

What is claimed is:

1. A method for determining a fraction of cell-free fetal nucleic acids in cell free nucleic acids in a peripheral blood sample from a pregnant woman, comprising:

5 (i) performing sequencing on the cell-free nucleic acids contained in said sample, so as to obtain a sequencing result consisting of a plurality of sequencing data;

(ii) determining the number of the cell-free nucleic acids in a length falling into a predetermined range in said sample based on the sequencing result; and

10 (iii) determining the fraction of the cell-free fetal nucleic acids based on the number of the cell-free nucleic acids in the length falling into the predetermined range, wherein

said predetermined range is 185bp -204bp; and

said determining of step (iii) is by means of the function $d=0.0334*p+1.6657$, where d represents the fraction of the cell-free fetal nucleic acids, and p represents said
15 percentage of cell-free nucleic acids in the predetermined range.

2. The method according to claim 1, wherein determining the number of the cell-free nucleic acids in a length falling into a predetermined range in said sample based on the sequencing result further comprises:

20 aligning the sequencing result to a reference genome, so as to construct a dataset consisting of a plurality of uniquely-mapped reads, where each read in the dataset can be mapped to a single position of the reference genome;

determining a length of the cell-free nucleic acid corresponding to each uniquely- mapped read in the dataset; and

25 determining the number of the cell-free nucleic acids in the length falling into the predetermined range.

3. The method according to claim 2, wherein determining the length of the cell-free nucleic acid corresponding to each uniquely- mapped read in the dataset further
30 comprises:

determining the length of each read uniquely mapped to the reference genome as the length of the cell-free nucleic acid corresponding to the read.

4. The method according to claim 2, wherein the cell-free nucleic acids in said sample are sequenced by paired-end sequencing, and determining the length of the cell-free nucleic acid corresponding to each uniquely-mapped read in the dataset further
5 comprises:

determining a position, corresponding to the reference genome, of the 5'-end of the cell-free nucleic acid, based on the sequencing data for the 5'-end of each uniquely-mapped read obtained in the paired-end sequencing;

determining a position, corresponding to the reference genome, of the 3'-end of
10 the cell-free nucleic acid, based on the sequencing data for the 3'-end of same uniquely-mapped read obtained in the paired-end sequencing; and

determining the length of the cell-free nucleic acid based on the positions of the 5'-end and the 3'-end of the cell-free nucleic acid.

15 5. A device for determining a fraction of cell-free fetal nucleic acids in accordance with any one of claims 1 to 4, comprising:

a sequencing apparatus, configured to sequence cell-free nucleic acids contained in a peripheral blood sample from a pregnant woman, so as to obtain a sequencing result consisting of a plurality of sequencing data;

20 a counting apparatus, connected to the sequencing apparatus and configured to determine the number of the cell-free nucleic acids in a length falling into a predetermined range in said sample based on the sequencing result; and

an apparatus for determining the fraction of the cell-free fetal nucleic acids, connected to the counting apparatus and configured to determine the fraction of the cell-
25 free fetal nucleic acids in accordance with step (iii) of claim 1.

6. A computer readable medium having stored therein instructions that, when executed by the device according to claim 5, causes the device to perform:

(i) sequencing on the cell-free nucleic acids contained in a peripheral blood
30 sample from a pregnant woman, so as to obtain a sequencing result consisting of a plurality of sequencing data;

(ii) determining the number of the cell-free nucleic acids in a length falling into a predetermined range in said sample based on the sequencing result; and

(iii) determining the fraction of the cell-free fetal nucleic acids based on the number of the cell-free nucleic acids in the length falling into the predetermined range,
5 wherein

said predetermined range is 185bp -204bp; and

said determining of step (iii) is by means of the function $d=0.0334*p+1.6657$, where d represents the fraction of the cell-free fetal nucleic acids, and p represents said percentage of cell-free nucleic acids in the predetermined range.

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7. A method for determining sex of twins, comprising:

(i) performing sequencing on cell-free nucleic acids contained in a peripheral blood sample obtained from a pregnant woman with twins, so as to obtain a sequencing result consisting of a plurality of sequencing data;

15 (ii) determining a first cell-free fetal DNA fraction based on the sequencing data, by the method according to any one of claims 1 to 4;

(iii) determining a second cell-free fetal DNA fraction based on a sequencing data derived from chromosome Y in the sequencing result; and

(iv) determining the sex of the twins based on the first cell-free fetal DNA
20 fraction and the second cell-free fetal DNA fraction.

8. The method according to claim 7, wherein the second cell-free fetal DNA fraction is determined according to the following formula:

$$fra.chry = (chry.ER\% - Female.chry.ER\%) / (Man.chry.ER\% - Female.chry.ER\%) * 100\%$$

25 , where *fra.chry* represents the second cell-free fetal DNA fraction, *chry.ER%* represents a percentage of the sequencing data derived from chromosome Y in the sequencing result to total sequencing data;

Female.chry.ER% represents an average percentage of sequencing data of cell-free nucleic acids derived from chromosome Y in a peripheral blood sample obtained
30 from a pregnant woman predetermined to be with a normal female foetus to total sequencing data thereof; and *Man.chry.ER%* represents an average percentage of

sequencing data of cell-free nucleic acids derived from chromosome Y in a peripheral blood sample obtained from a healthy man to total sequencing data thereof.

9. The method according to claim 7 or 8, wherein determining the sex of the twins based on the first cell-free fetal DNA fraction and the second cell-free fetal DNA fraction further comprises:

(a) determining a ratio of the second cell-free fetal DNA fraction to the first cell-free fetal DNA fraction; and

(b) determining the sex of the twins by comparing the ratio determined in (a) with a first threshold and a second threshold predetermined, wherein the first threshold has been determined based on a plurality of control samples obtained from pregnant women known with female twins, and the second threshold has been determined based on a plurality of control samples obtained from pregnant women known with male twins, and wherein

both foetuses of the twins are determined as female if the ratio of the second cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is lower than the first threshold,

both foetuses of the twins are determined as male if the ratio of the second cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is greater than the second threshold, and

the twins are determined as including a male foetus and a female foetus if the ratio of the second cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is equal to the first threshold or the second threshold, or between the first threshold and the second threshold.

10. The method according to claim 9, wherein the first threshold is 0.35 and the second threshold is 0.7.

11. A system for determining sex of twins in accordance with any one of claims 7 to 10, comprising:

a first cell-free fetal DNA fraction determining device, being the device for determining the fraction of cell-free fetal nucleic acids according to claim 5, and

configured to sequence cell-free nucleic acids contained in a peripheral blood sample obtained from a pregnant woman with twins, so as to obtain a sequencing result consisting of a plurality of sequencing data, and configured to determine a first cell-free fetal DNA fraction based on the sequencing data;

5 a second cell-free fetal DNA fraction determining device, configured to determine a second cell-free fetal DNA fraction based on a sequencing data derived from chromosome Y in the sequencing result; and

a sex determining device, configured to determine the sex of the twins based on the first cell-free fetal DNA fraction and the second cell-free fetal DNA fraction.

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12. A method for detecting a chromosome aneuploidy of twins, comprising:

(i) performing sequencing on cell-free nucleic acids contained in a peripheral blood sample obtained from a pregnant woman with twins, so as to obtain a sequencing result consisting of a plurality of sequencing data;

15 (ii) determining a first cell-free fetal DNA fraction, based on the sequencing data, by the method according to any one of claims 1 to 4;

(iii) determining a third cell-free fetal DNA fraction, based on a sequencing data derived from a predetermined chromosome in the sequencing result; and

(iv) determining whether the twins under detection have aneuploidy with respect
20 to the predetermined chromosome based on the first cell-free fetal DNA fraction and the third cell-free fetal DNA fraction.

13. The method according to claim 12, wherein the third cell-free fetal DNA fraction is determined according to the following formula:

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$$fra.chri = 2 * (chri.ER\% / adjust.chri.ER\% - 1) * 100\%,$$

where $fra.chri$ represents the third cell-free fetal DNA fraction, i represents a serial number of the predetermined chromosome, and i is any integer in the range of 1 to 22; $chri.ER\%$ represents a percentage of the sequencing data derived from the predetermined chromosome in the sequencing result to total sequencing data;
30 $adjust.chri.ER\%$ represents an average percentage of sequencing data of cell-free nucleic acids derived from the predetermined chromosome in a peripheral blood sample

obtained from a pregnant woman predetermined to be with normal twins to total sequencing data thereof.

14. The method according to claim 12 or 13, wherein determining whether
5 the twins under detection have aneuploidy with respect to the predetermined chromosome based on the first cell-free fetal DNA fraction and the third cell-free fetal DNA fraction further comprises:

(a) determining a ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction; and

10 (b) determining whether the twins under detection have aneuploidy with respect to the predetermined chromosome by comparing the ratio determined in (a) with a third threshold and a fourth threshold predetermined, and wherein the third threshold has been determined based on a plurality of control samples obtained from pregnant women with twins known not to have aneuploidy with respect to the predetermined
15 chromosome, and the fourth threshold has been determined based on a plurality of control samples obtained from pregnant women with twins known to have aneuploidy with respect to the predetermined chromosome, and wherein

both foetuses of the twins are determined to have no aneuploidy with respect to the predetermined chromosome if the ratio of the third cell-free fetal DNA fraction to
20 the first cell-free fetal DNA fraction is lower than the third threshold,

both foetuses of the twins are determined to have aneuploidy with respect to the predetermined chromosome if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is greater than the fourth threshold, and

one foetus of the twins is determined to have aneuploidy with respect to the
25 predetermined chromosome, while the other foetus of the twins is determined to have no aneuploidy with respect to the predetermined chromosome if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is equal to the third threshold or the fourth threshold, or between the third threshold and the fourth threshold.

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15. The method according to claim 14, wherein the third threshold is 0.35 and the fourth threshold is 0.7.

16. The method according to claim 12, wherein the predetermined chromosome is at least one selected from chromosomes 18, 21 and 23.

5 17. A system for determining a chromosome aneuploidy of twins in accordance with any one of claims 12 to 16, comprising:

a first cell-free fetal DNA fraction determining device, being the device for determining the fraction of cell-free fetal nucleic acids according to claim 5, and configured to sequence cell-free fetal nucleic acids contained in cell free nucleic acids
10 in a peripheral blood sample obtained from a pregnant woman with twins, so as to obtain a sequencing result consisting of a plurality of sequencing data, and configured to determine a first cell-free fetal DNA fraction based on the sequencing data;

a third cell-free fetal DNA fraction determining device, configured to determine a third cell-free fetal DNA fraction based on a sequencing data derived from a
15 predetermined chromosome in the sequencing result; and

a first aneuploidy determining device, configured to determine whether the twins under detection have aneuploidy with respect to the predetermined chromosome based on the first cell-free fetal DNA fraction and the third cell-free fetal DNA fraction.

20 18. A method for determining a chromosome aneuploidy of twins, comprising:

performing sequencing on cell-free nucleic acids contained in a peripheral blood sample obtained from a pregnant woman with twins, so as to obtain a sequencing result consisting of a plurality of sequencing data;

25 determining a fraction x_i of the number of sequencing data derived from chromosome i in the sequencing result to total sequencing data, where i represents a serial number of the chromosome, and i is any integer in the range of 1 to 22;

determining a T score of the chromosome i according to $T_i = (x_i - \mu_i) / \sigma_i$, where i represents the serial number of the chromosome and i is any integer in the range of 1 to
30 22, μ_i represents an average percentage of sequencing data of the chromosome i selected as a reference system in a reference database to total sequencing data thereof,

σ_i represents a standard deviation of percentages of the sequencing data of the chromosome i selected as the reference system in the reference database to total sequencing data thereof,

determining an L score of the chromosome i according to
 $L_i = \log(d(T_i, a)) / \log(d(T2_i, a))$, where i represents the serial number of the
 5 chromosome and i is any integer in the range of 1 to 22,
 $T2_i = (x_i - \mu_i * (1 + fra/2)) / \sigma_i$; $d(T_i, a)$ and $d(T2_i, a)$ represent t distribution
 probability density function, where a represents degree of freedom, fra represents a first
 cell-free fetal DNA fraction determined by the method according to any one of claims 1
 10 to 4,

plotting a four-quadrant diagram with T as vertical coordinate and L as
 horizontal coordinate by zoning with a first straight line where T =predetermined fifth
 threshold and a second straight line where L = predetermined sixth threshold, wherein
 both foetuses of the twins are determined to have trisome if a sample under detection is
 15 determined to be of the T score and the L score falling into a first quadrant;

one foetus of the twins is determined to have trisome and the other foetus of the
 twins is determined to be normal if a sample under detection is determined to be of the
 T score and the L score falling into a second quadrant;

both foetuses of the twins are determined to be normal if a sample under
 20 detection is determined to be of the T score and the L score falling into a third quadrant;
 and

the twins are determined to have a low fetal fraction if a sample under detection
 is determined to be of the T score and the L score falling into a fourth quadrant, such a
 result is not adopted.

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19. A system for determining a chromosome aneuploidy of twins in
 accordance with claim 18, comprising:

an x_i value determining device, configured to sequence cell-free nucleic acids
 contained in a peripheral blood sample obtained from a pregnant woman with twins, so
 30 as to obtain a sequencing result consisting of a plurality of sequencing data, and
 configured to determine a fraction x_i of the number of sequencing data derived from

chromosome i in the sequencing result to total sequencing data, where i represents a serial number of the chromosome and i is any integer in the range of 1 to 22;

a T score determining device, configured to determine a T score of the chromosome i according to $T_i = (x_i - \mu_i) / \sigma_i$, where i represents the serial number of the chromosome and is any integer in the range of 1 to 22, μ_i represents an average percentage of sequencing data of the chromosome i selected as a reference system in a reference database to total sequencing data thereof, σ_i represents a standard deviation of percentages of the sequencing data of the chromosome i selected as the reference system in the reference database to total sequencing data thereof,

an L score determining device, configured to determine an L score of the chromosome i according to $L_i = \log(d(T_i, a)) / \log(d(T2_i, a))$, where i represents the serial number of the chromosome and i is any integer in the range of 1 to 22, $T2_i = (x_i - \mu_i * (1 + fra/2)) / \sigma_i$; $d(T_i, a)$ and $d(T2_i, a)$ represent t distribution probability density function, where a represents degree of freedom, fra represents a cell-

free fetal DNA fraction determined by the method according to any one of claims 1 to 4,

a second aneuploidy determining device, configured to plot a four-quadrant diagram with T as vertical coordinate and L as horizontal coordinate by zoning with a fifth straight line where T=predetermined fifth threshold and a seventh straight line where L= predetermined sixth threshold,

wherein both foetuses of the twins are determined to have trisome if a sample under detection is determined to be of the T score and the L score falling into a first quadrant; and

one foetus of the twins is determined to have trisome and the other foetus of the twins is determined to be normal if a sample under detection is determined to be of the T score and the L score falling into a second quadrant;

both foetuses of the twins are determined to be normal if a sample under detection is determined to be of the T score and the L score falling into a third quadrant; and

the twins are determined to have a low fetal fraction if a sample under detection is determined to be of the T score and the L score falling into a fourth quadrant, such a result is not adopted.

20. A method for detecting fetal chimera, comprising:

performing sequencing on cell-free nucleic acids contained in a peripheral blood sample obtained from a pregnant woman with a foetus, so as to obtain a sequencing
5 result consisting of a plurality of sequencing data;

determining a first cell-free fetal DNA fraction, based on the sequencing data, by the method according to any one of claims 1 to 4;

determining a third cell-free fetal DNA fraction based on sequencing data derived from a predetermined chromosome in the sequencing result; and

10 determining whether the foetus under detection has fetal chimera with respect to the predetermined chromosome based on the first cell-free fetal DNA fraction and the third cell-free fetal DNA fraction.

21. The method according to claim 20, wherein the third cell-free fetal DNA
15 fraction is determined by the following formula:

$$fra.chri = 2 * (chri.ER\% / adjust.chri.ER\% - 1) * 100\%,$$

where $fra.chri$ represents the third cell-free fetal DNA fraction, i represents a serial number of the predetermined chromosome and i is any integer in the range of 1 to 22; $chri.ER\%$ represents a percentage of the sequencing data derived from the
20 predetermined chromosome in the sequencing result to total sequencing data; $adjust.chri.ER\%$ represents an average percentage of sequencing data of cell-free nucleic acids derived from the predetermined chromosome in a peripheral blood sample obtained from a pregnant woman predetermined to be with a normal foetus to total sequencing data thereof.

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22. The method according to claim 20 or 21, wherein determining whether the foetus under detection has fetal chimera with respect to the predetermined chromosome based on the first cell-free fetal DNA fraction and the third cell-free fetal DNA fraction further comprises:

30 (a) determining a ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction; and

(b) determining whether the foetus under detection has chimera with respect to the predetermined chromosome by comparing the ratio determined in (a) with a plurality of predetermined thresholds.

5 23. The method according to claim 22, wherein the plurality of predetermined thresholds comprises at least one selected from:

 a seventh threshold, determined based on a plurality of control samples with the predetermined chromosome known to have complete monosome,

 an eighth threshold, determined based on a plurality of control samples with the
10 predetermined chromosome known to have monosome chimera,

 a ninth threshold, determined based on a plurality of control samples with the predetermined chromosome known to be normal,

 a tenth threshold, determined based on a plurality of control samples with the predetermined chromosome known to have complete trisome,

15 and wherein,

 the predetermined chromosome of the foetus under detection is determined to have complete monosome, if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is lower than the seventh threshold;

 the predetermined chromosome of the foetus under detection is determined to
20 havemonosome chimera, if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is not lower than the seventh threshold and not greater than the eighth threshold;

 the predetermined chromosome of the foetus under detection determined to be normal, if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal
25 DNA fraction is greater than the eighth threshold and lower than the ninth threshold;

 the predetermined chromosome of the foetus under detection is determined to havetrisome chimera, if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is not lower than the ninth threshold and not greater than the tenth threshold; and

30 the predetermined chromosome of the foetus under detection is determined to havecomplete trisome, if the ratio of the third cell-free fetal DNA fraction to the first cell-free fetal DNA fraction is greater than the tenth threshold.

24. The method according to claim 23, wherein the seventh threshold is greater than -1 and lower than 0, for example is -0.85;

the eighth threshold is greater than the seventh threshold and lower than 0, for
5 example is -0.3;

the ninth threshold is greater than 0 and lower than 1, for example is 0.3;

the tenth threshold is greater than the ninth threshold and lower than 1, for
example is 0.85.

10 25. A system for detecting fetal chimera in accordance with any one of claims 20 to 24, comprising:

a first cell-free fetal DNA fraction determining device, being the device for determining the fraction of cell-free fetal nucleic acids according to claim 5, and configured to sequence cell-free nucleic acids contained in a peripheral blood sample
15 obtained from a pregnant woman with foetus, so as to obtain a sequencing result consisting of a plurality of sequencing data, and configured to determine a first cell-free fetal DNA fraction based on the sequencing data;

a third cell-free fetal DNA fraction determining device, configured to determine a third cell-free fetal DNA fraction based on sequencing data derived from a
20 predetermined chromosome in the sequencing result; and

a chimera determining device, configured to determine whether the foetus under detection has fetal chimera with respect to the predetermined chromosome based on the first cell-free fetal DNA fraction and the third cell-free fetal DNA fraction.

25 26. A method for detecting fetal chimera, comprising:

performing sequencing on cell-free nucleic acids contained in a peripheral blood sample obtained from a pregnant woman with a foetus, so as to obtain a sequencing result consisting of a plurality of sequencing data;

determining a fraction x_i of the number of sequencing data derived from
30 chromosome i in the sequencing result to total sequencing data, where i represents a serial number of the chromosome, and i is any integer in the range of 1 to 22;

determining a T score of the chromosome i according to $T_i = (x_i - \mu_i) / \sigma_i$, where i represents the serial number of the chromosome and i is any integer in the range of 1 to 22, μ_i represents an average value of percentages of sequencing data of the chromosome i selected as a reference system in a reference database to total sequencing data thereof, σ_i represents a standard deviation of percentages of the sequencing data of the chromosome i selected as the reference system in the reference database to total sequencing data thereof,

determining an L score of the chromosome i according to $L_i = \log(d(T_i, a)) / \log(d(T2_i, a))$, where i represents the serial number of the chromosome and i is any integer in the range of 1 to 22, $T2_i = (x_i - \mu_i * (1 + fra/2)) / \sigma_i$; $d(T_i, a)$ and $d(T2_i, a)$ represent t distribution probability density function, where a represents degree of freedom, fra represents a cell-free fetal DNA fraction determined by the method according to any one of claims 1 to 4; plotting a four-quadrant diagram with T as vertical coordinate and L as horizontal coordinate by zoning with a third straight line where T=predetermined eleventh threshold and a fourth straight line where L= predetermined twelfth threshold, when the T score is greater than 0,

wherein the foetus is determined to have complete monosome or monosome chimera with respect to the predetermined chromosome, if a sample under detection is determined to be of the T score and the L score falling into a first quadrant;

the foetus is determined to have monosome chimera with respect to the predetermined chromosome, if a sample under detection is determined to be of the T score and the L score falling into a second quadrant;

the foetus is determined to be normal with respect to the predetermined chromosome, if a sample under detection is determined to be of the T score and the L score falling into a third quadrant;

the foetus is determined to have a low fetal fraction if a sample under detection is determined to be of the T score and the L score falling into a fourth quadrant, such a result is not adopted,

plotting a four-quadrant diagram with T as vertical coordinate and L as horizontal coordinate by zoning with a first straight line where T=predetermined

thirteenth threshold and a second straight line where $L =$ predetermined fourteenth threshold, when the T score is greater than 0,

wherein the foetus is determined to have complete trisome or trisome chimera with respect to the predetermined chromosome, if a sample under detection is
5 determined to be of the T score and the L score falling into a first quadrant;

the foetus is determined to have trisome chimera with respect to the predetermined chromosome, if a sample under detection is determined to be of the T score and the L score falling into a second quadrant;

the foetus is determined to be normal with respect to the predetermined
10 chromosome, if a sample under detection is determined to be of the T score and the L score falling into a third quadrant;

the foetus is determined to have a low fetal fraction if a sample under detection is determined to be of the T score and the L score falling into a fourth quadrant, such a result is not adopted.

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27. The method according to claim 26, wherein the eleventh threshold and the thirteenth threshold are each independently 3, and the twelfth threshold and the fourteenth threshold are each independently 1.