

【CLAIMS】

1. A method of differentiating fetal sex and fetal sex chromosome abnormality including: (a) performing next-generation sequencing (NGS) on two or more platforms by
5 extracting DNA from maternal blood;

(b) aligning sequences (reads) that are sequenced for each platform to a reference genome database;

(c) extracting unique reads that are uniquely aligned on chromosome for each platform and calculating a percent
10 of unique reads (UR%) relative to the total reads;

(d) calculating data for each platform at the same time, setting a cut-off value, and organizing the UR(y)% values by normalization by correcting UR(y)% according to the following equation, the UR(y)% is calculated for each
15 platform:

$$\text{UR}(y)\% \text{ new} = (\text{UR}(y)\% \text{ original} - (\text{mean value of the three smallest UR}(y)\% \text{ values});$$

(e) classifying male and female using the organized UR(y)%;

20 (f) calculating partial reference chromosome (relative FGR) values using three or more autosomes that are similar in size to chromosomes X and Y; and

(g) calculating Z-score using the partial reference chromosome (relative FGR) values, and calculating G score
25 (Gscore) and M score (Mscore) based on the Z-score to

differentiate sex chromosome abnormality.

2. The method of claim 1, wherein the step (a) comprising the steps of:

- 5 (a) separating a plasma by centrifuging the collected biological sample;
- (b) extracting cfDNA from the separated plasma;
- (c) preparing a library using the extracted cfDNA; and
- (d) pooling the prepared library, and then decoding
- 10 the base sequence using NGS equipment.

3. The method of claim 1, wherein the step (c) comprising the steps of:

- (a) removing PCR duplication and ambiguous reads in
- 15 sequencing, aligning only perfectly matched reads, and extracting a read in which a GC content and mappability are more than or equal to the cut-off value in the aligned sequence; and

- (b) calculating a percentage (UR(x)%) of the read that
- 20 uniquely matches the chromosome X and a percentage (UR(y)%) of the read that uniquely matches the chromosome Y according to the following Equations, based on the extracted read:

 Equation 1) $UR(x)\% = \frac{\text{the number of chromosome X (chrX) reads}}{\text{total number of autosome reads}} \times 100$, and

25

Equation 2) $UR(y)\% = \frac{\text{the number of chromosome Y (chrY) reads}}{\text{total number of autosome reads}} \times 100$.

4. The method of claim 3, wherein the step (a) is performed by dividing each region into 100 kb bin regions, wherein the cut-off value of the GC content is 0.30 to 0.50, and the cut-off value of the mappability is 20% or more.

5. The method of claim 1, wherein the cut-off value of step (d) is 0.02 ~ 0.06.

6. The method of claim 1, wherein the gender classification of (e) is characterized in that when the organized $UR(y)\%$ value is more than or equal to the cut-off value, it is determined as a male, and when the organized $UR(y)\%$ value is smaller than the cut-off value, it is determined as a female.

7. The method of claim 1, wherein the step (f) comprising the steps of:

(a) selecting autosomes (chr7, chr8, chr9, and chr10) that are similar in size to sex chromosome X (chrX) as internal reference chromosomes and calculating each FGR by the following Equations:

Equation 4) $FGR(7) = (UR(x)\% + UR(y)\%) / UR(7)\%$

Equation 5) $FGR(8) = (UR(x)\% + UR(y)\%) / UR(8)\%$

Equation 6) $FGR(9) = (UR(x)\% + UR(y)\%) / UR(9)\%$

Equation 7) $FGR(10) = (UR(x)\% + UR(y)\%) / UR(10)\%$, and

5 (b) selecting autosomes (chr19, chr20, chr21, and chr22) that are similar in size to sex chromosome Y (chrY) as partial reference chromosomes and calculating each FGR by the following Equations:

Equation 8) $FGR(19) = (UR(x)\% + UR(y)\%) / UR(19)\%$

10 Equation 9) $FGR(20) = (UR(x)\% + UR(y)\%) / UR(20)\%$

Equation 10) $FGR(21) = (UR(x)\% + UR(y)\%) / UR(21)\%$

Equation 11) $FGR(22) = (UR(x)\% + UR(y)\%) / UR(22)\%$.

8. The method of claim 1, wherein the step (g) comprising
15 the steps of:

(a) differentiating sex chromosome abnormality by the following steps when the sex of the fetus is determined to be female;

a-1) calculating Z-score for each chromosome in the
20 following Equations:

Equation 12) $Zscore(7) = \{FGR(7) - (mean\ FGR(7))\} / (stdev\ FGR(7))$

Equation 13) $Zscore(8) = \{FGR(8) - (mean\ FGR(8))\} / (stdev\ FGR(8))$

25 Equation 14) $Zscore(9) = \{FGR(9) - (mean\ FGR(9))\} /$

(stdev FGR(9))

Equation 15) $Zscore(10) = \{FGR(10) - (mean\ FGR(10))\} /$
(stdev FGR(10))

a-2) calculating Gscore using the calculated Z-score
5 by the following Equation; and

Equation 16) $Gscore = \{Zscore(7) + Zscore(8) +$
 $Zscore(9) + Zscore(10)\} / 4$

a-3) determining that the sex chromosome is XXX when
the Gscore is more than or equal to 1.5, that the sex
10 chromosome is XX when the Gscore is more than -3 and less
than 1.5, and that the sex chromosome is XO when the Gscore
is less than or equal to -3;

(b) differentiating sex chromosome abnormality by the
following steps when the sex of the fetus is determined to
15 be male;

b-1) calculating Z-score for each chromosome in the
following Equations:

Equation 17) $Zscore(19) = \{FGR(19) - (mean\ FGR(19))\} /$
(stdev FGR(19))

20 Equation 18) $Zscore(20) = \{FGR(20) - (mean\ FGR(20))\} /$
(stdev FGR(20))

Equation 19) $Zscore(21) = \{FGR(21) - (mean\ FGR(21))\} /$
(stdev FGR(21))

Equation 20) $Zscore(22) = \{FGR(22) - (mean\ FGR(22))\} /$
25 (stdev FGR(22))

b-2) calculating Mscore using the calculated Z-score by the following Equation; and

Equation 21) $Mscore = \{Zscore(19) + Zscore(20) + Zscore(21) + Zscore(22)\} / 4$

5 b-3) determining that the sex chromosome is XXY when the Mscore is more than or equal to 0.7, that the sex chromosome is XY when the Mscore is more than -3 and less than 0.7.

10 9. The method of claim 1, wherein the biological sample is selected from the group consisting of blood, plasma, serum, urine, and saliva.

15 10. A computer system including a computer readable medium having a plurality of instructions encrypted for controlling a computing system so as to perform a method of differentiating fetal sex and sex chromosome abnormality using two or more NGS platforms from gDNA extracted from a maternal biological sample, wherein

20 the biological sample contains cell free nucleic acid molecules of a fetus, and

the method of differentiating fetal sex and sex chromosome abnormality includes:

aligning sequences (reads) that are sequenced for each
25 NGS platform to a reference genome database;

extracting unique reads unique to the chromosome for each NGS platform and calculating a percent of unique reads (UR%) relative to the total reads;

calculating data for each NGS platform at the same time, setting
5 a cut-off value, and organizing the UR(y)% values by normalization by correcting UR(y)% according to the following equation, the UR(y)% is calculated for each platform:

$$\text{UR(y)\% new} = (\text{UR(y)\% original} - (\text{mean value of the three smallest UR(y)\% values}));$$

10 classifying male and female using the organized UR(y)%;

calculating partial reference chromosomes (relative FGRs) using three or more autosomes that are similar in size to chromosomes X and Y; and

15 calculating Z-score using the relative FGR values, and calculating G score (Gscore) and M score (Mscore) based on the Z-score to differentiate sex chromosome abnormality.