

Claims

1. 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;

wherein each said normalizing chromosome for each of said twenty or more chromosomes of interest is determined by systematically calculating all possible chromosome doses for each of the twenty or more chromosomes of interest in a training set of samples comprising qualified samples comprising chromosomes that are present in known copy number and which are not aneuploid for each of the twenty or more chromosomes of interest, by using the number of sequence tags identified for each of the remaining chromosomes and the sum of the number of sequence tags identified for all possible combinations of the remaining chromosomes to determine which single chromosome or group of chromosomes results in the least variability of the chromosome dose for each of the twenty or more chromosomes of interest across said qualified samples.

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 any one of Claims 1-2, 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 twenty 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.

5. The method of any one of Claims 1-4, wherein said normalizing chromosome sequence is a single chromosome selected from chromosomes 1-22, X, and Y.

6. The method of any one of Claims 1-4, wherein said normalizing chromosome sequence is a group of chromosomes selected from chromosomes 1-22, X, and Y.

7. The method of any one of Claims 1-6, wherein said different complete chromosomal

aneuploidies are selected from complete chromosomal trisomies, complete chromosomal monosomies and complete chromosomal polysomies.

8. The method of any one of Claims 1-7, 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.

9. The method of any one of Claims 1-8, 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 twenty or more different complete fetal chromosomal aneuploidies in each of said samples.

10. The method of any one of Claims 1-9, 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 .

11. 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.

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

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

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

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

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

17. The method of any one of the preceding Claims, comprising, prior to step a), the step of preparing a sequencing library from said mixture of fetal and maternal nucleic acid molecules; wherein preparing said library comprises the consecutive steps of end-repairing, dA-tailing and adaptor ligating said nucleic acids, wherein said consecutive steps exclude purifying the end-repaired products prior to the dA-tailing step and exclude purifying the dA-tailing products prior to the adaptor-ligating step.

18. 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) preparing a sequencing library from said mixture of fetal and maternal nucleic acid molecules; wherein preparing said library comprises the consecutive steps of end-repairing, dA-tailing and adaptor ligating said nucleic acids, wherein said consecutive steps exclude purifying the end-repaired products prior to the dA-tailing step and exclude purifying the dA-tailing products prior to the adaptor-ligating step;

(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 a 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;

wherein each said normalizing chromosome for each of said four or more chromosomes of interest is determined by systematically calculating all possible chromosome doses for each of the four or more chromosomes of interest in a training set of samples comprising qualified samples comprising chromosomes that are present in known copy number and which are not aneuploid for each of the four or more chromosomes of interest, by using the number of sequence tags identified for each of the remaining chromosomes and the sum of the number of sequence tags identified for all possible combinations of the remaining chromosomes to determine which single chromosome or group of chromosomes results in the least variability of the chromosome dose for each of the four or more chromosomes of interest across said qualified samples.

19. The method of claim 18, wherein said consecutive steps comprise heat inactivating the end-repair reaction mixture prior to the dA-tailing step, and heat inactivating the dA-tailing reaction mixture prior to the adaptor-ligating step.

20. The method of Claim 18, wherein step (d) 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.

21. The method of any one of Claims 18-20, wherein step (d) 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 (c) 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 (c) 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.

22. The method of any one of Claims 18-21, 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.

23. The method of any one of Claims 18-22, 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.

24. The method of any one of Claims 18-23, wherein said normalizing chromosome sequence is a single chromosome selected from chromosomes 1-22, X, and Y.

25. The method of any one of Claims 18-24, wherein said normalizing chromosome sequence is a group of chromosomes selected from chromosomes 1-22, X, and Y.

26. A method for determining a fetal chromosomal aneuploidy in a maternal sample comprising a mixture of fetal and maternal nucleic acids molecules, said method comprising:

(a) preparing a sequencing library from said mixture of fetal and maternal nucleic acid molecules; wherein preparing said library comprises the consecutive steps of end-repairing, dA-tailing and adaptor ligating said nucleic acids, wherein said consecutive steps exclude purifying the end-repaired products prior to the dA-tailing step and exclude purifying the dA-tailing products prior to the adaptor-ligating step;

(b) sequencing at least a portion of said nucleic acid molecules in said sequencing library, thereby obtaining sequence information for a plurality of fetal and maternal nucleic acid molecules of a maternal sample; and

(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 a 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;

wherein each said normalizing chromosome segment for each of said one or more chromosomes of interest is determined by systematically calculating all possible chromosome doses for each of the one or more chromosomes of interest in a training set of samples comprising qualified samples comprising chromosomes that are present in known copy number and which are not aneuploid for each of the one or more chromosomes of interest, by using the number of sequence tags identified for segments of each of the remaining chromosomes and the sum of the number of sequence tags identified for all possible combinations of segments of the remaining chromosomes to determine which single segment or group of segments results in the least variability of the chromosome dose for each of the one or more chromosomes of interest across said qualified samples.

27. The method of claim 26, wherein said consecutive steps comprise heat inactivating the end-repair reaction mixture prior to the dA-tailing step, and heat inactivating the dA-tailing reaction mixture prior to the adaptor-ligating step.

28. 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 fetal and maternal nucleic acids, said method comprising:

(a) preparing a sequencing library from said mixture of fetal and maternal nucleic acid molecules; wherein preparing said library comprises the consecutive steps of end-repairing, dA-tailing and adaptor ligating said nucleic acids, wherein said consecutive steps exclude purifying the end-repaired products prior to the dA-tailing step and exclude purifying the dA-tailing products prior to the adaptor-ligating step;

(b) sequencing at least a portion of said nucleic acid molecules in said sequencing library, thereby obtaining sequence information for said fetal and maternal nucleic acid molecules 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,

wherein each said normalizing chromosome segment for each of said one or more segments of any one or more chromosomes of interest is determined by systematically

calculating all possible chromosome doses for each of the one or more segments of any one or more chromosomes of interest in a training set of samples comprising qualified samples comprising chromosomes that are present in known copy number and which are not aneuploid for each of the one or more chromosomes of interest, by using the number of sequence tags identified for segments of each of the remaining chromosomes and the sum of the number of sequence tags identified for all possible combinations of the segments of the remaining chromosomes to determine which single segment or group of segments results in the least variability of the chromosome dose for each of the one or more segments of any one or more chromosomes of interest across said qualified samples.