6_faster1	Yes	10557	INDI 002 PL	Lung	II	11741917	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7757.3_faster1	Yes	10580	INDI 030 PL	Colorectum	II	11752353	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7546.3_faster1		10561	INDI 007 PL	Lung	II	11877295	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7610.6_faster1		10692	INDI 156 PL	Colorectum	III	11879034	Positive	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Positive
7670.5_faster1		110981	INDI 649 PL	Colorectum	III	11893568	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7646.3_faster1		110880	INDI 613 PL	Breast	II	11896835	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7677.11_faster1		111038	PANCA 115	Pancreas	I	11899685	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7760.8_faster1	Yes	10681	INDI 143 PL	Colorectum	II	11955147	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6839_redo.7_faster1		109000	INDI 567 PL	Colorectum	II	11976877	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive	Positive
7756.4_faster1	Yes	10541	CRC 486 PL	Colorectum	I	12013803	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7307.7_faster1	Yes	108957	INDI 487 PL	Breast	II	12026717	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7303.9_faster1	Yes	10806	INDI 317 PL	Breast	II	12074956	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7543.5_faster1		110649	PANCA 103	Pancreas	II	12078995	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7549.7_faster1		108965	INDI 497 PL	Lung	II	12080651	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7012.11_faster1		108940	INDI 461 PL	Colorectum	III	12087006	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7760.6_faster1	Yes	10677	INDI 137 PL	Stomach	II	12102716	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative
7304.7_faster1	Yes	108997	INDI 559 PL	Breast	III	12106034	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7758.10_faster1	Yes	10639	INDI 092 PL	Stomach	II	12202185	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7673.4_faster1		111011	INDI 785 PL	Colorectum	I	12202785	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7756.10_faster1	Yes	10575	INDI 025 PL	Lung	I	12225917	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7758.12_faster1	Yes	10651	INDI 107 PL	Colorectum	II	12270627	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7760.4_faster1	Yes	10674	INDI 134 PL	Stomach	III	12288756	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7758.3_faster1	Yes	10615	INDI 066 PL	Breast	III	12290640	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6850.4_faster1		109059	INDI 711 PL	Breast	III	12330713	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7607.5_faster1	Yes	10812	INDI 326 PL	Lung	I	12357550	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7759.11_faster1	Yes	10670	INDI 130 PL	Stomach	II	12357763	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
6836.9_faster1		10565	INDI 012 PL	Lung	I	12388482	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7307.5_faster1	Yes	10838	INDI 361 PL	Breast	III	12406295	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7011.4_faster1		109127	INDI 859 PL	Esophagus	II	12414216	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7042.11_faster1		10534	CRC 465 PL	Colorectum	II	12423748	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7536.7_faster1		110691	PAPA 1343	Ovary	III	12457913	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7758.11_faster1	Yes	10647	INDI 101 PL	Colorectum	III	12471222	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7043.8_faster1		10542	CRC 489 PL	Colorectum	II	12501319	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7305.12_faster1		109139	INDI 887 PL	Breast	III	12506601	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Negative
7649.10_faster1		110916	INDI 704 PL	Breast	II	12508209	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7596.8_faster1		10609	INDI 060 PL	Breast	II	12527347	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7742.4_faster1		111080	CRC 501 PL	Colorectum	I	12534877	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7591.9_faster1		109101	INDI 798 PL	Ovary	II	12542225	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7304.9_faster1	Yes	108999	INDI 565 PL	Breast	III	12568286	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7760.9_faster1	Yes	10690	INDI 153 PL	Stomach	II	12571286	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
6839_redo.4_faster1		109103	INDI 800 PL	Liver	II	12571619	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7305.4_faster1	Yes	109032	INDI 668 PL	Breast	III	12597944	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7760.7_faster1	Yes	10680	INDI 141 PL	Stomach	III	12602463	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7596.9_faster1		10610	INDI 061 PL	Breast	II	12603504	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7043.12_faster1		10552	CRC 524 PL	Colorectum	II	12612680	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7644.7_faster1		110868	INDI 633 PL	Colorectum	II	12637868	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7757.9_faster1	Yes	10598	INDI 050 PL	Breast	II	12708371	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7042.5_faster1		109105	INDI 802 PL	Pancreas	II	12736111	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7543.12_faster1		110656	PANCA 105	Pancreas	II	12750761	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6839_redo.3_faster1		109094	INDI 786 PL	Liver	I	12774784	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7547.9_faster1		10768	INDI 247 PL	Lung	I	12774797	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7606.5_faster1	Yes	10587	INDI 038 PL	Colorectum	II	12779723	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7665.4_faster1		110950	INDI 677 PL	Colorectum	II	12822270	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7663.4_faster1		110930	INDI 378 PL	Colorectum	I	12844324	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7306.5_faster1		10600	INDI 052 PL	Breast	II	12876008	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7742.3_faster1		111079	CRC 500 PL	Colorectum	I	12878766	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7307.3_faster1	Yes	10829	INDI 348 PL	Breast	II	12894091	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7011.8_faster1		10666	INDI 126 PL	Stomach	III	12932905	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7757.5_faster1	Yes	10582	INDI 032 PL	Colorectum	III	12935921	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7739.5_faster1		111051	CRC 457 PL	Colorectum	II	12949574	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7307.8_faster1		108958	INDI 489 PL	Breast	II	13000860	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.6_faster1		109064	INDI 721 PL	Breast	II	13019464	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7563.7_faster1		109080	INDI 755 PL	Esophagus	III	13023659	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6839_redo.10_faster1		109012	INDI 614 PL	Colorectum	II	13025861	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7649.8_faster1		110914	INDI 673 PL	Breast	II	13086229	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7638.8_faster1		110814	INDI 421 PL	Colorectum	II	13095095	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7306.7_faster1		10603	INDI 055 PL	Breast	II	13118272	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

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Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
6835.10_faster1		110645	PANCA 103	Pancreas	II	13135905	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7549.4_faster1		108960	INDI 491 PL	Lung	I	13143161	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7756.8_faster1	Yes	10571	INDI 018 PL	Lung	III	13162781	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7663.9_faster1		110935	INDI 398 PL	Colorectum	II	13172563	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7670.7_faster1		110983	INDI 651 PL	Colorectum	III	13201680	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7306.9_faster1		10605	INDI 057 PL	Breast	III	13242175	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7304.6_faster1	Yes	109002	INDI 577 PL	Breast	I	13313274	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7042.8_faster1	Yes	10832	INDI 353 PL	Lung	II	13337284	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7011.11_faster1		108943	INDI 465 PL	Esophagus	II	13379186	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6836.6_faster1		10664	INDI 124 PL	Colorectum	II	13390707	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive
7305.8_faster1		109135	INDI 876 PL	Breast	II	13403320	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7304.8_faster1	Yes	108998	INDI 561 PL	Breast	III	13464530	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7666.6_faster1		110959	INDI 234 PL	Colorectum	I	13511305	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.9_faster1		109096	INDI 789 PL	Breast	II	13516563	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7666.10_faster1		110963	INDI 251 PL	Colorectum	II	13571140	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7666.7_faster1		110960	INDI 243 PL	Colorectum	I	13578246	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7043.4_faster1		10537	CRC 471 PL	Colorectum	III	13587546	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6839_redo.9_faster1		109008	INDI 591 PL	Colorectum	II	13660283	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive
7670.11_faster1		110987	INDI 660 PL	Colorectum	II	13703592	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7607.6_faster1	Yes	10844	INDI 367 PL	Liver	I	13774303	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6850.5_faster1		10813	INDI 327 PL	Colorectum	II	13817537	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7594.11_faster1		109148	INDI 911 PL	Esophagus	II	13867646	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
7305.9_faster1		109136	INDI 877 PL	Breast	I	13951918	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.8_faster1		110974	INDI 281 PL	Colorectum	I	13953579	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7303.6_faster1		10620	INDI 071 PL	Breast	II	13959147	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7610.8_faster1		10694	INDI 159 PL	Colorectum	II	13985715	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7740.12_faster1		111068	CRC 483 PL	Colorectum	I	14034018	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7757.4_faster1	Yes	10581	INDI 031 PL	Colorectum	II	14048988	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7596.3_faster1		10597	INDI 049 PL	Breast	III	14117560	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7757.11_faster1	Yes	10611	INDI 062 PL	Breast	II	14121104	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7760.5_faster1	Yes	10676	INDI 136 PL	Colorectum	II	14149268	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7537.7_faster1		109153	LCR 814 PL	Pancreas	II	14181633	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.5_faster1		110971	INDI 268 PL	Colorectum	III	14199962	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7610.7_faster1		10693	INDI 158 PL	Colorectum	I	14204452	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7638.7_faster1		110813	INDI 419 PL	Colorectum	I	14241999	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7757.6_faster1	Yes	10590	INDI 042 PL	Colorectum	I	14247573	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7612.8_faster1		10742	INDI 213 PL	Colorectum	III	14302511	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7740.3_faster1		111059	CRC 469 PL	Colorectum	II	14346493	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7643.3_faster1		110849	INDI 600 PL	Colorectum	II	14392099	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7565.5_faster1		10659	INDI 119 PL	Esophagus	I	14393602	Positive	Negative	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive
7305.7_faster1	Yes	109134	INDI 875 PL	Breast	II	14409097	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.7_faster1		110973	INDI 279 PL	Colorectum	II	14426072	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7592_combined.4_faste	Yes	10814	INDI 331 PL	Lung	III	14455257	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7307.6_faster1	Yes	10860	INDI 390 PL	Breast	II	14465100	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7534.10_faster1		110666	PAP 941 PL	Ovary	III	14471184	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7606.4_faster1	Yes	10569	INDI 016 PL	Lung	III	14478527	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7585.3_faster1		10710	INDI 177 PL	Liver	III	14516564	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7543.10_faster1		110654	PANCA 105	Pancreas	II	14534837	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7303.5_faster1		10618	INDI 069 PL	Breast	II	14572791	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
6835.6_faster1		110682	PAPA 1330	Ovary	III	14623813	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7759.6_faster1	Yes	10658	INDI 116 PL	Stomach	I	14651561	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7638.10_faster1		110816	INDI 423 PL	Colorectum	II	14681242	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7610.5_faster1		10691	INDI 155 PL	Colorectum	II	14715914	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6851.11_faster1		109086	INDI 770 PL	Colorectum	II	14739471	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7549.5_faster1		108961	INDI 492 PL	Lung	I	14747011	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6839_redo.6_faster1		10883	INDI 439 PL	Colorectum	III	14782970	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7602.4_faster1		10588	INDI 039 PL	Colorectum	III	14879768	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7608.12_faster1	Yes	109133	INDI 872 PL	Breast	III	14923030	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7678.5_faster1		111041	INDI 920 PL	Ovary	III	14942044	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7305.5_faster1	Yes	109081	INDI 756 PL	Breast	II	14975145	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7535.9_faster1		110683	PAPA 1331	Ovary	III	15069331	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7742.5_faster1		111081	CRC 503 PL	Colorectum	I	15091681	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7639.3_faster1		110819	INDI 429 PL	Colorectum	II	15107456	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7606.12_faster1	Yes	10791	INDI 287 PL	Breast	III	15110509	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7606.3_faster1	Yes	109150	INDI 928 PL	Stomach	II	15199986	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7565.3_faster1		10656	INDI 112 PL	Esophagus	II	15242109	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7645.4_faster1		110870	INDI 588 PL	Breast	III	15244435	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7043.5_faster1		10538	CRC 479 PL	Colorectum	II	15245644	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive

WO 2020/236625 PCT/US2020/033209

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7014.9_faster1		108977	INDI 518 PL	Colorectum	III	15270476	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7041.7_faster1		109023	INDI 643 PL	Colorectum	II	15328844	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7560.6_faster1		108988	INDI 538 PL	Lung	III	15413435	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.12_faster1		109114	INDI 828 PL	Breast	II	15523762	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7586.10_faster1		109083	INDI 760 PL	Liver	III	15527805	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7307.10_faster1		108976	INDI 517 PL	Breast	III	15624023	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7607.9_faster1	Yes	108956	INDI 485 PL	Esophagus	III	15637371	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7664.5_faster1		110941	INDI 515 PL	Colorectum	III	15688198	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7534.5_faster1		110680	PAP 962 PL	Ovary	III	15695598	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7611.8_faster1		10713	INDI 180 PL	Colorectum	III	15784294	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6858.3_faster1		10533	CRC 460 PL	Colorectum	II	15786364	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7566.10_faster1		10735	INDI 206 PL	Stomach	III	15861333	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7642.7_faster1		110843	INDI 583 PL	Colorectum	II	15884110	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7665.6_faster1		110952	INDI 682 PL	Colorectum	III	15898942	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7544.4_faster1		110658	PANCA 105	Pancreas	II	15938986	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6858.7_faster1		10545	CRC 499 PL	Colorectum	I	15947500	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7601.5_faster1		108952	INDI 478 PL	Breast	II	15952190	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7603.4_faster1		10640	INDI 093 PL	Colorectum	I	15978437	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7677.9_faster1		111035	INDI 840 PL	Breast	II	16140068	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7562.3_faster1		109099	INDI 792 PL	Stomach	I	16179994	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
7639.7_faster1		110823	INDI 436 PL	Colorectum	III	16208714	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7544.11_faster1		10559	INDI 004 PL	Lung	I	16262105	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
6838_redo.3_faster1		109005	INDI 585 PL	Esophagus	III	16300134	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7561.12_faster1		109117	INDI 843 PL	Esophagus	I	16335896	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7670.8_faster1		110984	INDI 654 PL	Colorectum	II	16345406	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7664.3_faster1		110939	INDI 506 PL	Colorectum	I	16419133	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7009.11_faster1		10578	INDI 028 PL	Colorectum	II	16523538	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7638.4_faster1		110810	INDI 311 PL	Colorectum	III	16541062	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative
7304.4_faster1	Yes	108948	INDI 473 PL	Breast	III	16558913	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7305.6_faster1	Yes	109082	INDI 757 PL	Breast	II	16672564	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7548.7_faster1		10794	INDI 291 PL	Lung	I	16675122	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7549.9_faster1		108969	INDI 503 PL	Lung	I	16752817	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7544.6_faster1		110660	PANCA 106	Pancreas	II	16838030	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Positive
7535.3_faster1		110676	PAP 956 PL	Ovary	III	16844133	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive

WO 2020/236625 PCT/US2020/033209

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7643.4_faster1		110850	INDI 605 PL	Colorectum	II	16865887	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7672.9_faster1		111000	INDI 724 PL	Colorectum	II	17102657	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7756.7_faster1	Yes	10570	INDI 017 PL	Lung	I	17102879	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7642.11_faster1		110847	INDI 598 PL	Colorectum	II	17127403	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7307.4_faster1	Yes	10831	INDI 352 PL	Breast	III	17185278	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7758.7_faster1	Yes	10635	INDI 086 PL	Stomach	I	17283778	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
6858.9_faster1		10550	CRC 520 PL	Colorectum	III	17374052	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7607.3_faster1		10793	INDI 289 PL	Breast	II	17378202	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7663.5_faster1		110931	INDI 379 PL	Colorectum	III	17447719	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7584.4_faster1		10627	INDI 078 PL	Liver	II	17497723	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6858.4_faster1		10539	CRC 480 PL	Colorectum	III	17512125	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7306.3_faster1		10596	INDI 048 PL	Breast	II	17518393	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7041.5_faster1		109019	INDI 635 PL	Colorectum	II	17523673	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7608.5_faster1	Yes	109049	INDI 696 PL	Lung	II	17541286	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7740.9_faster1		111065	CRC 477 PL	Colorectum	II	17644267	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7043.6_faster1		10540	CRC 481 PL	Colorectum	II	17728894	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7608.3_faster1	Yes	108972	INDI 511 PL	Colorectum	III	17764622	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7602.5_faster1		10589	INDI 040 PL	Colorectum	I	17912621	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7560.4_faster1		108983	INDI 532 PL	Lung	I	17926546	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7637.12_faster1		110808	INDI 307 PL	Colorectum	III	18031641	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
6850.9_faster1		108989	INDI 539 PL	Colorectum	II	18211342	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6858.11_faster1		10553	CRC 526 PL	Colorectum	III	18336045	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7613.12_faster1		109126	INDI 855 PL	Colorectum	II	18343702	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7542.3_faster1		110635	PANCA 101	Pancreas	II	18351665	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Positive
7011.6_faster1		109031	INDI 665 PL	Esophagus	III	18412737	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Positive
7536.11_faster1		110695	PAPA 1348	Ovary	III	18417631	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7543.6_faster1		110650	PANCA 104	Pancreas	II	18443523	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7303.7_faster1	Yes	109130	INDI 865 PL	Breast	III	18466940	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7610.11_faster1		10699	INDI 164 PL	Colorectum	I	18473786	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7590.7_faster1		109048	INDI 695 PL	Pancreas	II	18525122	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
7742.6_faster1		111082	CRC 505 PL	Colorectum	III	18541814	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7594.8_faster1		109145	INDI 907 PL	Esophagus	II	18542868	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7586.9_faster1		109033	INDI 669 PL	Liver	III	18602855	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7585.7_faster1		10737	INDI 208 PL	Liver	II	18613650	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7010.4_faster1		10644	INDI 097 PL	Colorectum	III	18645760	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7563.3_faster1		109124	INDI 853 PL	Esophagus	II	18770983	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7591.7_faster1		109076	INDI 745 PL	Pancreas	II	18782675	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7739.12_faster1		111058	CRC 467 PL	Colorectum	III	18793851	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7639.8_faster1		110824	INDI 437 PL	Colorectum	I	18869016	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7564.5_faster1		10719	INDI 187 PL	Stomach	I	18873266	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7308.5_faster1		109062	INDI 717 PL	Breast	I	18970952	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7664.7_faster1		110943	INDI 523 PL	Colorectum	I	18978926	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6850.6_faster1		108966	INDI 498 PL	Colorectum	II	18997615	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7534.4_faster1		110672	PAP 950 PL	Ovary	III	19030714	Positive	Negative	Positive	Positive	Negative	Negative	Negative	Positive	Negative	Positive
7637.6_faster1		110802	INDI 299 PL	Colorectum	I	19165467	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7591.6_faster1		109056	INDI 706 PL	Lung	I	19176306	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7613.3_faster1		10751	INDI 223 PL	Colorectum	I	19229885	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7742.11_faster1		111087	CRC 511 PL	Colorectum	II	19303564	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7611.4_faster1		10704	INDI 170 PL	Colorectum	I	19410722	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7306.4_faster1		10599	INDI 051 PL	Breast	III	19496002	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7561.11_faster1		10778	INDI 267 PL	Esophagus	II	19771902	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7536.5_faster1		110689	PAPA 1339	Ovary	I	19787879	Positive	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7640.9_faster1		110835	INDI 558 PL	Colorectum	I	19824684	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7739.7_faster1		111053	CRC 461 PL	Colorectum	I	19956732	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7665.11_faster1		110958	INDI 899 PL	Breast	I	19981298	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6851.8_faster1		109058	INDI 710 PL	Colorectum	III	20001862	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7592_combined.9_faste	Yes	10824	INDI 342 PL	Lung	III	20199922	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
6850.11_faster1		109037	INDI 675 PL	Colorectum	III	20230333	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7646.7_faster1		110883	INDI 630 PL	Breast	I	20320861	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7596.5_faster1		10601	INDI 053 PL	Breast	II	20810618	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7611.11_faster1		10726	INDI 195 PL	Colorectum	III	20853590	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
6850.7_faster1		108968	INDI 502 PL	Colorectum	III	20932649	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7013.4_faster1		109004	INDI 581 PL	Colorectum	II	20977458	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7639.5_faster1		110821	INDI 433 PL	Colorectum	II	21461355	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7586.3_faster1		108955	INDI 482 PL	Liver	II	21477232	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6839_redo.5_faster1		109109	INDI 817 PL	Liver	III	21545705	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7602.9_faster1		10593	INDI 045 PL	Colorectum	III	21580012	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Negative
7676.9_faster1		111020	INDI 794 PL	Breast	II	21787265	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7671.7_faster1		110993	INDI 761 PL	Colorectum	II	21899955	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7649.9_faster1		110915	INDI 697 PL	Breast	II	21937982	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.9_faster1		110924	INDI 338 PL	Colorectum	II	21995745	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7012.12_faster1		108944	INDI 466 PL	Colorectum	II	22048167	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7549.3_faster1		10879	INDI 417 PL	Lung	I	22199612	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7561.10_faster1		109111	INDI 824 PL	Esophagus	II	22217017	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7671.4_faster1		110989	INDI 746 PL	Colorectum	III	22277590	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7549.8_faster1		108967	INDI 499 PL	Lung	I	22290340	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7043.9_faster1		10547	CRC 504 PL	Colorectum	III	22408133	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7670.4_faster1		110980	INDI 648 PL	Colorectum	III	22510880	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive	Positive
7043.11_faster1		10549	CRC 512 PL	Colorectum	II	22667843	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
6835.5_faster1		110679	PAP 961 PL	Ovary	III	22676074	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Positive
7649.12_faster1		110918	INDI 714 PL	Breast	III	22684049	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7662.5_faster1		110920	INDI 322 PL	Colorectum	III	22726250	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6858.6_faster1		10544	CRC 497 PL	Colorectum	III	22727295	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7014.6_faster1		10852	INDI 380 PL	Colorectum	II	23010226	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
7010.7_faster1		10740	INDI 211 PL	Colorectum	II	23031581	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7009.3_faster1		110627	PANC 762 P	Pancreas	II	23155883	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
7012.5_faster1		108942	INDI 464 PL	Liver	III	23250231	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
7013.11_faster1		10822	INDI 340 PL	Colorectum	III	23297382	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7637.9_faster1		110805	INDI 304 PL	Colorectum	I	23389735	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
7594.12_faster1		109149	INDI 913 PL	Esophagus	III	23543415	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7612.7_faster1		10741	INDI 212 PL	Colorectum	III	23614346	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7010.10_faster1		108970	INDI 505 PL	Lung	II	23634307	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7606.7_faster1	Yes	10628	INDI 079 PL	Liver	II	23851041	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
7013.7_faster1		109017	INDI 625 PL	Colorectum	II	23884864	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
6837.5_faster1		108963	INDI 494 PL	Lung	III	23965761	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7544.12_faster1		10560	INDI 005 PL	Lung	I	23999311	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7542.6_faster1		110638	PANCA 101	Pancreas	II	24010534	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Positive
7594.7_faster1		109144	INDI 905 PL	Esophagus	II	24032028	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7562.8_faster1		109104	INDI 801 PL	Stomach	I	24096871	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7645.9_faster1		110875	INDI 601 PL	Breast	III	24221059	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Positive
7009.12_faster1		10583	INDI 034 PL	Colorectum	III	24367937	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7014.7_faster1		10866	INDI 397 PL	Colorectum	III	24491709	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive

Table 3.

Unique Name	Repeats	Subject ID	Sample Nar	Cancer Type	Stage	Coverage	Aneuploidy	AFP +(>21321)	CA-125 (577 U/ml)	CA 15-3 +(>98 U/ml)	CA19-9 (U/ml) + >92	CEA (pg/ml) >7507	HGF (pg/ml) +>899	OPN (pg/ml) + >157772	TIMP-1 (pg/ml) + > 176989	Mut+
7639.10_faster1		110826	INDI 444 PL	Colorectum	II	24690075	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7589.4_faster1		109046	INDI 693 PL	Lung	III	24743114	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7602.8_faster1		10592	INDI 044 PL	Colorectum	II	25416842	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7662.8_faster1		110923	INDI 329 PL	Colorectum	III	25554567	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7637.10_faster1		110806	INDI 305 PL	Colorectum	II	25620843	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive
7013.10_faster1		10809	INDI 320 PL	Colorectum	II	25848426	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
6835.4_faster1		110671	PAP 949 PL	Ovary	III	26009990	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7610.3_faster1		10687	INDI 150 PL	Colorectum	II	26190880	Positive	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Positive
7663.10_faster1		110936	INDI 400 PL	Colorectum	II	26205126	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7013.3_faster1		108946	INDI 470 PL	Colorectum	III	26290647	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive
7678.3_faster1		111039	INDI 917 PL	Liver	III	26418631	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7740.8_faster1		111064	CRC 476 PL	Colorectum	II	26527000	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7665.3_faster1		110949	INDI 670 PL	Colorectum	III	26759855	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
6857.11_faster1		110662	PANCA 115	Pancreas	II	26760223	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive
6839_redo.11_faster1		109018	INDI 626 PL	Colorectum	II	26767307	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
7673.5_faster1		111012	INDI 788 PL	Colorectum	II	27075277	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
6837.10_faster1		10665	INDI 125 PL	Esophagus	II	27099980	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7535.10_faster1		110684	PAPA 1332	Ovary	I	27521236	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7011.10_faster1		108936	INDI 457 PL	Esophagus	II	27665822	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive
7677.5_faster1		111031	INDI 831 PL	Breast	I	27831412	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7537.4_faster1		110699	PAPA 1355	Ovary	III	27866616	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7638.3_faster1		110809	INDI 309 PL	Colorectum	II	27979944	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6838_redo.9_faster1		10747	INDI 219 PL	Liver	III	28101753	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7566.3_faster1		10675	INDI 135 PL	Stomach	III	28195882	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Positive
7640.4_faster1		110830	INDI 467 PL	Colorectum	II	28285870	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7561.8_faster1		109093	INDI 784 PL	Stomach	I	28355787	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7602.10_faster1		10594	INDI 046 PL	Colorectum	II	28577142	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
7041.6_faster1		109022	INDI 640 PL	Colorectum	II	28800336	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7567.5_faster1		10884	INDI 440 PL	Esophagus	II	29087757	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
6851.9_faster1		109025	INDI 647 PL	Colorectum	III	29099826	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7644.5_faster1		110866	INDI 631 PL	Colorectum	II	29183100	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7042.10_faster1		10532	CRC 458 PL	Colorectum	II	29493266	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7609.6_faster1		10663	INDI 123 PL	Colorectum	II	29592186	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7593.5_faster1		10837	INDI 360 PL	Lung	II	29802114	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive

Table 3.

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7011.5_faster1		109029	INDI 663 PL	Esophagus	II	30466055	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
7677.6_faster1		111032	INDI 832 PL	Breast	II	31310565	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7041.9_faster1		109026	INDI 657 PL	Colorectum	II	31653547	Positive	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Positive
6857.3_faster1		109063	INDI 720 PL	Colorectum	III	31915897	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7010.6_faster1		10723	INDI 191 PL	Colorectum	I	32107486	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative
7542.5_faster1		110637	PANCA 101	Pancreas	III	32393583	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Negative
7592_combined.8_faste	Yes	10819	INDI 336 PL	Lung	III	32529369	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7586.8_faster1		109028	INDI 661 PL	Liver	III	32614452	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7560.3_faster1		108979	INDI 522 PL	Lung	I	32694027	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7667.12_faster1		110977	INDI 716 PL	Colorectum	III	32862529	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6851.7_faster1		109054	INDI 702 PL	Lung	II	33124065	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7673.9_faster1		111015	INDI 808 PL	Colorectum	III	33215174	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7640.11_faster1		110837	INDI 562 PL	Colorectum	I	33273561	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative
7566.12_faster1		10642	INDI 095 PL	Stomach	II	33345327	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6838_redo.7_faster1		10714	INDI 182 PL	Liver	III	33731393	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
7563.9_faster1		109131	INDI 867 PL	Esophagus	II	33771332	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative
7664.8_faster1		110944	INDI 525 PL	Colorectum	I	33945588	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7589.11_faster1		10843	INDI 366 PL	Pancreas	II	33972894	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
7759.10_faster1	Yes	10669	INDI 129 PL	Liver	II	34138426	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7564.3_faster1		10716	INDI 184 PL	Esophagus	II	34571164	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive
7560.5_faster1		108986	INDI 535 PL	Lung	I	35034507	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7646.4_faster1		110879	INDI 607 PL	Breast	II	35885421	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7592_combined.3_faste	Yes	10868	INDI 403 PL	Lung	III	35927206	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7742.12_faster1		111088	CRC 513 PL	Colorectum	II	36090588	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7594.9_faster1		109146	INDI 908 PL	Esophagus	II	36616898	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive
7548.3_faster1		10779	INDI 269 PL	Lung	III	36802049	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7649.11_faster1		110917	INDI 712 PL	Breast	I	37318457	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7585.6_faster1		10734	INDI 205 PL	Liver	III	37490440	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive
7042.12_faster1		10535	CRC 468 PL	Colorectum	III	38370073	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7014.5_faster1		10851	INDI 377 PL	Colorectum	II	38574612	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7566.5_faster1		10678	INDI 139 PL	Stomach	III	38605637	Positive	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Positive
7567.3_faster1		10649	INDI 103 PL	Stomach	II	39168812	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7562.12_faster1		109123	INDI 850 PL	Esophagus	II	39489197	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive
7740.4_faster1		111060	CRC 472 PL	Colorectum	II	39783916	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative

WO 2020/236625 PCT/US2020/033209

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7676.7_faster1		111028	INDI 837 PL	Colorectum	II	41486671	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7563.5_faster1		10697	INDI 162 PL	Stomach	III	41846036	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
6837.3_faster1		10769	INDI 252 PL	Lung	II	41868738	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7589.9_faster1		109142	INDI 893 PL	Pancreas	II	42034627	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
7596.6_faster1		10602	INDI 054 PL	Breast	II	43241792	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7584.3_faster1		10626	INDI 077 PL	Liver	II	43439264	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
7612.12_faster1		10750	INDI 222 PL	Colorectum	II	44049554	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive
7541.3_faster1		110623	PANC 677 F	Pancreas	II	44382768	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7672.6_faster1		111006	INDI 777 PL	Colorectum	II	44818555	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7671.9_faster1		110995	INDI 765 PL	Colorectum	I	45084409	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive
7012.10_faster1		10801	INDI 310 PL	Colorectum	II	45238888	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive
7642.6_faster1		110842	INDI 582 PL	Colorectum	II	45318338	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative
7739.4_faster1		111050	CRC 456 PL	Colorectum	I	45555883	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7645.3_faster1		110869	INDI 578 PL	Breast	I	46297286	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7643.11_faster1		110857	INDI 618 PL	Colorectum	III	46621165	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive
7593.9_faster1		10848	INDI 372 PL	Lung	II	47063234	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7678.8_faster1		111044	INDI 923 PL	Esophagus	I	48092734	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7609.3_faster1		10653	INDI 109 PL	Colorectum	III	48226837	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7537.6_faster1	Yes	109152	LCR 812 PL	Pancreas	II	51896117	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Positive	Positive
7547.4_faster1		10752	INDI 224 PL	Lung	III	51931089	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
7603.3_faster1	Yes	10638	INDI 090 PL	Colorectum	II	52478584	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7567.4_faster1		108951	INDI 476 PL	Esophagus	II	52566550	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Negative
7590.12_faster1		109073	INDI 740 PL	Pancreas	II	53237733	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7644.6_faster1		110867	INDI 632 PL	Colorectum	II	55764474	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7009.4_faster1		110628	PANC 765 F	Pancreas	II	56998190	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Positive
7591.3_faster1		109074	INDI 741 PL	Pancreas	III	57886079	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Positive
7666.3_faster1		110967	INDI 902 PL	Breast	III	59817502	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive
7613.4_faster1		10753	INDI 225 PL	Colorectum	I	68078206	Positive	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Positive

WHAT IS CLAIMED IS:

1. A method of testing for the presence of aneuploidy in a genome of a mammal comprising:

a) amplifying a plurality of chromosomal sequences in a DNA sample obtained from a mammal with a single pair of primers complementary to the chromosomal sequences to form a plurality of amplicons, wherein the primer pair amplifies from about 50,000 to about 1,000,000 unique amplicons comprising short interspersed nucleotide elements (SINEs), and wherein the plurality of amplicons comprises sequences from a plurality of different chromosome;

b) determining at least a portion of the nucleic acid sequence of the plurality of amplicons to generate amplicon sequences;

c) mapping the amplicon sequences to a reference genome;

d) dividing the amplicon sequences into a plurality of genomic intervals;

e) quantifying read counts for the amplicon sequences mapped to the genomic intervals; and

f) comparing the read counts of the amplicon sequences in the genomic intervals on a chromosome arm to an expected distribution;

thereby testing for the presence of aneuploidy in the genome of the mammal.

2. The method of claim 1, wherein the DNA sample is a biological sample from a subject.

3. The method of claim 2, wherein the biological sample is a liquid sample.

4. The method of claim 2, wherein the biological sample is a blood sample, a plasma sample, a serum sample, or a tissue sample.

5. The method of any one of claims 2-4, wherein the biological sample comprises cell-free DNA.

6. The method of any one of claims 1 to 5, wherein the mammal is a human.

7. The method of any one of claims 1 to 6, wherein the method detects aneuploidy in a sample comprising about 0.01 picograms to about 500 picograms DNA.

8. The method of any one of claims 1 to 7, wherein the pair of primers comprises a first primer with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1 and a second primer with at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10.

9. The method of any one of claims 1 to 8, wherein the plurality of amplicons have an average size of 10 to 110 basepairs.

10. The method of any one of claims 1 to 9, wherein the primer pair amplifies from about 200,000 to about 1,000,000 unique amplicons.

11. The method of claim 10, wherein the primer pair amplifies from about 300,000 to about 1,000,000 unique amplicons.

12. The method of any one of claims 1 to 11, wherein the genomic intervals comprise from about 100 nucleotides to about 125,000,000 nucleotides.

13. The method of any one of claims 1 to 12, wherein the expected distribution is a normal distribution.

14. The method of any one of claims 1 to 13, wherein the read counts of the amplicon sequences in the genomic intervals on a chromosome arm are compared to the expected distribution to obtain a Z-score for the chromosome arm.

15. The method of claim 14, wherein a Z-score outside of a predetermined significance threshold indicates that the read counts of the amplicon sequences in the genomic intervals on a chromosome arm do not follow the expected distribution.

16. The method of any one of claims 1-15, comprising performing supervised machine learning to classify the sample according to aneuploidy status.

17. The method of claim 16, wherein the supervised machine learning is performed to make a genome-wide aneuploidy call.

18. The method of claim 16 or claim 17, wherein the supervised machine learning is performed using Z-scores for a plurality of chromosome arms, wherein obtaining the Z-score for each chromosome arm of the plurality of chromosome arms comprises comparing the read counts of the amplicon sequences in the genomic intervals on the chromosome arm to an expected distribution.

19. The method of any one of claims 1 to 18, wherein the read counts of the amplicon sequences in the genomic intervals on a chromosome arm are compared to read counts of the genomic intervals on the chromosome arm in a reference sample.

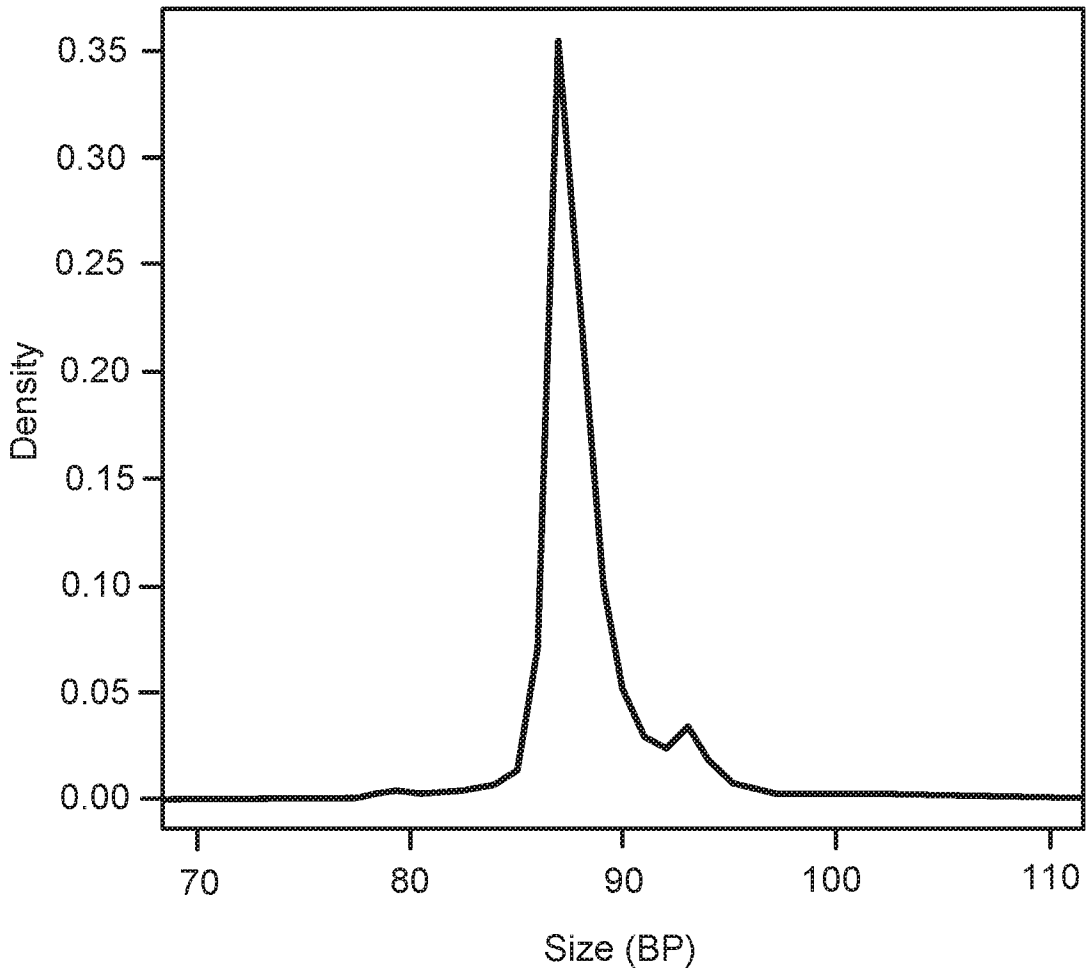


FIG. 1A

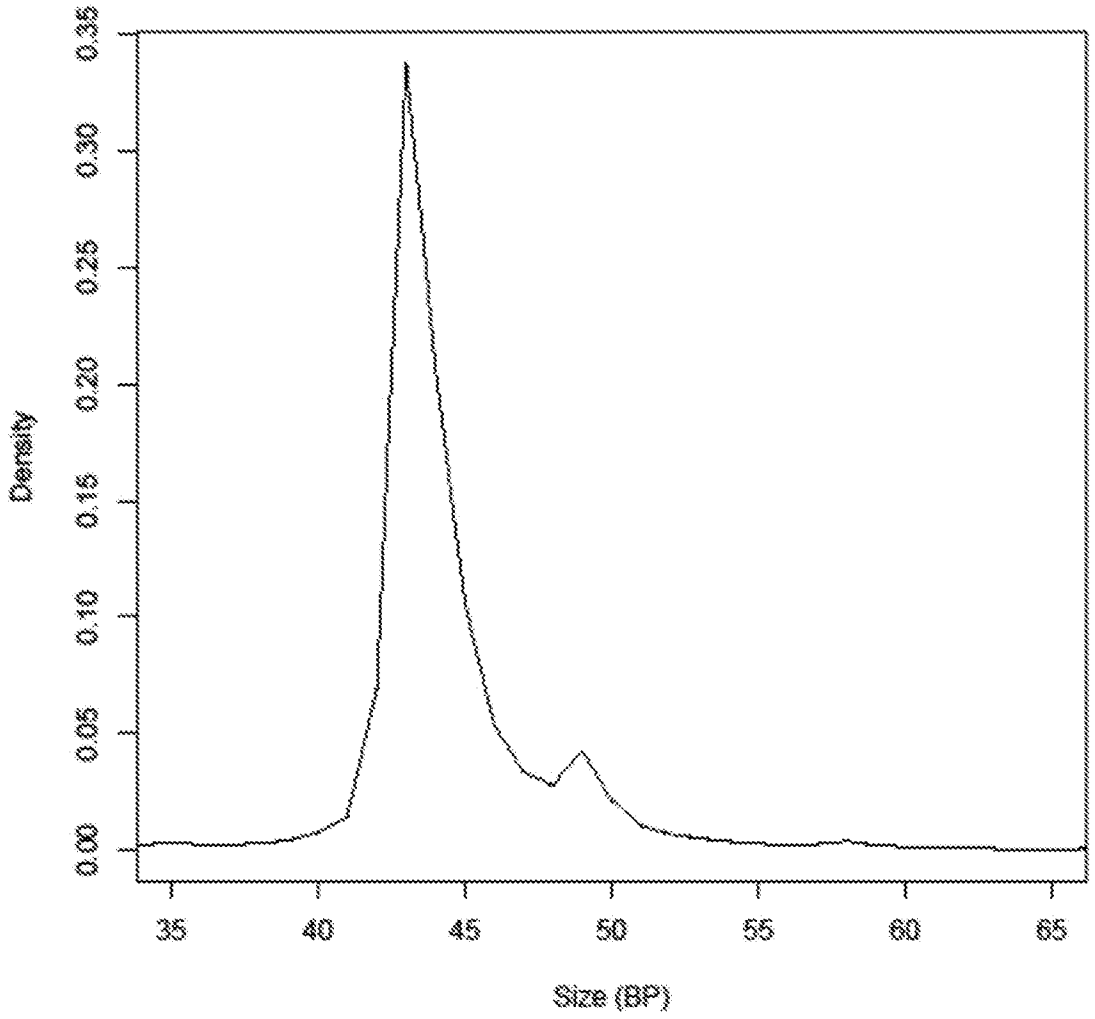


FIG. 1B

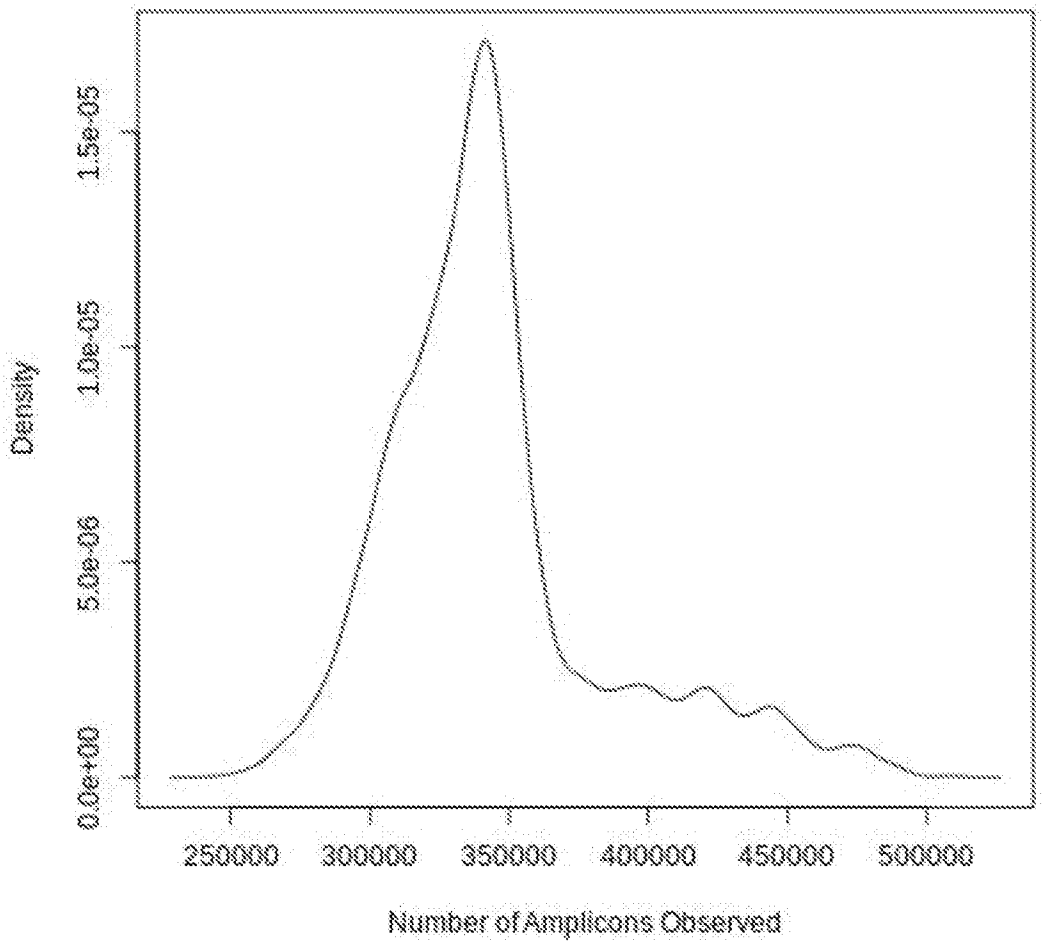


FIG. 1C

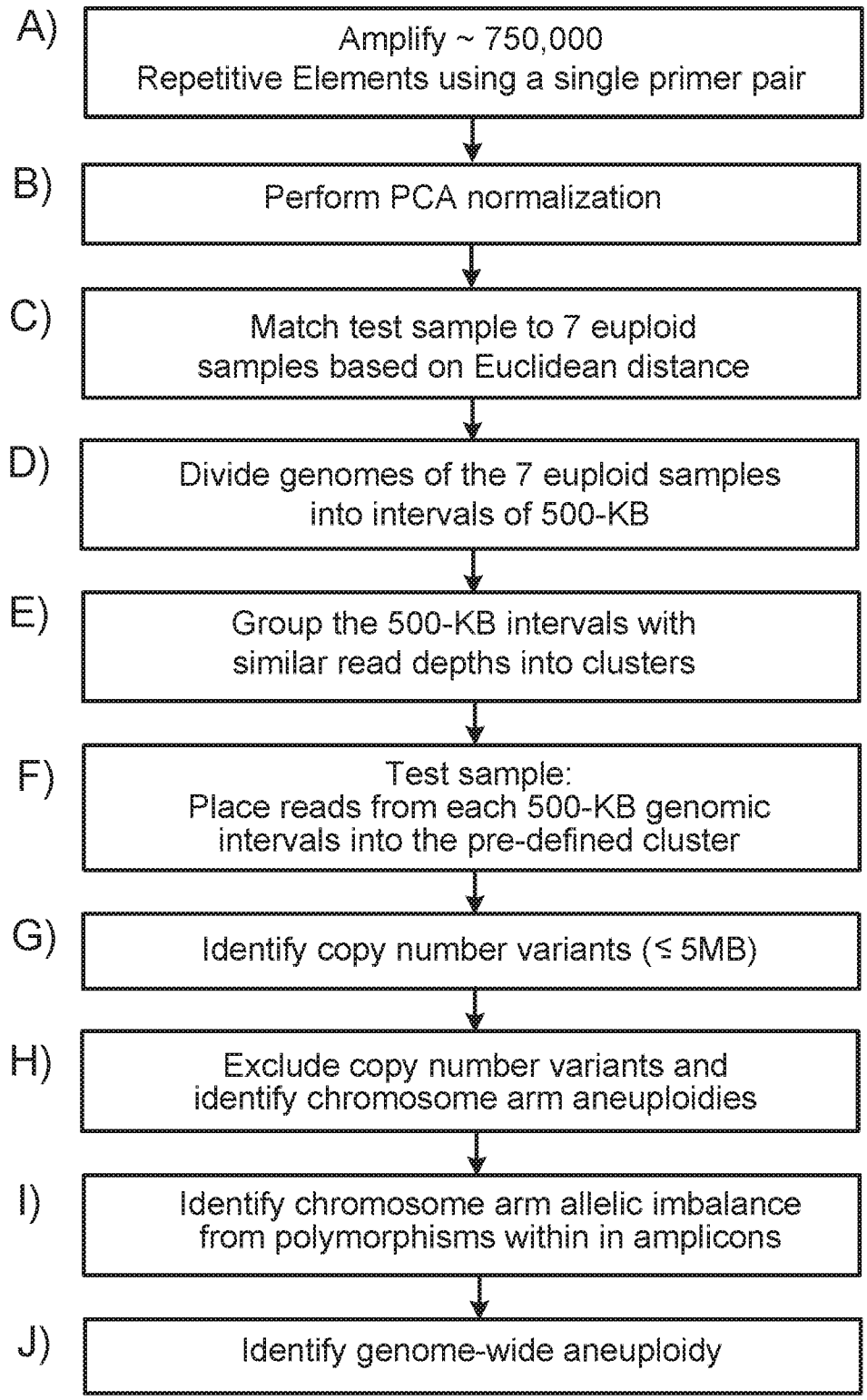
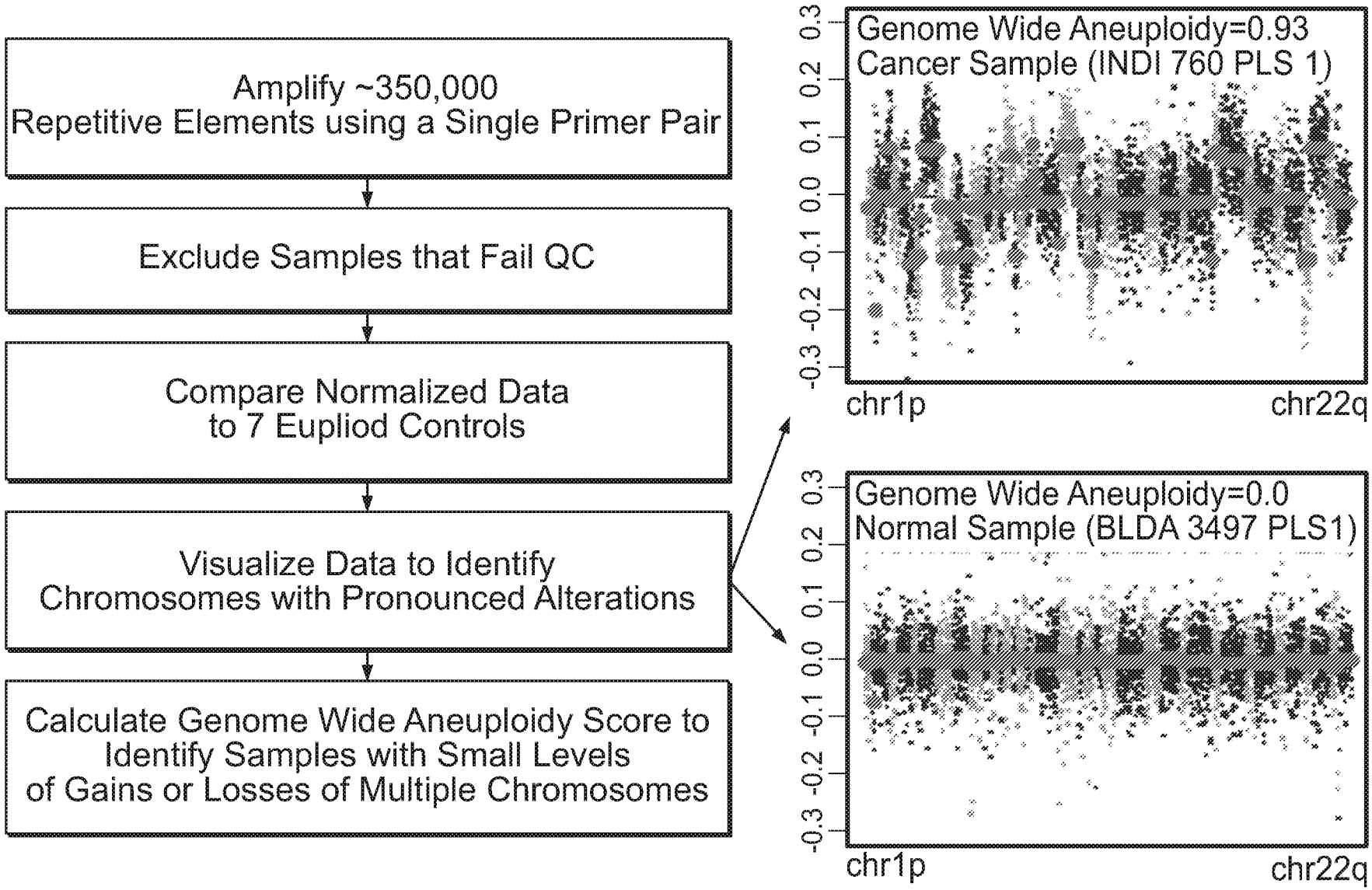


FIG. 2A


Amplify ~350,000
Repetitive Elements using a Single Primer Pair
Exclude Samples that Fail QC
Compare Normalized Data
to 7 Eupliod Controls
Visualize Data to Identify
Chromosomes with Pronounced Alterations
Calculate Genome Wide Aneuploidy Score to
Identify Samples with Small Levels
of Gains or Losses of Multiple Chromosomes
Genome Wide Aneuploidy=0.93
Cancer Sample (INDI 760 PLS 1)
Genome Wide Aneuploidy=0.0
Normal Sample (BLDA 3497 PLS1)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
chr1p
chr22q

FIG, 2B

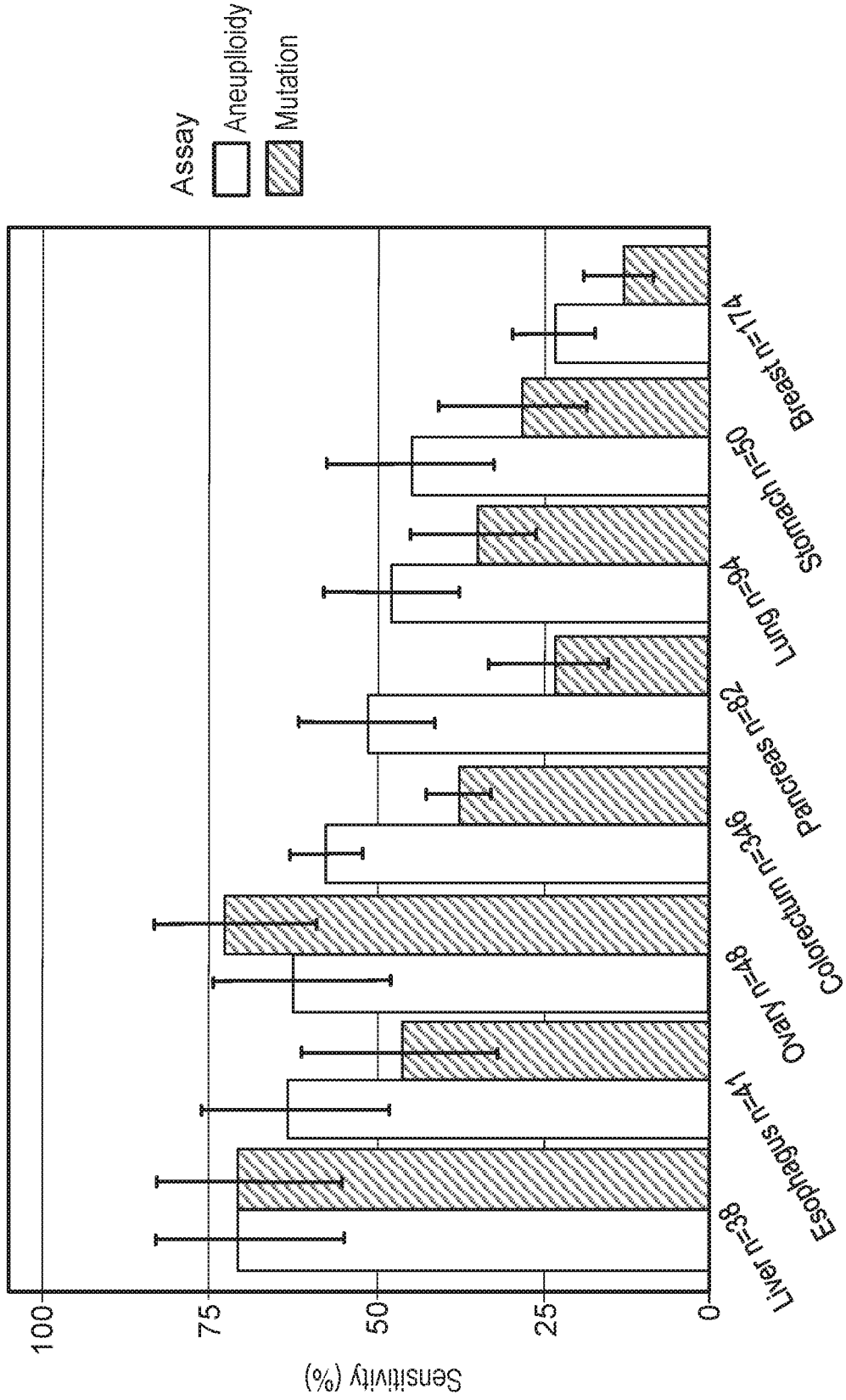


FIG. 3

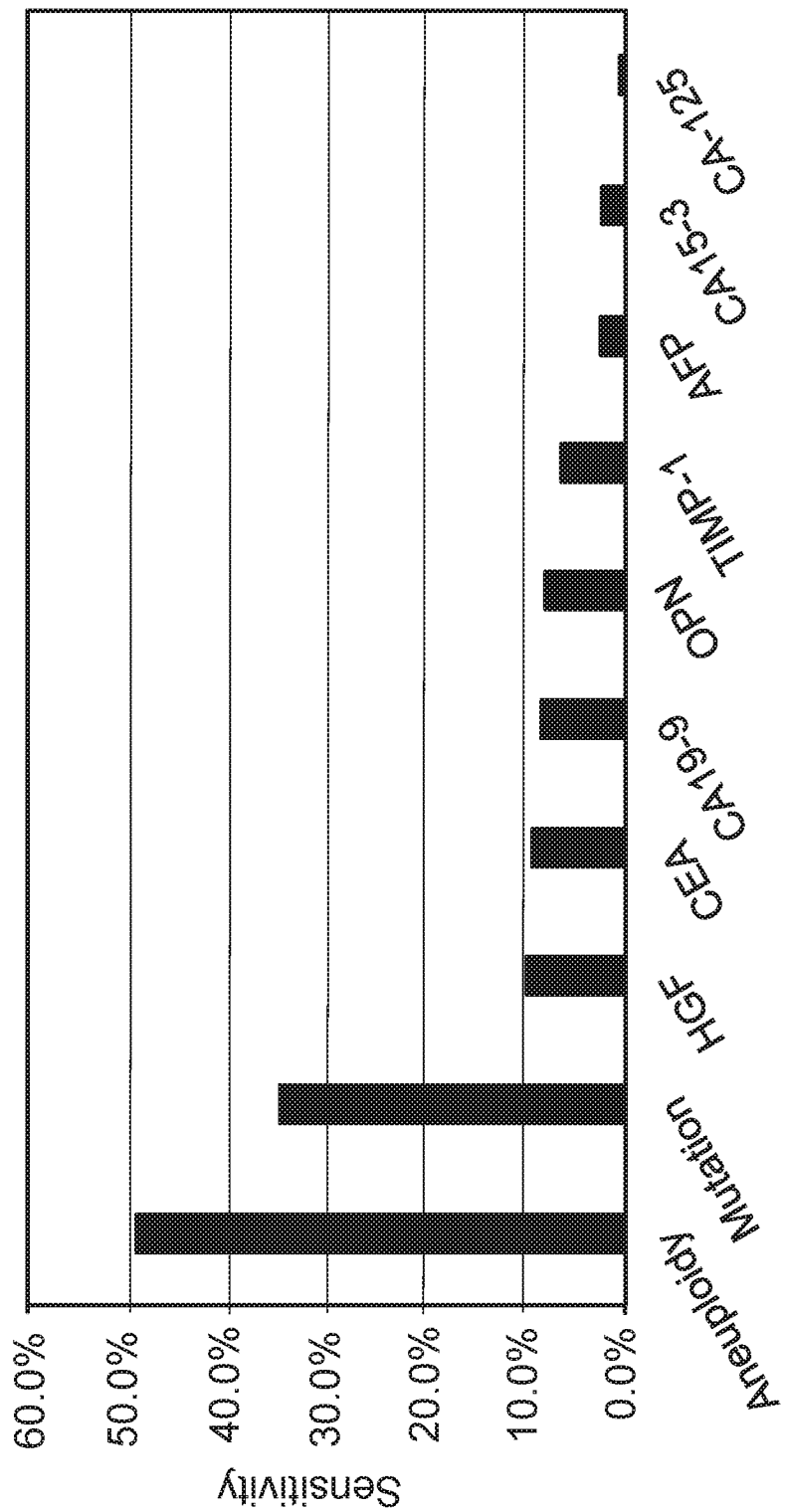


FIG. 4

```
<- number of normal plasma samples used
d <- desired degree of aneuploidy (number arms altered)
f <- desired neoplastic cell fraction
r <- chromosome arm (1p..22q)
r_type<-arm alteration type (gain or loss)
p(r) <- probability that an arm is gained or lost in cancer (Estimated from
{Douville, #3})
rho <- desired unique read depth of synthetic sample (10M default)
j<- desired number of repeats
For j=1:5
    For i=1:N
        s <- sample[i]
        U <- get reads from s
        For f in 1:F
            h <- new synthetic sample
            For d in (10,15,20) #desired numbers of altered arms
                t <- copy of s
                For a in 1:d #select alteration types and spike-in to t
                    r, r_type <- weighted random selection where the
                    weights [p(r)] are the likelihood that an arm is
                    gained or loss in cancer
                    u_all <- get all Reads that that map to chr arm r
                    if r_type== gain
                        t <- add 50% of u_all reads
                    else (r_type == loss)
                        t <- subtract 50% of u_all reads
                    Endif
                    h_normal<-randomselect (1-f)*rho reads from s
                    h_aneuploid<-randomselect f*rho reads from t
                    h<-h_normal+h_aneuploid
                End
            End
        End
    End
End
```

FIG. 5

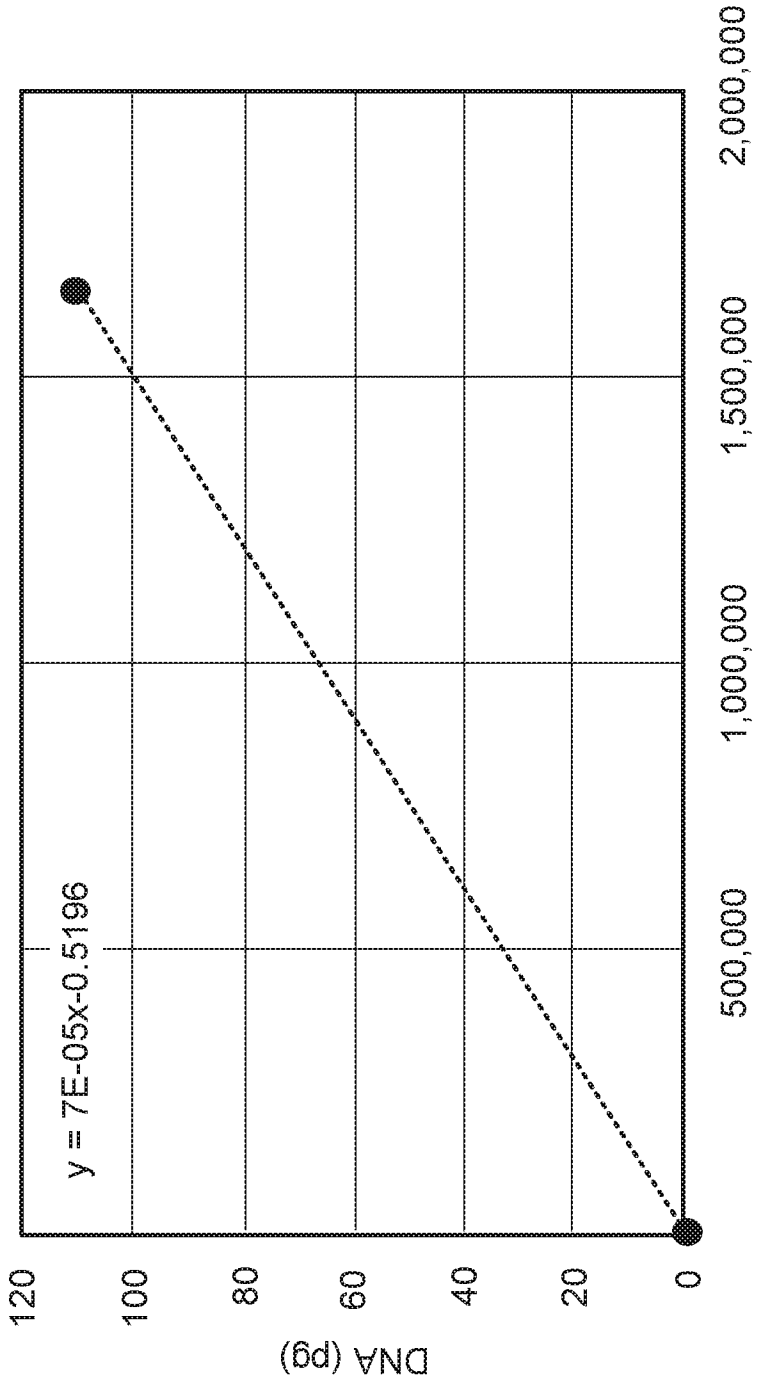


FIG. 6

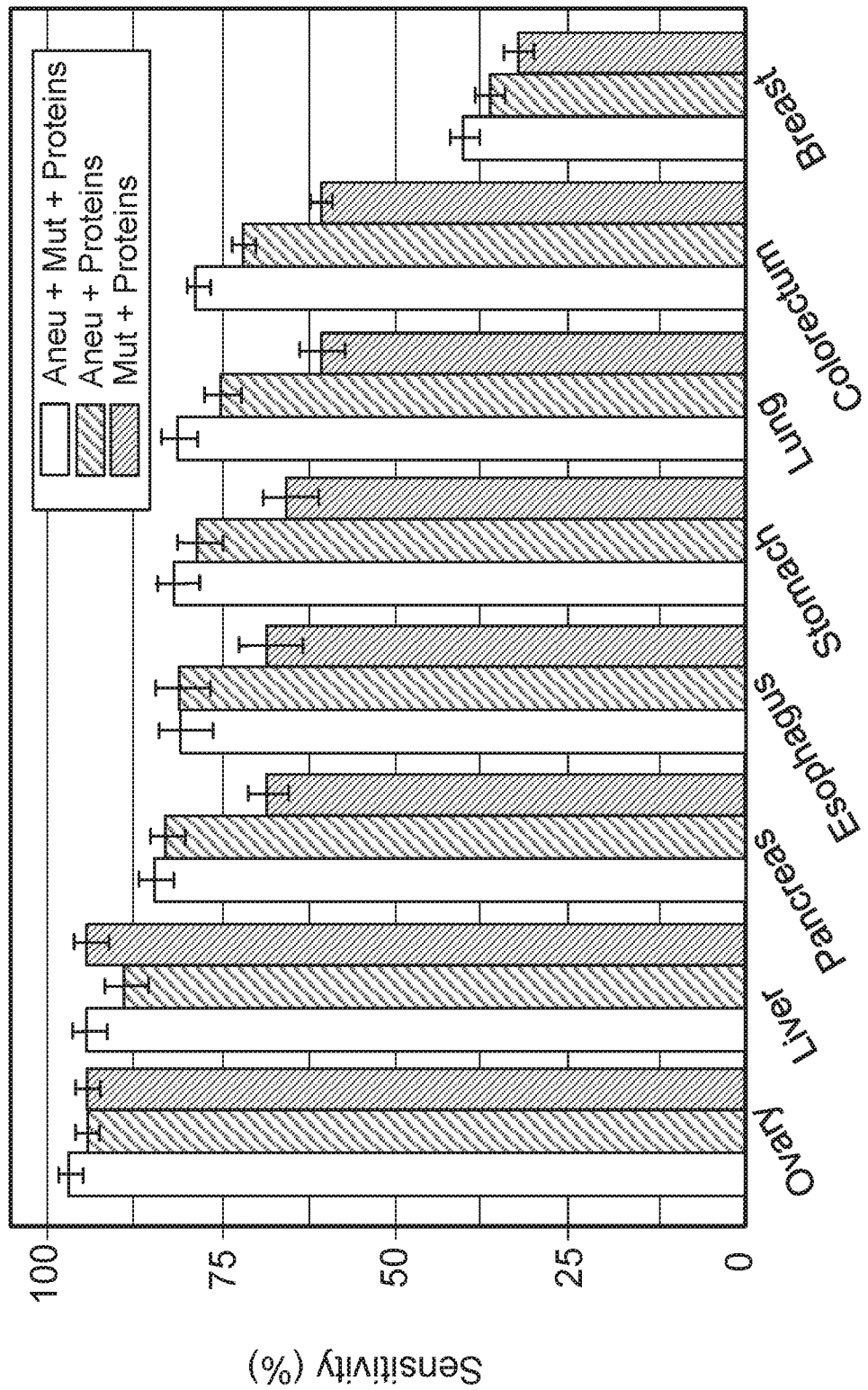


FIG. 7A

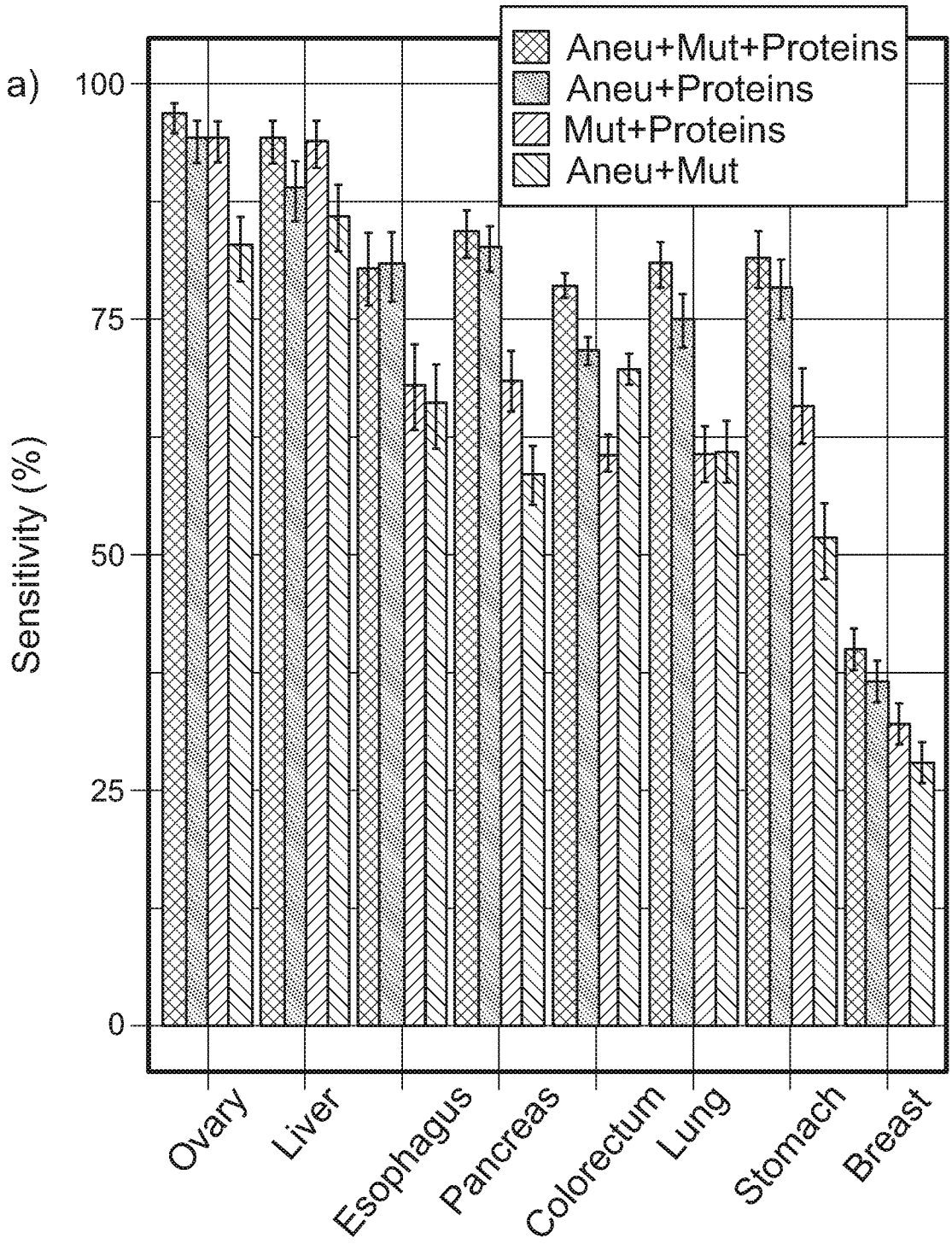


FIG. 7B

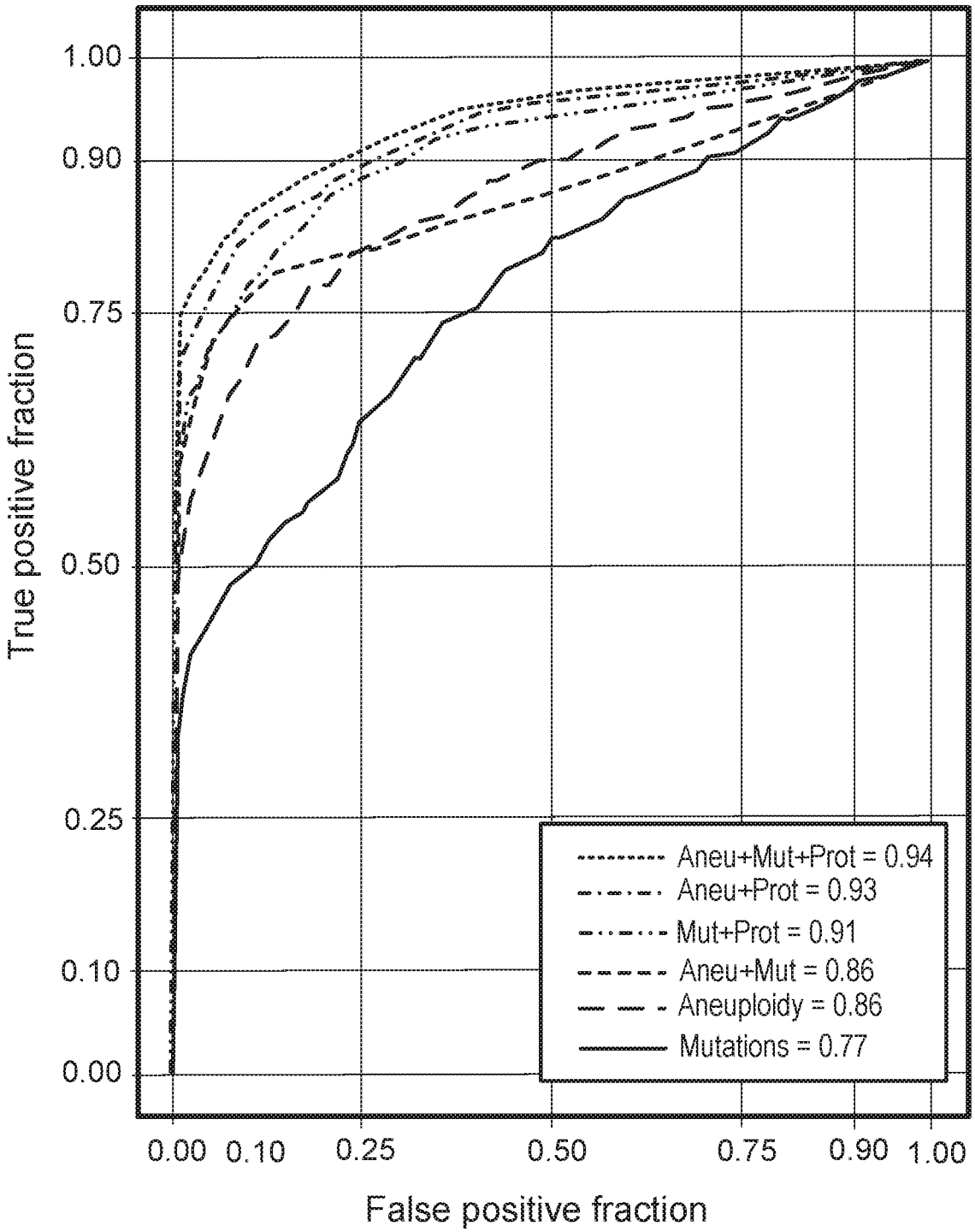


FIG. 8

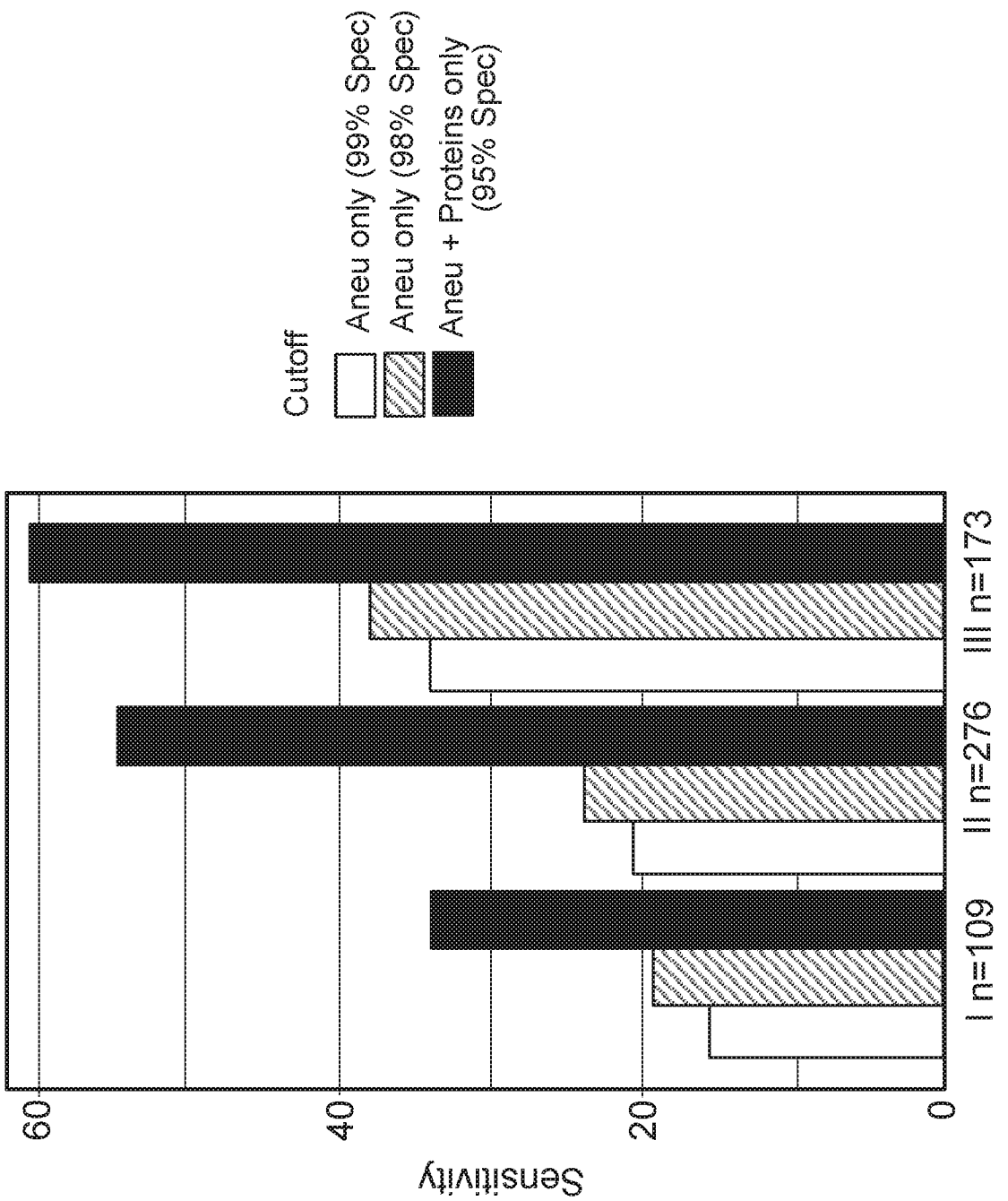


FIG. 9

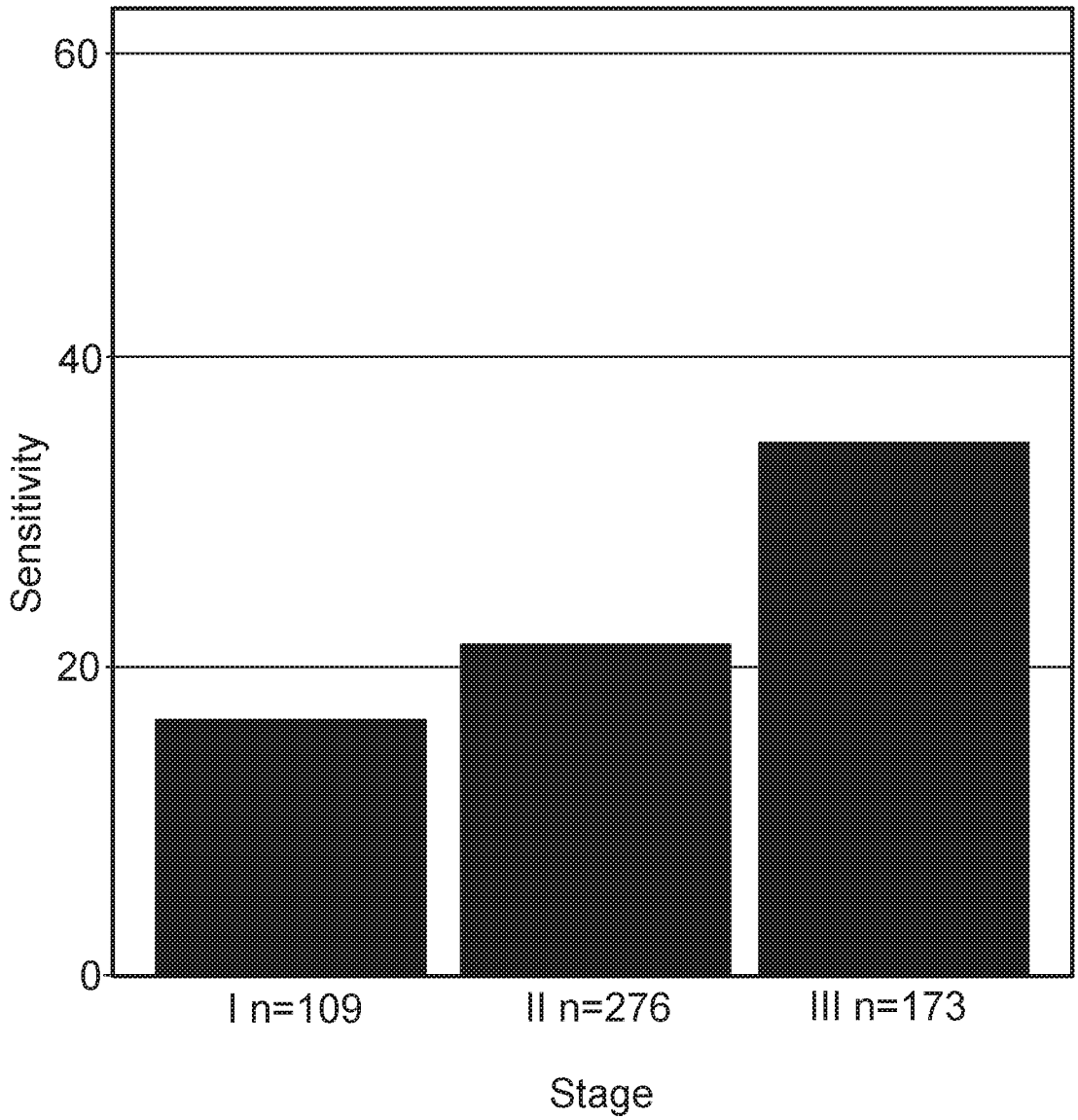


FIG. 10

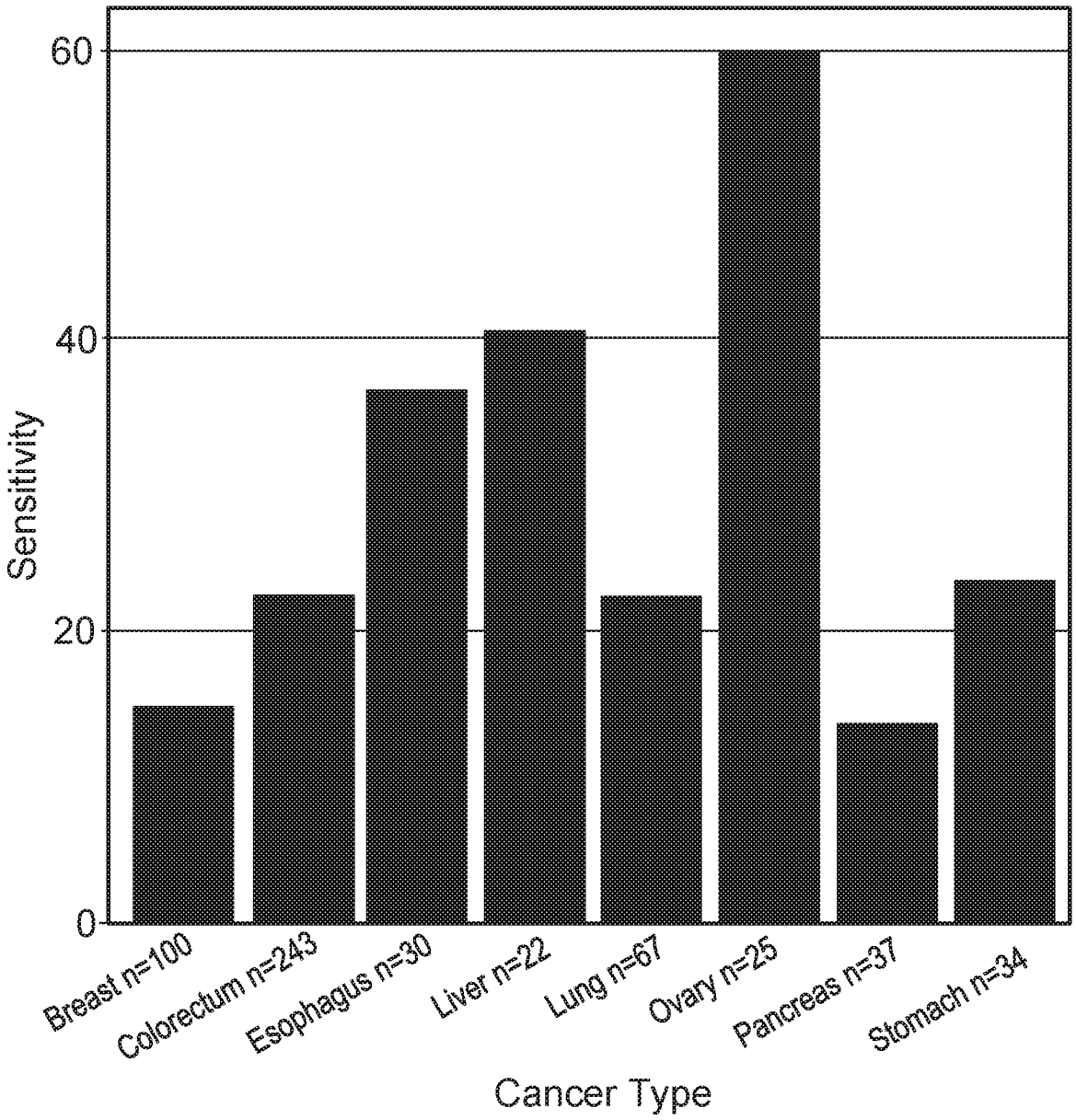


FIG. 11

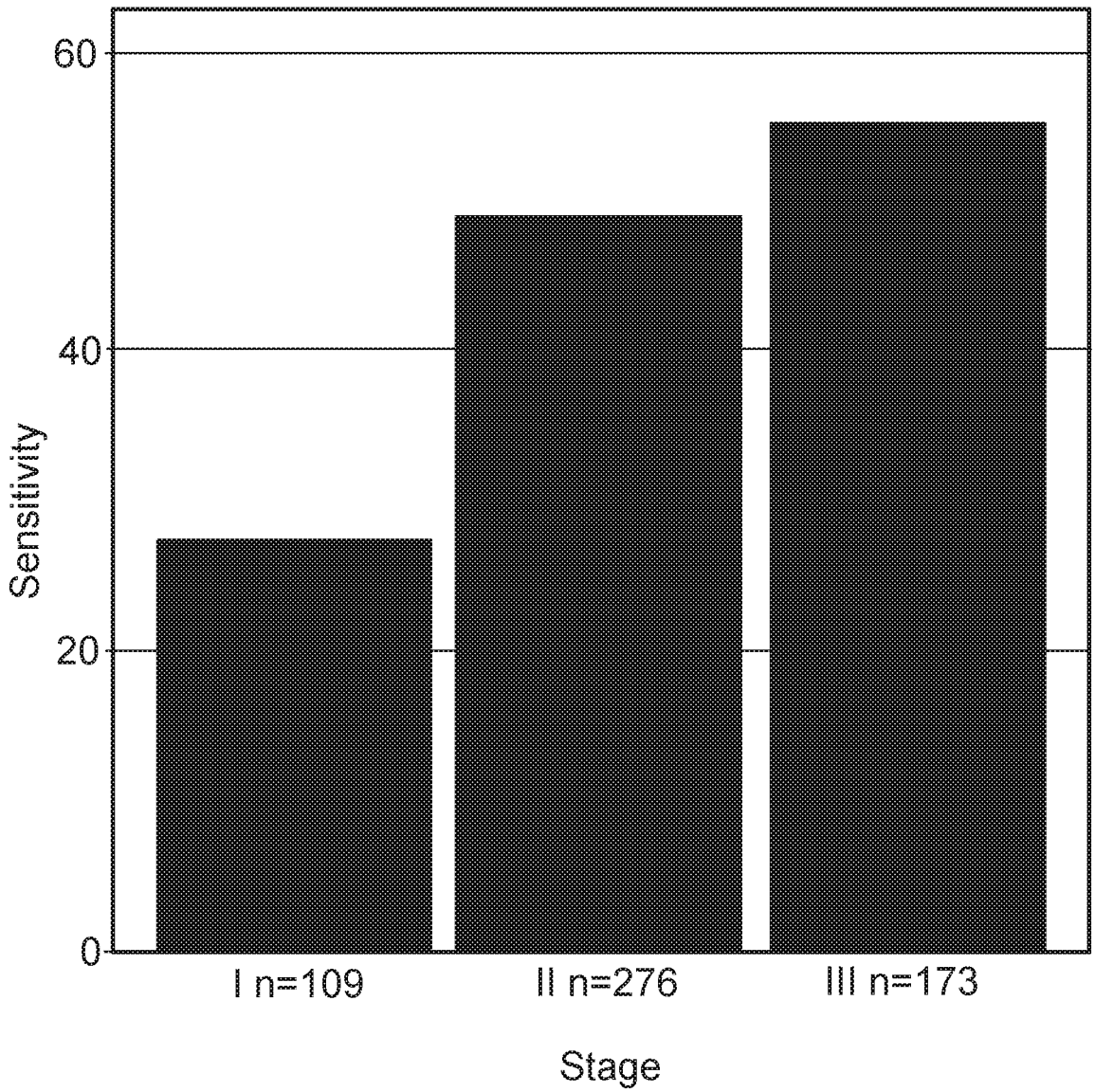


FIG. 12

In Silico simulated samples with monosomy or trisomy of one chromosomal arm

```
N <- number of normal samples used
f <- desired neoplastic cell fraction
r <- chromosome arm (1p..22q)
r_type<- alteration type (gain or loss)
rho <- desired unique read depth of synthetic sample
j<- desired number of repeats
For j=1:5
    For i=1:N
        s <- sample[i]
        s fraction<-vector that contains the fraction of reads that map to each of the 39 chr arm
            for sample s
        For f in 1:F
            For r in 1p:22q
                For r type in gain loss
                    h <- new in silico sample
                    t <- copy of s
                    t_fraction<- vector that contains the fraction of reads that map
                        to each of the 39 chr arm for sample t
                    if r_type== gain
                        t_fraction[r]<-t_fraction[r]*3/2 ###trisomy on arm r
                    else (r_type == loss)
                        t_fraction[r]<-t_fraction[r]*1/2 ###monosomy on arm r
                    Endif
                    h_normal<-weighted random select (1-f)*rho where the
                        weights are r_fraction
                    h_aneuploid<-weighted random select f*rho where the weights
                                                        are t_fraction
                                                    h<-h_normal+h_aneuploid
                                                End
                                            End
                                        End
                                    End
                                End
```

FIG. 13

In Silico simulated samples with alterations of many chromosome arms

```
N <- number of normal plasma samples used
d <- desired degree of aneuploidy (number arms altered)
f <- desired neoplastic cell fraction
r <- chromosome arm (1p..22q)
r_type<-arm alteration type (gain or loss)
p(r) <- probability that an arm is gained or lost in cancer (Estimated from (11))
rho <- desired unique read depth of in silico simulated sample (10M default)
j<- desired number of repeats
For j=1:5
    For i=1:N
        s <- sample[i]
        U <- get reads from s
        For f in 1:F
            h <- new in sillico sample
            For d in (10,15,20) #desired numbers of altered arms
                t <- copy of s
                For a in 1:d #select alteration types and spike-in to t
                    r, r_type <- weighted random selection where the weights [p(r)]
                    are the likelihood that an arm is gained or loss in cancer
                    u_all <- get all Reads that that map to chr arm r
                    if r_type== gain
                        t <- add 50% of u_all reads
                    else (r_type == loss)
                        t <- subtract 50% of u_all reads
                    Endif
                    h_normal<-randomselect (1-f)*rho reads from s
                    h_aneuploid<-randomselect f*rho reads from t
                    h<-h_normal+h_aneuploid

                End
            End
        End
    End
End
```

FIG. 14

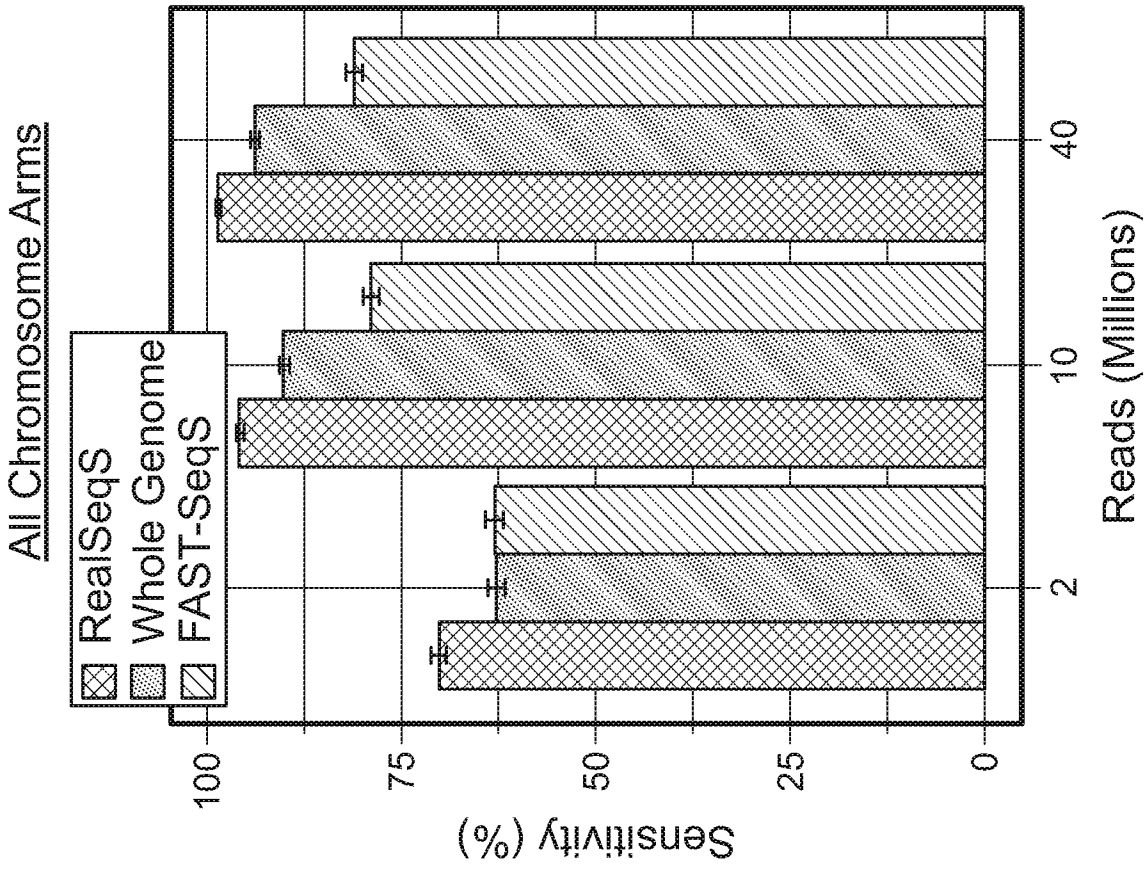


FIG. 15A

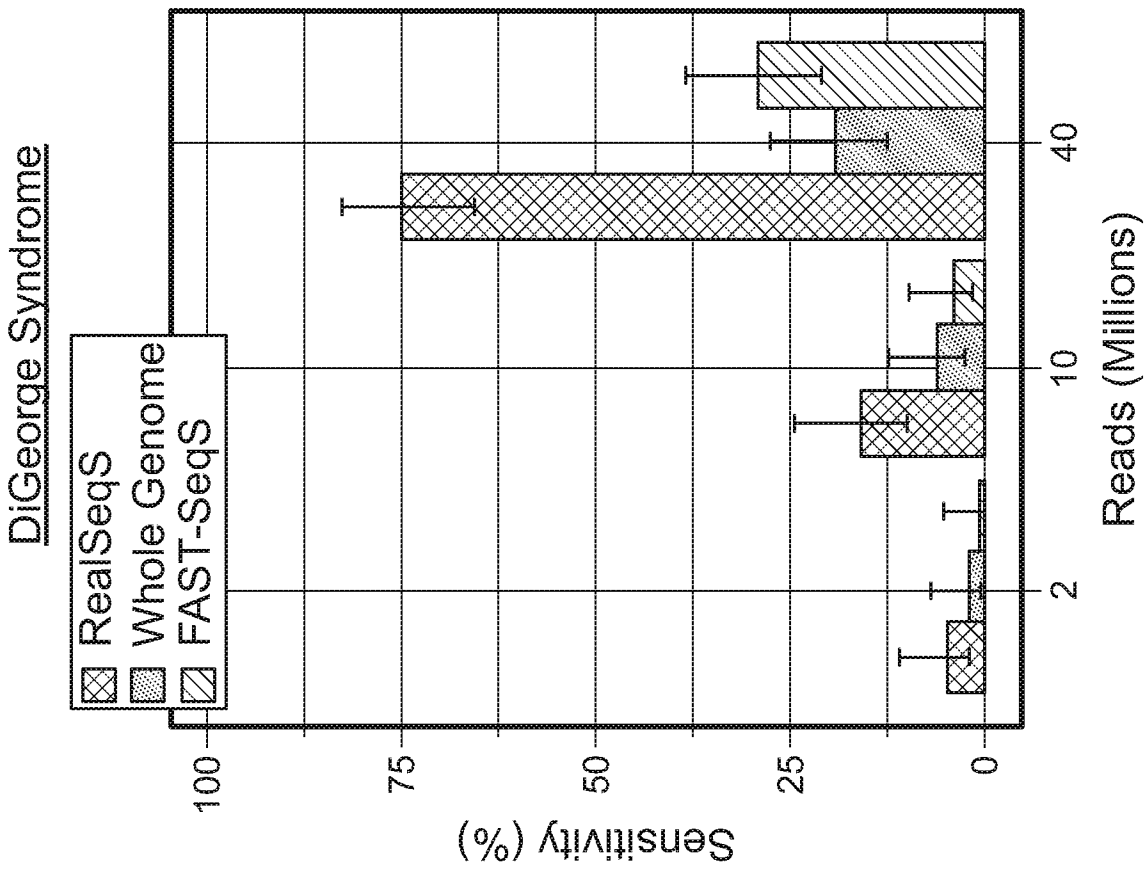


FIG. 15B

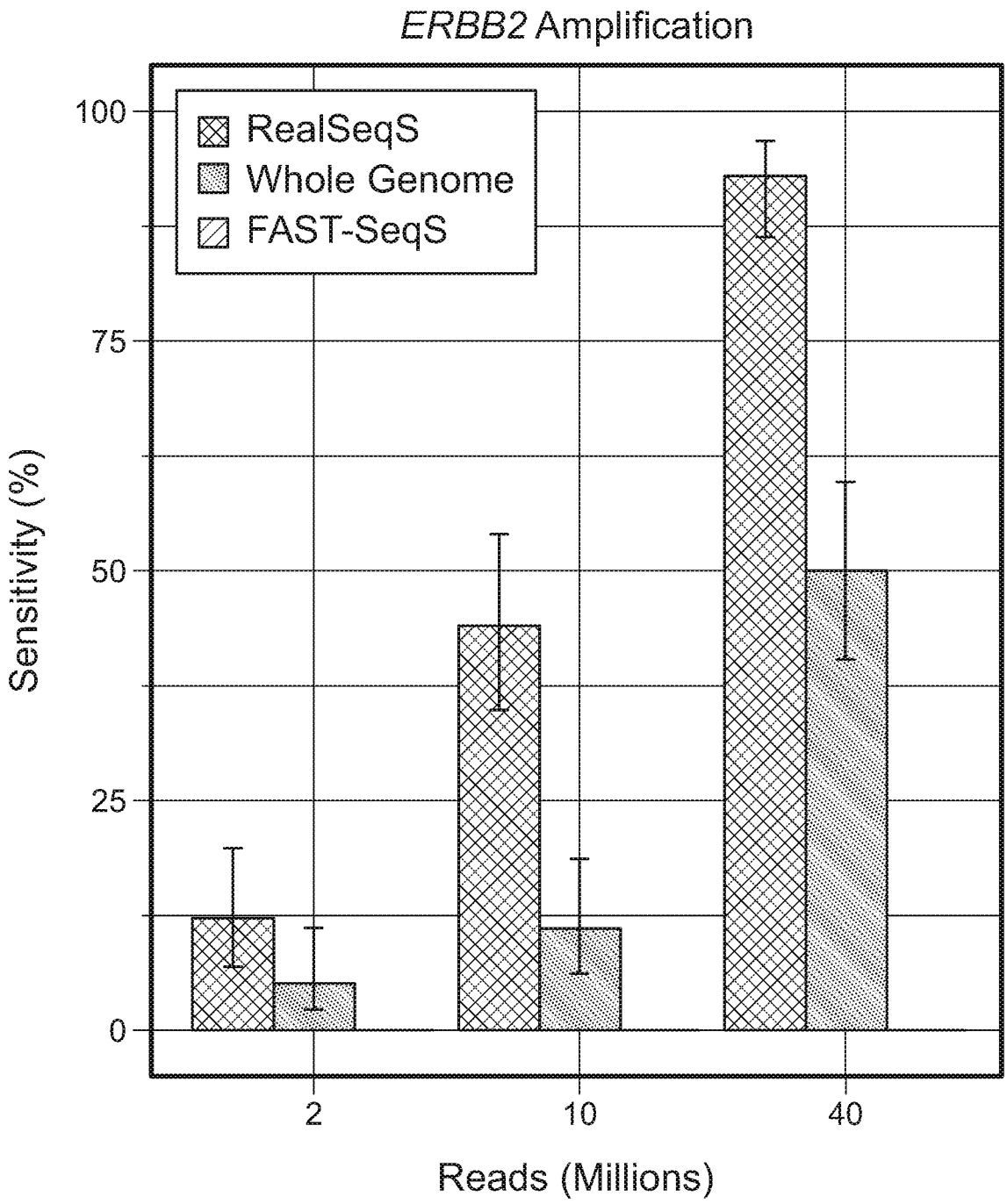


FIG. 15C

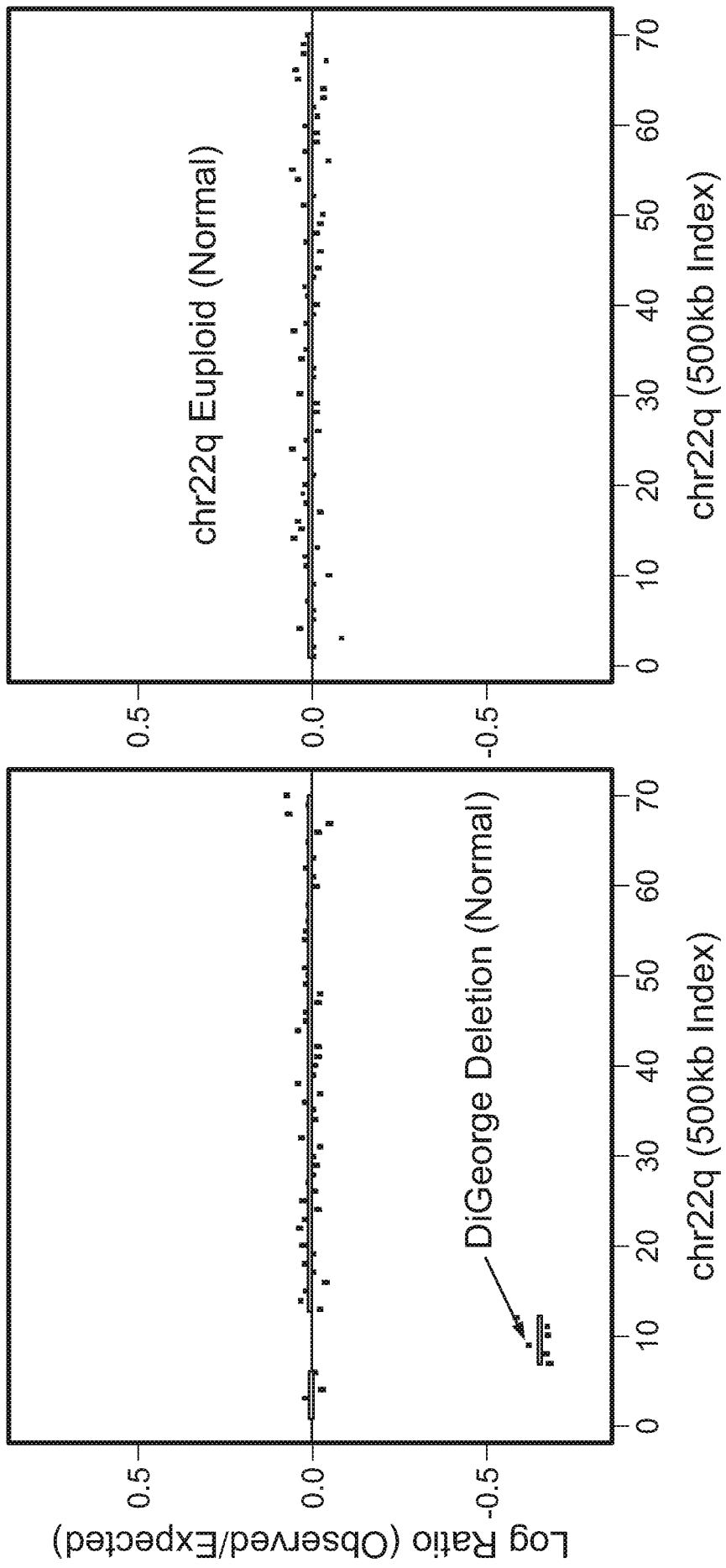


FIG. 16A

FIG. 16B

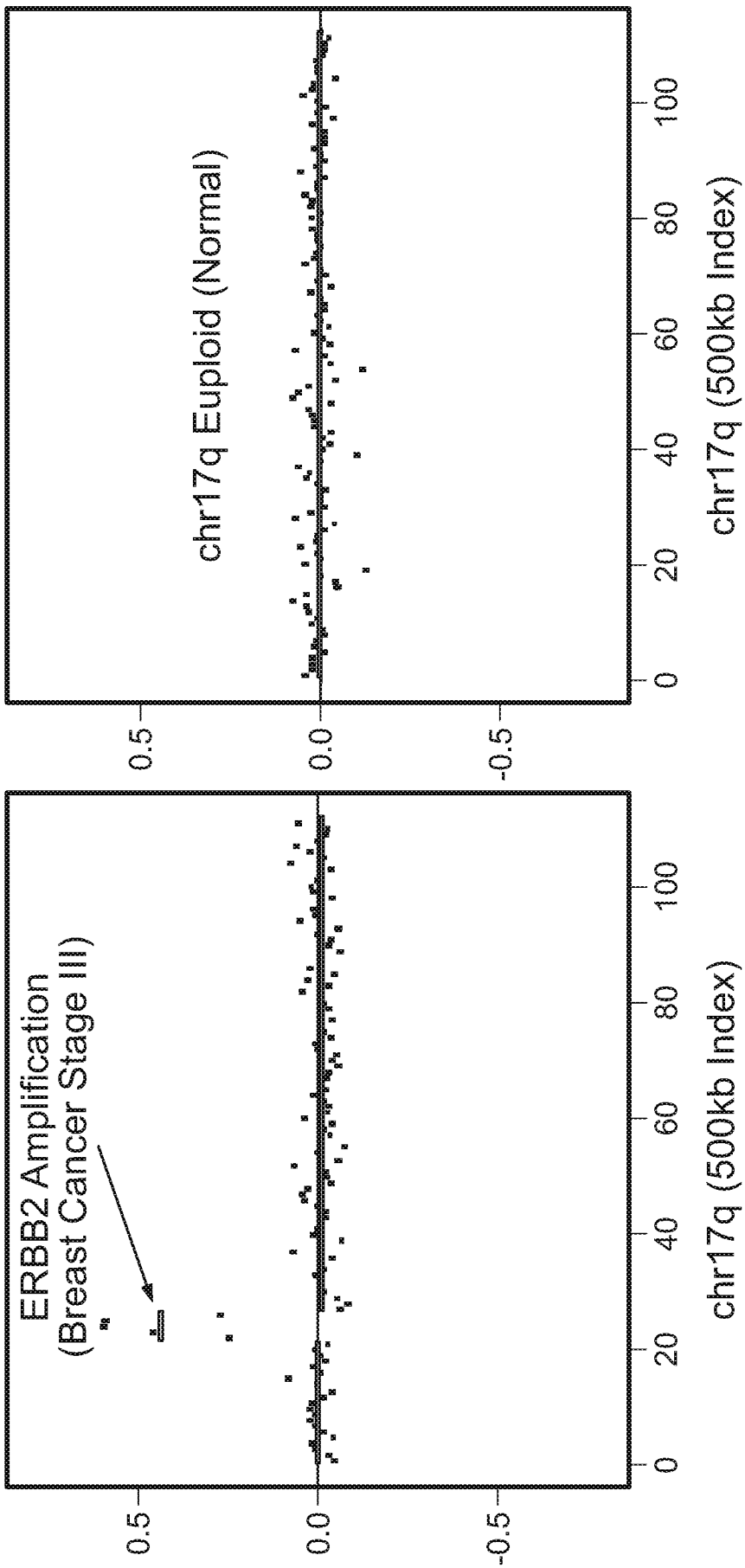


FIG. 17B

FIG. 17A

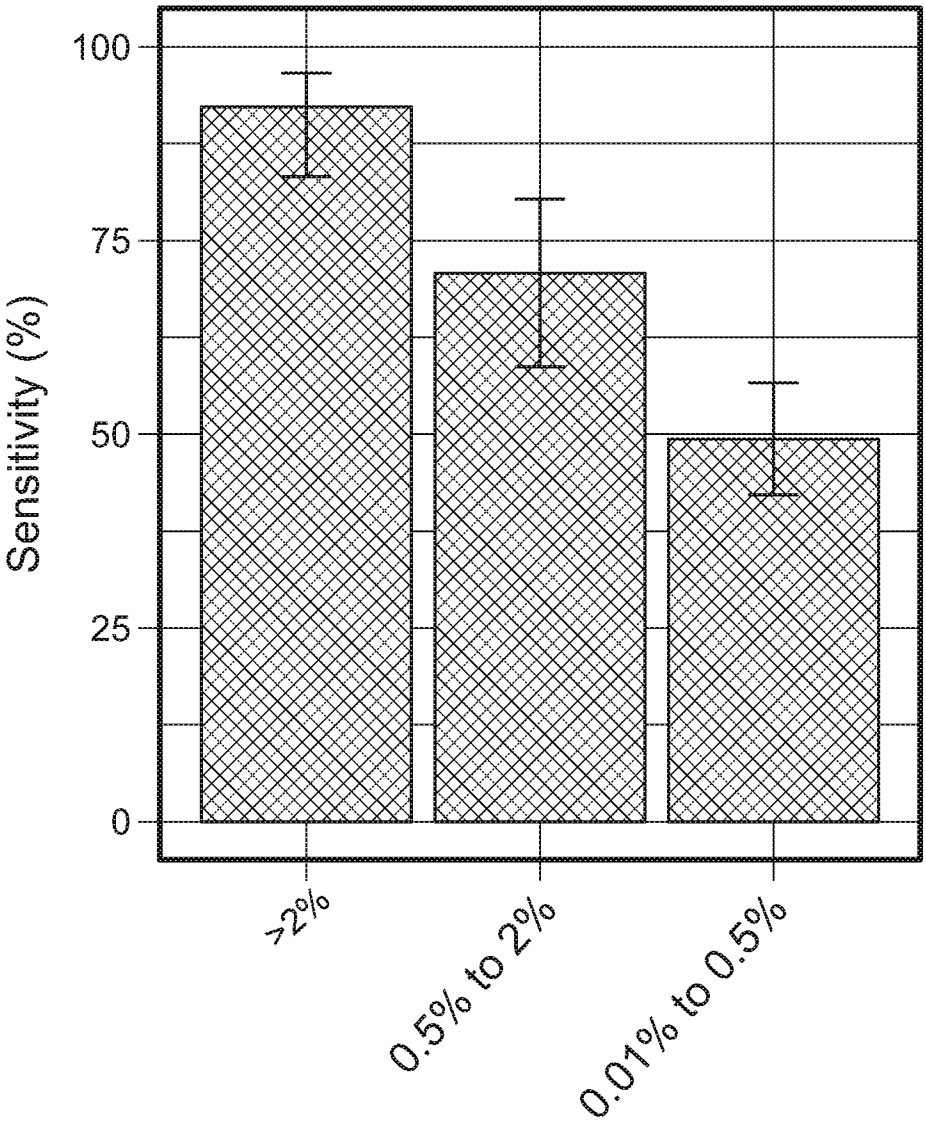


FIG. 18

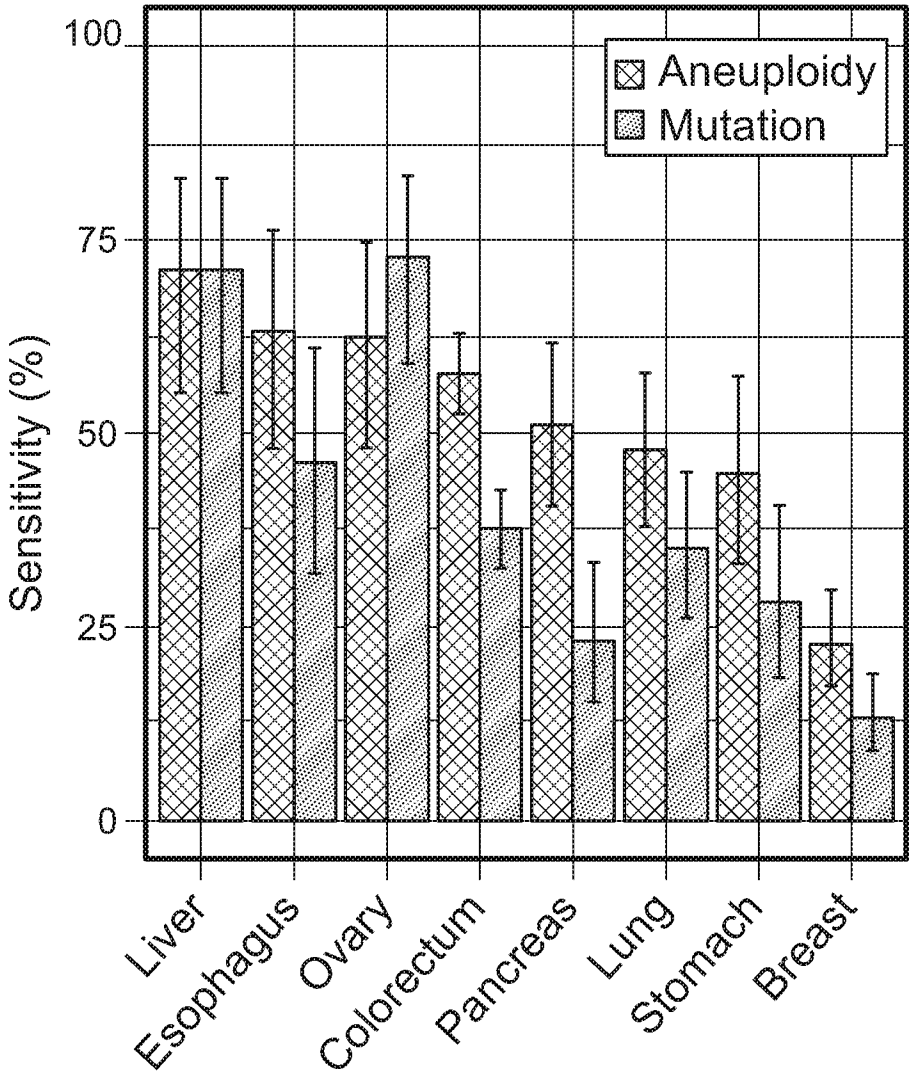


FIG. 19A

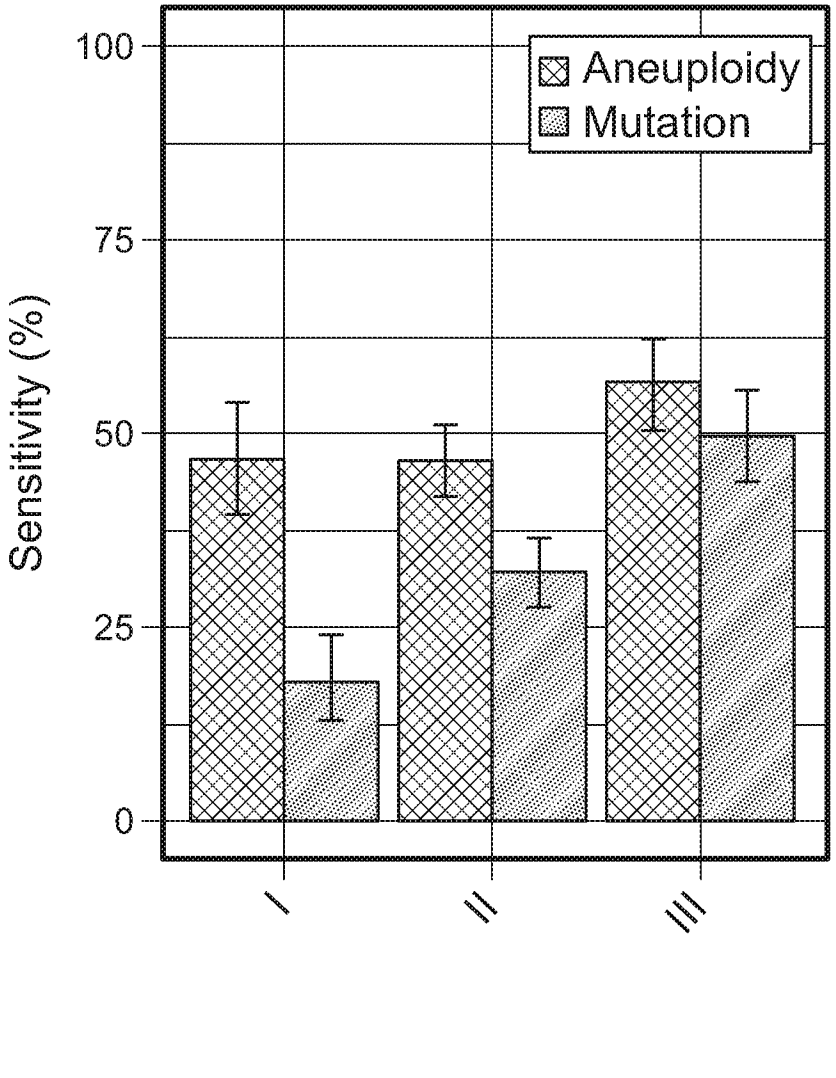


FIG. 19B

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