

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 Down's 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 examples, 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 examples, 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 examples, the amplified unique repetitive elements average less than 100 basepairs (bp) in size. In some examples, an approach (called WALDO for **W**ithin-**S**ample-**A**neup**L**oidy-**D**etect**I**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 example, 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
5 100,000 amplicons are formed in the step of amplifying (e.g., the plurality of amplicons can
include ~745,000 amplicons).

In some examples, the method is performed in vitro. In some examples, 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-
10 200,000 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
15 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 examples, the plurality of amplicons comprises about 50,000 amplicons;
about 100,000 amplicons; about 150,000 amplicons; about 200,000 amplicons; about
20 250,000 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.

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

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

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

In some examples, the average length of the amplicons is about 100 basepairs or less. In some examples, 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 examples, 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 examples, the amplicons comprise one or more long amplicons where the average length is 1000 basepairs or greater. In some examples, the long amplicons comprise DNA from a contaminating cell. In some examples, the contaminating cell is a leukocyte. In some examples, 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 example, 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.

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

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

15 In an example, the method comprises sequencing one or more subgenomic intervals or amplicons comprising the genetic biomarkers. In an example, the method comprises analyzing one or more genomic sequences for aneuploidy. In an example, the method comprises, contacting a protein biomarker with a detection reagent. In an example, 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.

20 In an example, 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 example, 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 examples, the method is performed in vitro.

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

In an example 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).

5 In an example 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 example, an increased sensitivity of detection, e.g., about
10 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 examples, the plurality of amplicons comprise about 1,000,000 amplicons,
15 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
20 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 examples, the plurality of amplicons comprises about 50,000 amplicons;
25 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
30 amplicons; about 950,000 amplicons; or about 1,000,000 amplicons.

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

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

In some examples, 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
5 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 examples, 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 examples, the average length of the amplicons is about 100 basepairs or less.

10 In some examples, 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,
15 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-
20 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 examples, 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
25 about 110bp.

In some examples, 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 examples, the radiologic scanning of an organ or body region characterizes the cancer. In some examples, the radiologic scanning of an organ or body region identifies the location of the cancer. In some
30 examples, the radiologic scan is a PET-CT scan. In some

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

In one aspect, the present disclosure provides 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 with a single pair of primers complementary to the chromosomal sequences to form a plurality of amplicons, wherein the primer pair 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 amplicons 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 a number of amplicons sufficient to detect aneuploidy are formed in the step of amplifying, thereby testing for the presence of aneuploidy in the genome of the mammal.

In another example, 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 examples, the method is performed in vitro.

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

In some examples, the sample comprises about 0.01 picogram (pg) to 500 pg of DNA. In some examples, 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 examples, 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 examples, the sample comprises 1 pg DNA. In some examples, the sample comprises 2 pg DNA. In some examples, the sample comprises 3 pg DNA. In some examples, the sample comprises 4 pg DNA. In some examples, the sample comprises 5 pg DNA. In some examples, the sample comprises 10 pg DNA.

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

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

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

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

In some examples, the method is used for forensics.

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

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

In some examples, 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 examples, 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 examples, 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 examples, a common polymorphism is present in a repetitive element, e.g., as described herein. In some examples, methods disclosed in Example 8 can be used to identify and/or distinguish the sample.

In another example, 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 one aspect, the present disclosure provides a pair of primers 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 another aspect, the present disclosure provides a kit for the amplification of a plurality of amplicons from a DNA sample comprising a pair of primers, wherein a first primer of the primer pair comprises SEQ ID NO: 1 and a second primer of the primer pair comprises SEQ ID NO: 10.

In yet another example, 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 examples 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 examples, the shared amplicon feature is the number of the mapped amplicons.

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

In some examples 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
15 primer comprising a sequence that is at least 80% identical to SEQ ID NO: 10. In some examples, the sequence of the first primer is at least 90% identical to SEQ ID NO. 1. In some examples, the sequence of the first primer is at least 95% identical to SEQ ID NO. 1. In some examples, the sequence of the first primer is 100% identical to SEQ ID NO. 1. In some examples, the sequence of the second primer is at least 90% identical to SEQ ID NO.
20 10. In some examples, the sequence of the second primer is at least 95% identical to SEQ ID NO. 10. In some examples, the sequence of the second primer is 100% identical to SEQ ID NO. 10. In some examples, 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
25 comprises SEQ ID NO: 10, or a sequence at least 80% identical thereto.

In another example, 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
30 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 examples, the method can include at least 100,000 amplicons formed in the step of amplifying. In some examples, the cancer can be a Stage I cancer. In some examples, 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 examples, the method is an in vitro method.

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

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

In some examples 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 examples, 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 examples 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 examples, 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 examples, 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 examples.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific examples of the disclosure
5 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,
10 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,
15 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
20
 - (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);
25
 - (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;
30and optionally wherein, the aneuploidy is associated with the presence, or risk, of a cancer of the plurality of cancers;

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 with a single pair of primers complementary to the chromosomal sequences to form a plurality of amplicons, wherein the primer pair 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 amplicons 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 a number of amplicons sufficient to detect aneuploidy are formed in the step of amplifying,
- thereby testing for the presence of aneuploidy in the genome of the mammal.
2. The method of claim 1, wherein the DNA sample comprises at least one of the following:
 - i) a plurality of euploid DNA samples, or ii) a plurality of test DNA samples of unknown ploidy.
3. The method of claim 1, wherein the DNA sample is from plasma or serum.
4. The method of claim 1, wherein the DNA sample comprises cell free DNA.
5. The method of any one of claims 1 to 4, wherein the mammal is a human.

6. The method of any one of claims 1 to 5, wherein the pair of primers comprises a first primer comprising SEQ ID NO: 1 and a second primer comprising SEQ ID NO: 10, or 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.

7. The method of any one of claims 1 to 6, wherein said amplicons comprise one or more repetitive elements shown in Table 1.

8. The method of claim 7, wherein said amplicons comprise unique short interspersed nucleotide elements (SINEs).

9. The method of any one of claims 1 to 8, wherein the plurality of amplicons comprises sequences on a plurality of 2 or more, different chromosomes.

10. The method of any one of claims 1 to 9, wherein the genomic intervals comprise from about 100 nucleotides to about 125,000,000 nucleotides.

11. The method of any one of claims 1 to 10, wherein quantifying amplicons mapped to genomic intervals comprises identifying a plurality of genomic intervals with one or more shared amplicon features.

12. The method of claim 11, wherein the shared amplicon feature is the number of the mapped amplicons, and/or the average length of the mapped amplicons.

13. The method of claim 11 or 12, wherein the plurality of genomic intervals with shared amplicon features are grouped into one or more clusters.

14. The method of claim 13, wherein each cluster comprises about two hundred genomic intervals, and optionally, said clusters are predefined clusters.

15. The method of any one of claims 1 to 14, wherein the comparison of the genomic intervals further comprises matching one or more genomic intervals from test samples to predefined clusters.

5

16. A pair of primers 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.

10

17. The pair of primers of claim 16, wherein the sequence of the first primer is or comprises a sequence that is 100% identical to SEQ ID NO. 1 and/or the sequence of the second primer is or comprises a sequence that is 100% identical to SEQ ID NO. 10.

15 18. A kit for the amplification of a plurality of amplicons from a DNA sample comprising a pair of primers, wherein a first primer of the primer pair comprises SEQ ID NO: 1 and a second primer of the primer pair comprises SEQ ID NO: 10.

19. The method of any one of claims 1 to 15, wherein at least 10,000 amplicons are formed
20 in the step of amplifying using said single primer pair.

20. The method of any one of claims 1 to 15, wherein at least 300,000 amplicons are formed in the step of amplifying using said single primer pair.

25