CLAIMS

WHAT IS CLAIMED IS:

1. A method for determining the presence or absence of any four or more different complete fetal chromosomal aneuploidies in a maternal test sample comprising fetal and maternal nucleic acids, said method comprising:

(a) obtaining sequence information for said fetal and maternal nucleic acids in said sample;

(b) using said sequence information to identify a number of sequence tags for each of any four or more chromosomes of interest selected from chromosomes 1-22, X, and Y and to identify a number of sequence tags for a normalizing chromosome sequence for each of said any four or more chromosomes of interest;

(c) using said number of sequence tags identified for each of said any four or more chromosomes of interest and said number of sequence tags identified for each said normalizing chromosome sequence to calculate a single chromosome dose for each of said any four or more chromosomes of interest; and

(d) comparing each of said single chromosome doses for each of said any four or more chromosomes of interest to a threshold value for each of said four or more chromosomes of interest, and thereby determining the presence or absence of any four or more complete different fetal chromosomal aneuploidies in said sample.

2. The method of Claim 1, wherein step (c) comprises calculating a single chromosome dose for each of said chromosomes of interest as the ratio of the number of sequence tags identified for each of said chromosomes of interest and the number of sequence tags identified for said normalizing chromosome sequence for each of said chromosomes of interest.

3. The method of Claim 1 or 2, wherein step (c) comprises:

(i) calculating a sequence tag density ratio for each of said chromosomes of interest, by relating the number of sequence tags identified for each of said chromosomes of interest in step (b) to the length of each of said chromosomes of interest;

(ii) calculating a sequence tag density ratio for each said normalizing chromosome by relating the number of sequence tags identified for said normalizing chromosome sequence in step (b) to the length of each said normalizing chromosome; and(iii) using the sequence tag density ratios calculated in steps (i) and (ii) to calculate a single chromosome dose for each of said chromosomes of interest, wherein said chromosome dose is calculated as the ratio of the sequence tag density ratio for

each of said chromosomes of interest and the sequence tag density ratio for said normalizing chromosome sequence for each of said chromosomes of interest.

4. The method of any one of Claims 1-3, wherein said any four or more chromosomes of interest selected from chromosomes 1-22, X, and Y comprise at least twenty chromosomes selected from chromosomes 1-22, X, and Y, and wherein the presence or absence of at least twenty different complete fetal chromosomal aneuploidies is determined.

5. The method of any one of Claims 1-3, wherein said any four or more chromosomes of interest selected from chromosomes 1-22, X, and Y is all of chromosomes 1-22, X, and Y, and wherein the presence or absence of complete fetal chromosomal aneuploidies of all of chromosomes 1-22, X, and Y is determined.

6. The method of any one of Claims 1-5, wherein said normalizing chromosome sequence is a single chromosome selected from chromosomes 1-22, X, and Y.

7. The method of any one of Claims 1-5, wherein said normalizing chromosome sequence is a group of chromosomes selected from chromosomes 1-22, X, and Y.

8. A method for determining the presence or absence of any one or more different complete fetal chromosomal aneuploidies in a maternal test sample comprising fetal and maternal nucleic acids, said method comprising:

(a) obtaining sequence information for said fetal and maternal nucleic acids in said sample;

(b) using said sequence information to identify a number of sequence tags for each of any one or more chromosomes of interest selected from chromosomes 1-22, X and Y and to identify a number of sequence tags for a normalizing segment sequence for each of said any one or more chromosomes of interest;

(c) using said number of sequence tags identified for each of said any one or more chromosomes of interest and said number of sequence tags identified for said normalizing segment sequence to calculate a single chromosome dose for each of said any one or more chromosomes of interest; and

(d) comparing each of said single chromosome doses for each of said any one or more chromosomes of interest to a threshold value for each of said one or more chromosomes of interest,

and thereby determining the presence or absence of one or more different complete fetal chromosomal aneuploidies in said sample.

9. The method of Claim 8, wherein step (c) comprises calculating a single chromosome dose for each of said chromosomes of interest as the ratio of the number of sequence tags identified for each of said chromosomes of interest and the number of sequence tags identified for said normalizing segment sequence for each of said chromosomes of interest.

10. The method of Claim 8 or 9, wherein said any one or more chromosomes of interest selected from chromosomes 1-22, X, and Y comprise at least twenty chromosomes selected from chromosomes 1-22, X, and Y, and wherein the presence or absence of at least twenty different complete fetal chromosomal aneuploidies is determined.

11. The method of any one of Claims 8 or 9, wherein said any one or more chromosomes of interest selected from chromosomes 1-22, X, and Y is all of chromosomes 1-22, X, and Y, and wherein the presence or absence of complete fetal chromosomal aneuploidies of all of chromosomes 1-22, X, and Y is determined.

12. The method of any one of Claims 1-11, wherein said different complete chromosomal aneuploidies are selected from complete chromosomal trisomies, complete chromosomal monosomies and complete chromosomal polysomies.

13. The method of any one of Claims 1-12, wherein said different complete fetal chromosomal aneuploidies are selected from trisomy 2, trisomy 8, trisomy 9, trisomy 21, trisomy 13, trisomy 16, trisomy 18, trisomy 22, 47,XXY, 47,XXX, 47,XYY, and monosomy X.

14. The method of any one of Claims 1-13, wherein steps (a)-(d) are repeated for test samples from different maternal subjects, and wherein the method comprises determining the presence or absence of any four or more different complete fetal chromosomal aneuploidies in each of said samples.

15. The method of any one of Claims 1-14, further comprising calculating a normalized chromosome value (NCV), wherein said NCV relates said chromosome dose to the mean of the corresponding chromosome dose in a set of qualified samples as:

$$NCV_{ij} = \frac{x_{ij} - \hat{\mu}_j}{\hat{\sigma}_j}$$

where $\hat{\mu}_j$ and $\hat{\sigma}_j$ are the estimated mean and standard deviation, respectively, for the j-th chromosome dose in a set of qualified samples, and x_{ij} is the observed j-th chromosome dose for test sample i.

16. A method for determining the presence or absence of different partial fetal chromosomal aneuploidies in a maternal test sample comprising a fetal and maternal nucleic acids, said method comprising:

(a) obtaining sequence information for said fetal and maternal nucleic acids in said sample;

(b) using said sequence information to identify a number of sequence tags for each of any one or more segments of any one or more chromosomes of interest selected from chromosomes 1-22, X, and Y and to identify a number of sequence tags for a normalizing segment sequence for each of said any one or more segments of any one or more chromosomes of interest;

(c) using said number of sequence tags identified for each of said any one or more segments of any one or more chromosomes of interest and said number of sequence tags identified for said normalizing segment sequence to calculate a single chromosome dose for each of said any one or more segments of any one or more chromosomes of interest; and

(d) comparing each of said single segment doses for each of said any one or more segments of any one or more chromosomes of interest to a threshold value for each of said any one or more chromosomal segments of any one or more chromosome of interest, and thereby determining the presence or absence of one or more different partial fetal chromosomal aneuploidies in said sample.

17. The method of Claim 16, wherein step (c) comprises calculating a single segment dose for each of said any one or more segments of any one or more chromosomes of interest as the ratio of the number of sequence tags identified for each of said any one or more segments of any one or more chromosomes of interest and the number of sequence tags identified for said normalizing segment sequence for each of said any one or more segments of any one or more chromosomes of interest.

18. The method of Claim 16 or 17, further comprising calculating a normalized segment value (NSV), wherein said NSV relates said segment dose to the mean of the corresponding segment dose in a set of qualified samples as:

$$NSV_{ij} = \frac{x_{ij} - \hat{\mu}_j}{\hat{\sigma}_j}$$

where $\hat{\mu}_j$ and $\hat{\sigma}_j$ are the estimated mean and standard deviation, respectively, for the j-th segment dose in a set of qualified samples, and x_{ij} is the observed j-th segment dose for test sample i.

19. The method of any one of Claims 8-18, wherein said normalizing segment sequence is a single segment of any one or more of chromosomes 1-22, X, and Y.

20. The method of any one of Claims 8-18, wherein said normalizing segment sequence is a group of segments of any one or more of chromosomes 1-22, X, and Y.

21. The method of any one of Claims 16-20, wherein said different partial fetal chromosomal aneuploidies are selected from partial duplications, partial multiplications, partial insertions and partial deletions.

22. The method of any one of Claims 16-21, wherein said partial fetal aneuploidies are selected from partial monosomy of chromosome 1, partial monosomy of chromosome 4, partial monosomy of chromosome 5, partial monosomy of chromosome 7, partial monosomy of chromosome 11, partial monosomy of chromosome 15, partial monosomy of chromosome 17, partial monosomy of chromosome 18, and partial monosomy of chromosome 22.

23. The method of any one of Claims 16-22, wherein steps (a)-(d) are repeated for test samples from different maternal subjects, and wherein the method comprises determining the presence or absence of different partial fetal chromosomal aneuploidies in each of said samples.

24. The method of any one of the preceding Claims, wherein step (a) comprises sequencing at least a portion of said nucleic acid molecules of said test sample to obtain said sequence information for said fetal and maternal nucleic acid molecules of said test sample.

25. The method of any one of the preceding Claims, wherein said test sample is a maternal sample selected from blood, plasma, serum, urine and saliva samples.

26. The method of any one of the preceding Claims, wherein said nucleic acid molecules are a mixture of fetal and maternal cell-free DNA molecules.

27. The method of any one of the preceding Claims, wherein said sequencing is next generation sequencing (NGS).

28. The method of any one of the preceding Claims, wherein said sequencing is massively parallel sequencing using sequencing-by-synthesis with reversible dye terminators.

29. The method of any one of the preceding Claims, wherein said sequencing is sequencing-by-ligation.

30. The method of any one of the preceding Claims, wherein said sequencing comprises an amplification.

31. The method of any one of the preceding Claims, wherein said sequencing is single molecule sequencing.

32. A method for determining the presence or absence of any twenty or more different complete fetal chromosomal aneuploidies in a maternal plasma test sample comprising a mixture of fetal and maternal cell-free DNA molecules, said method comprising:

(a) sequencing at least a portion of said cell-free DNA molecules to obtain sequence information for said fetal and maternal cell-free DNA molecules in said sample;

(b) using said sequence information to identify a number of sequence tags for each of any twenty or more chromosomes of interest selected from chromosomes 1-22, X, and Y and to identify a number of sequence tags for a normalizing chromosome for each of said twenty or more chromosomes of interest;

(c) using said number of sequence tags identified for each of said twenty or more chromosomes of interest and said number of sequence tags identified for each said normalizing chromosome to calculate a single chromosome dose for each of said twenty or more chromosomes of interest; and

(d) comparing each of said single chromosome doses for each of said twenty or more chromosomes of interest to a threshold value for each of said twenty or more chromosomes of interest, and thereby determining the presence or absence of any twenty or more different complete fetal chromosomal aneuploidies in said sample.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for determining the presence or absence of any four or more different complete fetal chromosomal aneuploidies for each of four or more chromosomes of interest in a maternal test sample comprising fetal and maternal nucleic acids, said method comprising:

(a) identifying a normalizing chromosome for each of said four or more chromosomes of interest in a qualified sample, wherein each of said normalizing chromosomes for each of said four or more chromosomes of interest:

(i) displays a variability in the number of sequence tags that are mapped to the normalizing chromosome that best approximates the number of sequence tags that map to the chromosome of interest in the qualified sample; and/or

(ii) provides the greatest statistical difference between the distribution of chromosome doses for the chromosome of interest in the qualified sample and the distribution of chromosome doses for the chromosome of interest in the test sample;

(b) obtaining sequence information for said fetal and maternal nucleic acids in said sample;

(c) using said sequence information to identify a number of sequence tags for each of said any four or more chromosomes of interest, wherein the four or more chromosomes of interest are selected from chromosomes 1-22, X, and Y and to identify a number of sequence tags for the normalizing chromosome for each of said any four or more chromosomes of interest;

(d) using said number of sequence tags identified for each of said any four or more chromosomes of interest and said number of sequence tags identified for each said normalizing chromosome to calculate a single chromosome dose for each of said any four or more chromosomes of interest; and

(e) comparing each of said single chromosome doses for each of said any four or more chromosomes of interest to a threshold value for each of said four or more chromosomes of interest, and thereby determining the presence or absence of any four or more complete different fetal chromosomal aneuploidies in said sample.

2. The method of Claim 1, wherein step (c) comprises calculating a single chromosome dose for each of said chromosomes of interest as the ratio of the number of sequence tags identified for each of said chromosomes of interest and the number of sequence tags identified for said normalizing chromosome sequence for each of said chromosomes of interest.

3. The method of Claim 1 or 2, wherein step (c) comprises:

(i) calculating a sequence tag density ratio for each of said chromosomes of interest, by relating the number of sequence tags identified for each of said chromosomes of interest in step (b) to the length of each of said chromosomes of interest;

(ii) calculating a sequence tag density ratio for each said normalizing chromosome by relating the number of sequence tags identified for said normalizing chromosome sequence in step (b) to the length of each said normalizing chromosome; and(iii) using the sequence tag density ratios calculated in steps (i) and (ii) to calculate a single chromosome dose for each of said chromosomes of interest, wherein said chromosome dose is calculated as the ratio of the sequence tag density ratio for each of said chromosomes of interest and the sequence tag density ratio for said normalizing chromosome sequence for each of said chromosomes of interest.

4. The method of any one of Claims 1-3, wherein said any four or more chromosomes of interest selected from chromosomes 1-22, X, and Y comprise at least twenty chromosomes selected from chromosomes 1-22, X, and Y, and wherein the presence or absence of at least twenty different complete fetal chromosomal aneuploidies is determined.

5. The method of any one of Claims 1-3, wherein said any four or more chromosomes of interest selected from chromosomes 1-22, X, and Y is all of chromosomes 1-22, X, and Y, and wherein the presence or absence of complete fetal chromosomal aneuploidies of all of chromosomes 1-22, X, and Y is determined.

6. The method of any one of Claims 1-5, wherein said normalizing chromosome sequence is a single chromosome selected from chromosomes 1-22, X, and Y.

7. The method of any one of Claims 1-5, wherein said normalizing chromosome sequence is a group of chromosomes selected from chromosomes 1-22, X, and Y.

8. A method for determining the presence or absence of any one or more different complete fetal chromosomal aneuploidies for said one or more chromosomes of interest in a maternal test sample comprising fetal and maternal nucleic acids, said method comprising:

(a) identifying a normalizing segment sequence for each of said one or more chromosomes of interest in a qualified sample, wherein said normalizing segment sequence for each of said one or more chromosomes of interest:

(i) displays a variability in the number of sequence tags that are mapped to the normalizing segment sequence that best approximates the number of sequence tags that map to the chromosome of interest in the qualified sample; and/or

(ii) provides the greatest statistical difference between the distribution of chromosome doses for the chromosome of interest in the qualified sample and the distribution of chromosome doses for the chromosome of interest in the test sample;

(b) obtaining sequence information for said fetal and maternal nucleic acids in said sample;

(c) using said sequence information to identify a number of sequence tags for each of any one or more chromosomes of interest, wherein the one or more chromosomes of interest are selected from chromosomes 1-22, X and Y and to identify a number of sequence tags for the normalizing segment sequence for each of said any one or more chromosomes of interest;

(d) using said number of sequence tags identified for each of said any one or more chromosomes of interest and said number of sequence tags identified for said normalizing segment sequence to calculate a single chromosome dose for each of said any one or more chromosomes of interest; and

(e) comparing each of said single chromosome doses for each of said any one or more chromosomes of interest to a threshold value for each of said one or more chromosomes of interest, and thereby determining the presence or absence of one or more different complete fetal chromosomal aneuploidies in said sample.

9. The method of Claim 8, wherein step (c) comprises calculating a single chromosome dose for each of said chromosomes of interest as the ratio of the number of sequence tags identified for each of said chromosomes of interest and the number of sequence tags identified for said normalizing segment sequence for each of said chromosomes of interest.

10. The method of Claim 8 or 9, wherein said any one or more chromosomes of interest selected from chromosomes 1-22, X, and Y comprise at least twenty chromosomes selected from chromosomes 1-22, X, and Y, and wherein the presence or absence of at least twenty different complete fetal chromosomal aneuploidies is determined.

11. The method of any one of Claims 8 or 9, wherein said any one or more chromosomes of interest selected from chromosomes 1-22, X, and Y is all of chromosomes 1-22, X, and Y, and wherein the presence or absence of complete fetal chromosomal aneuploidies of all of chromosomes 1-22, X, and Y is determined.

12. The method of any one of Claims 1-11, wherein said different complete chromosomal aneuploidies are selected from complete chromosomal trisomies, complete chromosomal monosomies and complete chromosomal polysomies.

13. The method of any one of Claims 1-12, wherein said different complete fetal chromosomal aneuploidies are selected from trisomy 2, trisomy 8, trisomy 9, trisomy 21, trisomy 13, trisomy 16, trisomy 18, trisomy 22, 47,XXY, 47,XXX, 47,XYY, and monosomy X.

14. The method of any one of Claims 1-13, wherein steps (a)-(d) are repeated for test samples from different maternal subjects, and wherein the method comprises determining the presence or absence of any four or more different complete fetal chromosomal aneuploidies in each of said samples.

15. The method of any one of Claims 1-14, further comprising calculating a normalized chromosome value (NCV), wherein said NCV relates said chromosome dose to the mean of the corresponding chromosome dose in a set of qualified samples as:

$$NCV_{ij} = \frac{x_{ij} - \hat{\mu}_j}{\hat{\sigma}_j}$$

where $\hat{\mu}_j$ and $\hat{\sigma}_j$ are the estimated mean and standard deviation, respectively, for the j-th chromosome dose in a set of qualified samples, and x_{ij} is the observed j-th chromosome dose for test sample i.

16. A method for determining the presence or absence of different partial fetal chromosomal aneuploidies for one or more segments of any one or more chromosomes of interest in a maternal test sample comprising a fetal and maternal nucleic acids, said method comprising:

(a) identifying a normalizing segment sequence for each of said one or more segments of the one or more chromosomes of interest in a qualified sample, wherein each of said normalizing segment sequence for each of the one or more segments of the one or more chromosomes of interest:

(i) displays a variability in the number of sequence tags that are mapped to the normalizing segment sequence that best approximates the number of sequence tags that map

to the one or more segments of the one or more chromosomes of interest in the qualified sample; and/or

(ii) provides the greatest statistical difference between the distribution of chromosome doses for the one or more segments of the one or more chromosomes of interest in the qualified sample and the distribution of chromosome doses for the one or more segments of the one or more chromosomes of interest in the test sample

(b) obtaining sequence information for said fetal and maternal nucleic acids in said sample;

(c) using said sequence information to identify a number of sequence tags for each of any one or more segments of any one or more chromosomes of interest, wherein the one or more segments are selected from chromosomes 1-22, X, and Y and to identify a number of sequence tags for a normalizing segment sequence for each of said any one or more segments of any one or more chromosomes of interest;

(d) using said number of sequence tags identified for each of said any one or more segments of any one or more chromosomes of interest and said number of sequence tags identified for said normalizing segment sequence to calculate a single chromosome dose for each of said any one or more segments of any one or more chromosomes of interest; and

(e) comparing each of said single segment doses for each of said any one or more segments of any one or more chromosomes of interest to a threshold value for each of said any one or more chromosomal segments of any one or more chromosome of interest, and thereby determining the presence or absence of one or more different partial fetal chromosomal aneuploidies in said sample.

17. The method of Claim 16, wherein step (c) comprises calculating a single segment dose for each of said any one or more segments of any one or more chromosomes of interest as the ratio of the number of sequence tags identified for each of said any one or more segments of any one or more chromosomes of interest and the number of sequence tags identified for said normalizing segment sequence for each of said any one or more segments of any one or more chromosomes of interest.

18. The method of Claim 16 or 17, further comprising calculating a normalized segment value (NSV), wherein said NSV relates said segment dose to the mean of the corresponding segment dose in a set of qualified samples as:

$$NSV_{ij} = \frac{x_{ij} - \hat{\mu}_j}{\hat{\sigma}_j}$$

where $\hat{\mu}_j$ and $\hat{\sigma}_j$ are the estimated mean and standard deviation, respectively, for the j-th segment dose in a set of qualified samples, and x_{ij} is the observed j-th segment dose for test sample i.

19. The method of any one of Claims 8-18, wherein said normalizing segment sequence is a single segment of any one or more of chromosomes 1-22, X, and Y.

20. The method of any one of Claims 8-18, wherein said normalizing segment sequence is a group of segments of any one or more of chromosomes 1-22, X, and Y.

21. The method of any one of Claims 16-20, wherein said different partial fetal chromosomal aneuploidies are selected from partial duplications, partial multiplications, partial insertions and partial deletions.

22. The method of any one of Claims 16-21, wherein said partial fetal aneuploidies are selected from partial monosomy of chromosome 1, partial monosomy of chromosome 4, partial monosomy of chromosome 5, partial monosomy of chromosome 7, partial monosomy of chromosome 11, partial monosomy of chromosome 15, partial monosomy of chromosome 17, partial monosomy of chromosome 18, and partial monosomy of chromosome 22.

23. The method of any one of Claims 16-22, wherein steps (a)-(d) are repeated for test samples from different maternal subjects, and wherein the method comprises determining the presence or absence of different partial fetal chromosomal aneuploidies in each of said samples.

24. The method of any one of the preceding Claims, wherein step (a) comprises sequencing at least a portion of said nucleic acid molecules of said test sample to obtain said sequence information for said fetal and maternal nucleic acid molecules of said test sample.

25. The method of any one of the preceding Claims, wherein said test sample is a maternal sample selected from blood, plasma, serum, urine and saliva samples.

26. The method of any one of the preceding Claims, wherein said nucleic acid molecules are a mixture of fetal and maternal cell-free DNA molecules.

27. The method of any one of the preceding Claims, wherein said sequencing is next generation sequencing (NGS).

28. The method of any one of the preceding Claims, wherein said sequencing is massively parallel sequencing using sequencing-by-synthesis with reversible dye terminators.

29. The method of any one of the preceding Claims, wherein said sequencing is sequencing-by-ligation.

30. The method of any one of the preceding Claims, wherein said sequencing comprises an amplification.

31. The method of any one of the preceding Claims, wherein said sequencing is single molecule sequencing.