An Efficient Computational Method to Simulate Autosomal Chromosomes with Trisomy Aberrations

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Abstract. Noninvasive prenatal test (NIPT) has been widely used for screening trisomy on chromosomes 13 (T13), 18 (T18), and 21 (T21). However, the false negative rate of NIPT algorithms has not been thoroughly evaluated due to the lack of positive samples. In this study, we present an efficient computational approach to simulate positive samples with autosomal trisomy from negative samples. We applied the method to create a dataset of 1440 positive samples with T13, T18, and T21 aberrations for both mosaic and non-mosaic conditions. We also introduced VINIPT algorithm, an improvement of the widely-used algorithm WisecondorX. We examined the performance of WisecondorX and VINIPT on both negative and positive datasets. Experiments showed that WisecondorX and VINIPT were able to detect all non-mosaic samples with T13, T18, and T21 aberrations (i.e., the sensitivity of 100%). For mosaic samples, WisecondorX and VINIPT have the overall sensitivity of 99.7% on detecting T13, T18, and T21 aneuploidy. WisecondorX has a specificity of 98.5% for the non-mosaic analysis, but a considerably lower specificity of 95% for the mosaic analysis. VINIPT has a much better specificity than WisecondorX, i.e., 99.9% for the non-mosaic analysis, and 98.2% for the mosaic analysis. The results suggest that VINIPT can play as a powerful tool for detecting autosomal trisomy.

Keywords: Noninvasive prenatal test (NIPT), data simulation, autosomal chromosome aneuploidy, low coverage data, mosaicism

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1 INTRODUCTION

The abnormality in the number of chromosomes results in different disorders in both males and females. The well-known chromosome abnormalities are the trisomy 13 (T13 or Patau syndrome due to an extra copy of chromosome 13), trisomy 18 (T18 or Edwards syndrome caused by an additional copy of chromosome 18), and trisomy 21 (T21 or Down syndrome because of an extra copy of chromosome 21). The non-invasive prenatal test (NIPT) has been widely applied to screen the autosomal disorders [1]–[6]. The NIPT analyzes cell-free DNA (cfDNA) which is a mixture of maternal DNA and a small percentage (i.e., around 10%) of fetal DNA in the blood of mother to detect chromosomal aberrations. The cfDNA from mother and fetus are mixed and not feasibly separated.

A number of computational algorithms have been developed to predict numerical chromosomal aneuploid based on genomic data sequenced from cfDNA such as NIFTY [7], NIPTeR [8], Wisecondor [9], and WisecondorX [10]. Experiments on a limited number of clinically validated positive samples showed that the NIPT algorithms have high sensitivity and specificity on screening T13, T18, and T21 aberrations [6], [11], [12], e.g., the sensitivity of 100% for T13, 98.2% for T18, and 99.1% for T21[6]; and the specificity of around 99.9% for determining negative samples.

The specificity of the NIPT algorithms can be measured from large-scale and timely retrospective studies. However, the sensitivity (or the false negative rate) of the NIPT algorithms, one of the most important indicators, is not easily measured due to the lack of positive samples. A large number of negative (normal) NIPT samples can be gathered from genetic testing centers; however, positive datasets are normally small and not available for scientific community.

Simulation can play as a reasonable approach to create positive NIPT samples. Although a number of computational methods have been proposed to generate genomic data [13], no computational method has been specifically designed to simulate NIPT data. Simulating NIPT data (i.e., short-reads from both maternal and fetal genomes) is very challenging due to complicated genomic characteristics of the NIPT data such as DNA artifacts, fragment size distribution, short-read quality and distribution, sequencing errors, or GC-content variation.

In this paper, we present an efficient computational method to simulate positive samples with autosomal trisomy from negative samples. Instead of generating additional short-reads to create an extra copy of a chromosome of interest h, we will remove short-reads of the other chromosomes such that the read coverage of chromosome h in comparison with that of the other chromosomes reflexes an extra copy of chromosome h in the fetal genome. We describe how to simulate positive samples from negative samples for both non-mosaic and mosaic conditions. We examined the performance of the widely-used open-source algorithm WisecondorX and our algorithm VINIPT (some

NIPT algorithms are implemented in commercial software and not available for testing).

The remainder of this paper is organized as follows: In the METHODS section, we will describe NIPT data used to simulate positive samples; NIPT procedure including both wet-lab and bioinformatics steps; NIPT algorithms to detect chromosomal aberrations; and algorithms to simulate positive samples from negative samples for both mosaic and non-mosaic conditions. The RESULTS section will report the sensitivity and specificity of NIPT algorithms on both positive and negative samples; and the z-score distributions of clinical positive samples and simulated positive samples with T13, T18, and T21 aberrations. Finally, discussions and remarks are given in the DISCUSSION section.

2 METHODS

2.1 Data

We collected a dataset of 1500 normal samples from singleton pregnancies with clear negative predictions from NIPT and no customer reports about false results. The NIPT samples were sequenced by the MGISEQ-200 platform with 50 bp single-end reads. We divided the 1500 negative samples into two datasets: dataset **P** of 1200 samples for evaluating the specificity of NIPT algorithms and dataset **S** of 300 samples used to create positive samples. Samples with small fetal DNA fractions (i.e., $< 5\%$) or containing a low number of short-reads (i.e., < 5 million) were removed from the study. As a result, the dataset **P** consists of 1058 normal samples and the dataset **S** contains 240 normal samples.

Fig. 1 shows the distribution of fetal DNA fractions and read coverages of the samples. The samples have the fetal DNA fractions ranging from 5% to 22%; while the read coverages are from 5M reads (i.e., ~0.08x) to 25M reads (i.e., ~0.4x). Most of the samples have fetal DNA fractions from 8 to 12 (i.e., the mean fetal DNA fraction of 9.45) and the read coverages from 9M reads to 15M reads (i.e., the mean read coverage of 11.8M).

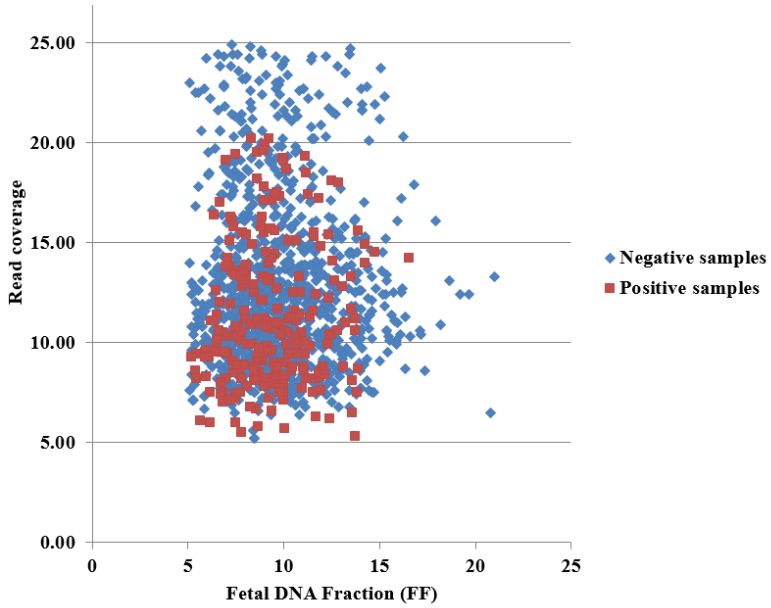

Fig. 1. The distribution of fetal DNA fractions and read coverages of negative and simulated positive samples.

2.2 NIPT procedure

Non-invasive prenatal test (NIPT) provides a safe and efficient method for screening aberrations in the genome of developing fetus. The NIPT method collects blood from mother and performs a series of both wet-lab and bioinformatics steps (see Fig. 2) to determine numerical chromosomal aberrations in the fetal genome.

Wet-lab steps: The steps include blood collection from pregnant women; cfDNA extraction from the maternal blood; and genome sequencing.

– *Blood collection:* The blood from pregnant woman contains a mixture of circular-free DNA (cfDNA) from genomes of both mother and developing fetus. A small amount of blood is collected from the pregnant woman, typically as early as the 10th week of pregnancy. The longer pregnancy contains the more fetal cfDNA in the maternal blood.
– *cfDNA extraction:* The cfDNA from the maternal blood are extracted. The extracted cfDNA are a mixture of DNA from genomes of both mother and fetus, i.e., ~90% from the maternal genome and ~10% from the fetal genome.
– *Genome sequencing:* The cfDNA are sequenced by high-throughput sequencing platforms. The step results in multi-million short reads. In practice, the read coverage of a NIPT sample typically ranges from 0.1x to 1x.

Bioinformatics steps: The steps include data processing; fetal DNA fraction estimation; and chromosomal aberrations detection.

- *Data processing:* The short-reads undergo a number of bioinformatics steps including sequence alignments and sequence cleaning. Mapping short-reads to the reference genome to determine their positions in the genome using genome aligner such as Bowtie2 [14] or BWA [15]. The short-reads ambiguously mapped to multiple positions might be removed; duplications should be also eliminated except one, to obtain high quality data for downstream analyses.
- *Fetal DNA fraction estimation:* Let f be the fetal DNA fraction of the sample, i.e., the percentage of reads from the fetus. For example, $f = 9\%$ indicates that 9% of reads come from the genome of fetus, and the remaining 91% of reads come from the mother's genome. The fetal DNA fraction f can be estimated by computational methods [16] of which SeqFF [17] can estimate fetal DNA fractions for both male and female samples. If the fetal DNA fraction is not enough (e.g., smaller than 5%), we have to re-collect blood sample and re-sequence data to obtain a higher fetal DNA fraction (the longer pregnancy time leads to the higher fetal DNA fraction).
- *Chromosomal aberrations detection:* Detecting chromosomal aberration by comparing the read coverage of test sample with that of normal (reference) samples. A number of NIPT algorithms have been proposed to detect numerical chromosome aberrations. If the fetus has an additional copy of a chromosome h, the read percentage of chromosome h will increase in comparison with that in the normal sample. Detecting aberrations in fetal DNA genome is a challenging problem because the fetal DNA contributes only a small part in the NIPT data.

2.3 NIPT algorithms

NIPT algorithms typically compare the read coverage on chromosomes of the test sample with that of normal (reference) samples to detect chromosomal aberrations [7], [8], [10], [18]. The workflow of the read coverage-based NIPT algorithms includes following steps:

- *Binning* step: The chromosomal aberration is determined from aberrations at small regions (called bins) in the chromosomes. The genome is normally divided into equally sized bins with the length from thousands to millions of base pairs such that each bin contains a sufficient number of reads. The read coverage percentages of bins are calculated and used to determine aberrations of the bins.
- *Constructing reference panel step:* The read coverage percentages of bins in reference (normal) samples are analyzed to construct a reference panel; and used as the base to compare with that in the test sample. If the read coverage of a bin b in the test sample considerably deviates from that in the reference panel, the bin b is considered as abnormal.

– *Estimating z*-scores: The NIPT algorithms use different statistical tests to assign a z-score for each bin in the genome of test sample. The overall z-score of a chromosome is estimated from the z-scores of its bins. A chromosome h is predicted abnormal if its overall z-score exceeds a predefined z-score threshold. Otherwise, it is considered as normal.

Comparing the read coverage of a test sample with that of reference samples requires that short-reads of the test sample and reference samples should be sequenced at the same conditions. This requirement might be not completely fulfilled in practice. To overcome the strictly technical requirements, within-sample copy number aberration detection algorithms Wisecondor [9] and its improvement WisecondorX [10] have been proposed. For each bin b in a chromosome h, the within-sample algorithm determines a set of reference bins in other chromosomes that have similar read coverage as b in reference samples. The bin b and its reference bins are sequenced at the same condition. The read coverage of each bin b in the test sample will be compared with that of its reference bins to calculate a z-score of b.

WisecondorX has a number of limitations. First, it results in a considerable number of false positive samples when using the overall z-score to predict chromosomal aberrations. The second limitation of WisecondorX is its sensitivity to the reference panel. We developed a software of the so-called VINIPT, an improvement of Wisecondor and WisecondorX algorithms, optimized for the low coverage data. First, we determined proper reference samples to build several reference panels that help consistently predict the chromosomal aberrations. Second, the VINIPT algorithm combines both Wisecondor and WisecondorX algorithms to determine if a chromosome is truly abnormal when its overall z-score is greater than a predefined z-score threshold. The improvements help reduce the false positive rate of the VINIPT algorithm.

2.4 Simulating positive non-mosaic samples with autosomal trisomy

A normal sample s has two copies of every autosomal chromosome. The sample s has a trisomy at a chromosome h if the fetus has three copies of h. Given a negative sample, instead of simulating additional reads to create an extra copy of chromosome h for the fetus, we remove available reads in the other chromosomes such that the read percentage of h in comparison with that of the other chromosomes will be equivalent to the case that the fetus has three copies of h. Let C_i be the read percentage (read coverage) mapped to chromosome i, e.g., $C_1 = 0.08$ means that the number of short-reads mapped to chromosome 1 is about 8% of all short-reads sequenced. The reads mainly come from the DNA of mother and a small fraction from the DNA of fetus.

For a singleton non-mosaic sample, introducing an additional copy of chromosome h for the fetus will increase the read percentage C_h to $C'_h = C_h(1 + 0.5 \times f)$. Technically, increasing the read percentage C_h to C'_h is equivalent to decreasing the read

coverage C_i of chromosome i ($i \neq h$) to $C'_i = \frac{C_i}{1+0.5 \times f}$. The NIPT simulation algorithm includes four steps described as follows:

NIPT simulation algorithm:

Input: A normal sample s including millions of short reads and an autosomal chromosome h.

Output: An abnormal sample s' with trisomy aberration on chromosome h.

- Step 1: Aligning short reads with the reference genome (e.g., GRCh37) using genome aligners such as BWA or Bowtie2. The GC content correction and other filtering methods might be applied to remove noise data.
- Step 2: Calculating the fetal DNA fraction f of sample s using a computational method such as SeqFF. If f is not greater than a pre-defined threshold (e.g., 5%), stopping the simulation process due to the low volume of fetal DNA in the maternal blood.
- Step 3: For every chromosome $i \neq h$, reducing its coverage C_i to $C'_i = \frac{C_i}{1+0.5 \times f}$. Technically, for each read r mapped to chromosome i, removing r with probability of $(1 - \frac{C'_i}{C_i})$.
- Step 4: Returning remained reads as an abnormal sample s' with trisomy aberration on chromosome h. The abnormal sample s'can be used to examine the sensitivity of read count-based NIPT algorithms.

2.5 Simulating positive mosaic samples with autosomal trisomy

There are different types of mosaicism (a number of cells in the fetal genome have chromosomal aberrations) that strongly affect the accuracy of the NIPT methods [16]. In this study, we simulate a common mosaic case (placenta mosaicism) that half of fetal cells are normal with 46 chromosomes and the other half are abnormal with autosomal trisomy. The read percentage C_h of a positive mosaic sample will be increased to $C''_h = C_h(1 + 0.25 \times f)$. In the mosaic case, the difference between C''_h and C_h is only half of the difference between C'_h and C_h. Thus, detecting aberrations in mosaic samples is much more difficult than that in the non-mosaic samples. Given a negative sample s and a chromosome h, for every chromosome i ($i \neq h$), we remove reads such that the read percentage C_i will be reduced to $C''_i = \frac{C_i}{1+0.25 \times f}$. The NIPT simulation algorithm described above is modified to create positive samples with mosaic condition. Technically, the probability of removing a read r in Step 3 is $\left(1 - \frac{C''_i}{C_i}\right)$ for the mosaic condition instead of $\left(1 - \frac{C'_i}{C_i}\right)$ for the non-mosaic condition.

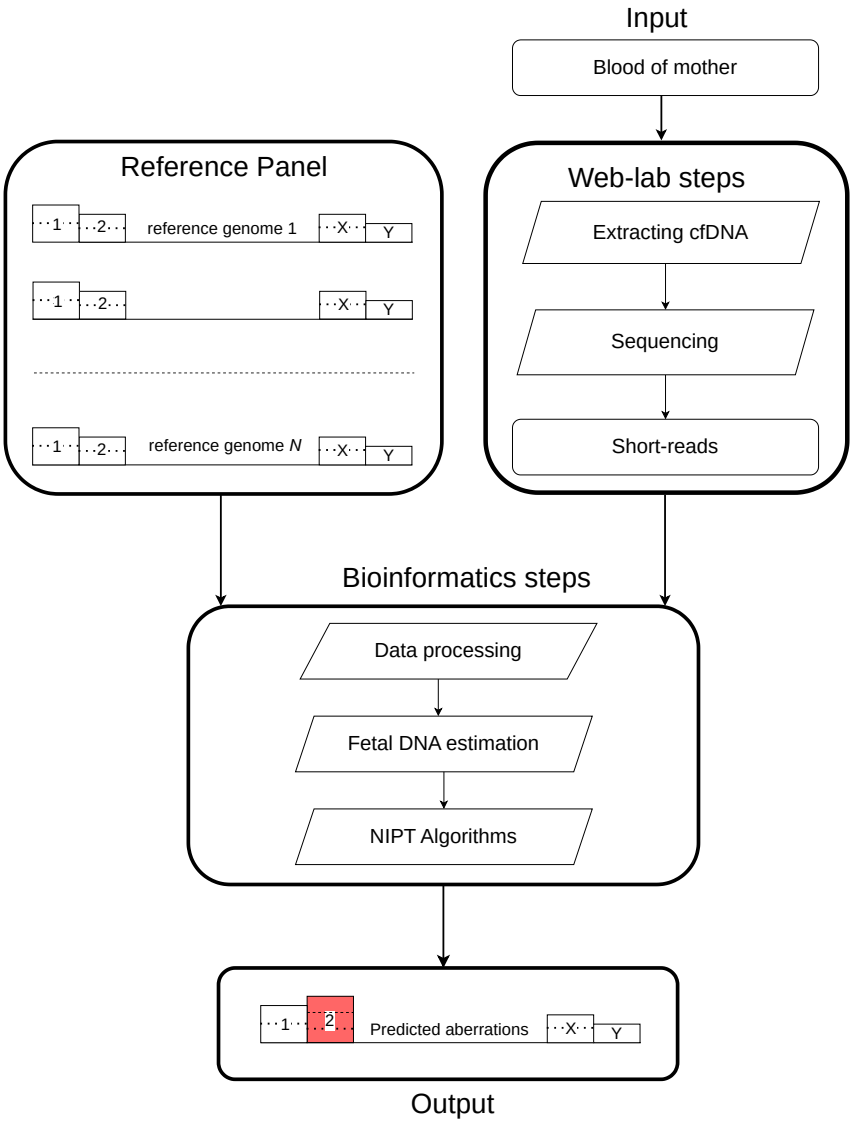

Fig. 2. The NIPT procedure to detect numerical chromosomal aberrations from the blood of mother. The procedure includes both web-lab and bioinformatics steps.

3 RESULTS

We used 240 negative samples to simulate positive samples under both non-mosaic and mosaic conditions. We obtained 1440 positive samples with T13, T18, and T21 aberrations for testing the NIPT algorithms (240 positive samples for each simulation

setting). We reported the performance of WisecondorX and VINIPT software on 1440 simulated positive samples as well as 1058 negative samples. We note that some NIPT algorithms such as NIFTY were implemented in commercial software and not publicly available for testing. We also examined the open-source algorithm NIPTeR [8]. However, preliminary results showed that NIPTeR did not work well on the low coverage data. The performance of NIPTeR should be further investigated, therefore, we eliminated its results from the report.

We analyzed the results from WisecondorX and VINIPT on negative samples and positive samples from non-mosaic samples (see Fig. 3). For the non-mosaic analysis, we set the z-score threshold of 3.3 for both WisecondorX and VINIPT. The z-scores of negative samples form one group and most of them smaller than 3.3; while the z-scores of positive samples create another separated group and most of them are greater than 3.3. Both WisecondorX and VINIPT could determine all T13, T18, and T21 aberrations on the positive samples, i.e., the sensitivity of 100%. The WisecondorX resulted in 16 false positive samples (i.e., 16 negatives samples were predicted as positive samples). The false positive rate of WisecondorX for non-mosaic samples is about 1.5% or the specificity of about 98.5%. The VINIPT falsely assigned only one negative sample as a positive sample with T13. The false positive rate of VINIPT is smaller than 0.1% (i.e., specificity greater than 99.9%) and much less than that of WisecondorX.

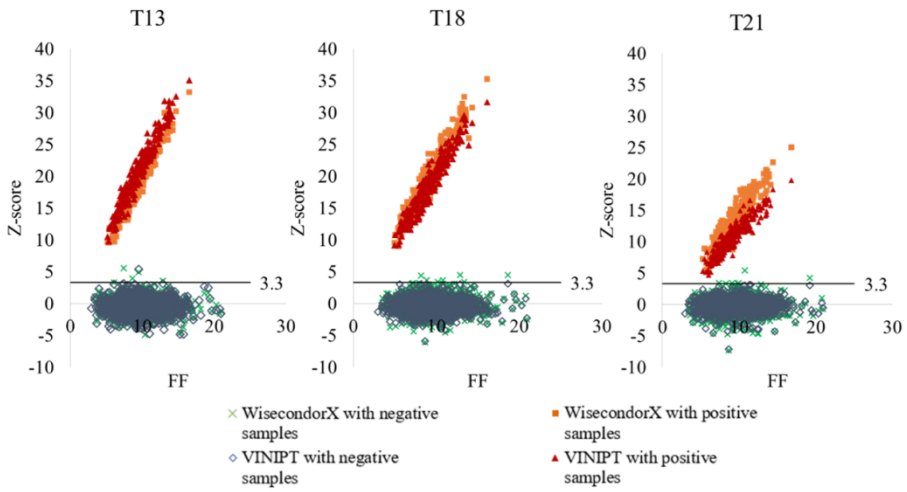

Fig. 3. The z-scores of negative and positive non-mosaic samples. The left figure for T13, middle figure for T18, and right figure for T21. FF: fetal fraction.

We now examined the results from WisecondorX and VINIPT for positive mosaic samples. It is much more difficult to detect aberrations from mosaic samples than from the non-mosaic samples. Fig. 4 shows that the z-scores of negative samples and that of positive mosaic samples are slightly overlapped.

The z-score threshold of 3.3 resulted in a number of false negative results. To avoid a high false negative rate, we lowered the z-score threshold from 3.3 to 2.58 for both WisecondorX and VINIPT for the mosaic analysis. Both WisecondorX and VINIPT were able to detect positive samples with T13 and T18 aberrations from the mosaic samples. WisecondorX and VINIPT algorithms falsely assigned two positive samples with T21 aberration as negative samples. The overall sensitivity of detecting T13, T18, and T21 aberrations of both WisecondorX and VINIPT are 99.7%. The fetal DNA fractions of the two false negative samples are considerably low (i.e., 5.8% and 6.1%). Lowering the z-score threshold helped reduce the false negative rate, however, it increased the false positive rate of both algorithms. WisecondorX resulted in 53 false positive samples, while VINIPT produced 19 false positive results. The false positive rates of WisecondorX and VINIPT for the mosaic analysis are about 5% and 1.8%, respectively.

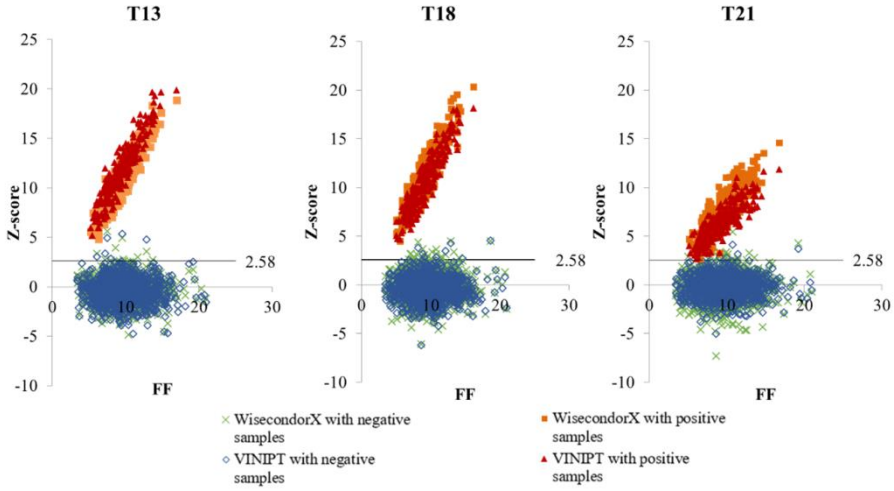

Fig. 4. The z-scores of negative samples and positive mosaic samples. The left figure for T13, middle figure for T18, and right figure for T2. FF: fetal fraction.

Finally, we collected 100 positive samples with T13, T18, and T21 aberrations from our genetic testing center. They are called clinical positive samples to differentiate from the simulated positive samples. Table 1 shows that the z-scores of the clinical positive samples and simulated positive samples for both non-mosaic and mosaic conditions. The z-scores from simulated positive non-mosaic samples are similar to that from clinical positive samples; and much higher than the z-scores from the simulated positive mosaic samples. The results confirm that it is much more difficult for NIPT algorithms to detect chromosomal aberrations from mosaic samples than from non-mosaic samples.

Table 1. The z-score distributions of 100 clinical positive samples and 1440 generated positive samples with T13, T18, and T21 aberrations.

	T13	T18	T21
Clinical positive samples	16.3 (8.5)	20.8 (5.9)	15.2 (6.8)
WisecondorX non-mosaicism	17.9 (4.8)	19.2 (5.2)	12.7 (3.8)
VINIPT non-mosaicism	19.5 (5.1)	17.8 (4.7)	10.7 (2.9)
WisecondorX mosaicism	10.5 (2.7)	11.2 (3.0)	7.5 (2.3)
VINIPT mosaicism	11.6 (2.9)	10.3 (2.8)	6.3 (1.7)

4 DISCUSSION

The NIPT has been widely used as a powerful screening method to detect numerical abnormality of chromosomes. As positive NIPT samples are rare, the positive NIPT datasets used to evaluate NIPT algorithms are typically small and not publicly available for scientific community. This prevents the development of new NIPT algorithms for different NIPT data types such as the low coverage NIPT data or the NIPT data from mosaic samples.

A large number of negative NIPT samples are available from hospitals or genetic testing centers. In this paper, we present a simple method to create positive samples with autosomal trisomy from negative samples. Our simulation method does not introduce new short-reads, therefore, the simulated positive samples can play as real as clinical positive NIPT samples. We applied the simulation method to create 1440 positive samples with T13, T18, and T21 aberrations for both non-mosaic and mosaic conditions.

We used the 1440 simulated positive samples to evaluate the performance of WisecondorX and VINIPT software. Experiments showed that both software could determine almost all positive samples. They failed to detect a few positive mosaic samples that have considerably low fetal DNA fractions. The low amount of DNA from the developing fetus makes the NIPT algorithms more difficult to detect chromosomal aberrations from mosaic samples. For the mosaic analysis, we should use a lower z-score threshold; and require a high fetal DNA fraction to reduce the false negative rate.

The positive detection rate of NIPT, i.e., the percentage of cases reported positive by NIPT, ranges from 0.5% to 1.5% depending on a number of factors such as sequencing platforms, read coverage per sample and NIPT algorithms used to call aberrations. Different large-scale retrospective studies have showed that the positive predictive values of NIPT are considerable low. Therefore, positive results from NIPT must be carefully interpreted and explained by genetic consultants for pregnant women. Other methods should be implemented to monitor the development of fetus that have positive results from NIPT.

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