

The Progress of Next Generation Sequencing in Preimplantation Genetic Testing

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Abstract

Preimplantation genetic testing (PGT), the determination of an embryo's constituency before implantation, is becoming more and more common in clinic and there has been a gradual shift in technology towards next generation sequencing (NGS). Numerous biopsy samples such as a blastomere of cleavage stage embryo, trophoctoderm of blastocyst and polar bodies as well as non-invasive or low-invasive samples such as blastocoe fluid and embryo's culture medium can be analyzed with different NGS platforms namely Roche, Illumina and Ion torrent. NGS technology has been validated and is now clinically applied to detect aneuploidies, partial or segmental aneuploidies, chromosomal aberrations including imbalanced translocations, inversion, deletion, duplication insertion and mosaicism, triploidy and single gene disorder which are often responsible for infertility and can be associated with spontaneous abortions and fetal malformations or diseases. Comprehensive chromosomal screening for all 24 chromosomes together with single gene disorder detection can be achieved at the same time in a single run using NGS and the analysis of multiple samples with different indication can be done on a single chip. NGS has the ability to detect numerical, structural and genetic abnormalities at a low cost with high efficiency and accuracy.

Keywords: Next generation sequencing; Preimplantation genetic testing; Preimplantation genetic diagnosis; Preimplantation genetic screening

1. Introduction

Preimplantation genetic testing (PGT) is the determination of an embryo's chromosomal constituency before implantation. It has been given many different names according to its use. Preimplantation genetic diagnosis (PGD) is often used with parents diagnosed with known single gene mutation and chromosome number or structure abnormalities who want to have an unaffected child. Preimplantation genetic screening (PGS) is the screening for aneuploidy especially in patients or couples who have a history of recurrent spontaneous abortions, recurrent implantation failure and advanced maternal age [1]. The goal of all of the above is to have an unaffected child, avoid the termination of pregnancy or recurrent miscarriages.

28 years ago, the first case of a live birth of a normal offspring from PGD for a recessive X chromosome-linked disease was born [2]. Nowadays, PGT is routinely used in clinics with a range of uses including single gene disorder and both structural and numerical chromosomal abnormalities. The success of PGT has led to over 10,000 babies born worldwide.

Since its adoption in clinics, a number of techniques have emerged from the cytogenetic molecular analysis. Fluorescent *in situ* hybridization (FISH) was the first diagnostic method used. However, FISH has several technical limitations including the number of probes required to obtain reliable results and the requirement of specific parent karyotyping prior to testing [3-5]. It analyzes a limited number of chromosomes at a time and thus misses aneuploidies in other chromosomes. These constraints have led to the development of new and improved 24 chromosomal copy number analysis technologies, including comparative genomic hybridization (CGH), array comparative genomic hybridization (aCGH), digital-PCR, real-time quantitative PCR, SNP microarray and next generation sequencing (NGS), which is the newest method to date. All of these different technologies have their own advantages and disadvantages according to the time of analysis, the cost, the resolution, the labor needed, the procedures and the abnormalities which can be detected¹. This review is a summary of the advances in preimplantation diagnosis with NGS.

1.1 Different platforms used in NGS

There are currently three major NGS platforms, namely Roche (also known as 454), Illumina and Ion torrent. Life technologies/Ion torrent and Roche/454 life sciences, both use emulsion PCR as their amplification method. Illumina on the other hand, uses bridge PCR. As for their sequencing techniques, Roche/454 life sciences use pyrosequencing with a read length of up to 1000bp. But this method has a high error rate in detecting insertions and deletions in homopolymers. Illumina uses sequencing by synthesis with reversible terminator. The newer platforms of Illumina can yield about 24million short fragments of DNA sequence per run or "read" which gives it an ultra-high-throughput and makes it cost effective. The main limitation of Illumina is in the increased base substitution error rate. Lastly, we have Life technologies/Ion torrent which uses ion semiconductor sequencing and can read up to 200bp. It has high error rates in calling insertions and deletions. Although it takes days to have one genome sequence with Illumina, it is the most widely used on the market due to its cost effectiveness, good sequencing throughput and accuracy [6]. Other emerging sequencing techniques still need work done in terms of cost, quantity,

quality and time.

2. Different biopsy sites

2.1 Blastomere of cleavage stage embryo VS Trophectoderm of blastocyst

After fertilization, each cleavage of the zygote yields two undifferentiated cells called blastomeres. For the purpose of PGT, in some centers, Day 3 cleavage cell blastomeres are usually biopsied to be analyzed. Another, most commonly used, stage at which the biopsy is taken, is the blastocyst stage at day 5 or day 6. Most specifically, cells from the outer layer of the blastocyst are taken, which are known as the trophectoderm after gastrulation. Many researchers dispute over which stage of the embryo yields better outcomes. In 2015, Lukaszuk et al. [7] published a prospective case control study in which the Day 3 blastomeres were used for aneuploidy analysis by NGS in implantation failure patients. Then, fresh day 5 embryo transfer was performed and the clinical pregnancy rates were compared to a control group in which no ploidy status analysis was done. They concluded that the clinical pregnancy rate was more than two times higher in the NGS-blastomere group compared to the control group and the results were statistically significant. But more and more studies are pointing towards and suggesting trophectoderm biopsy as a better choice. Blastomere biopsy has been shown to have several disadvantages. Scott et al. [8,9] have shown that there is a decreased rate of implantation when the biopsy is performed at the blastomere stage. Mertzaniidou et al. [10] revealed that at the cleavage stage, mosaicism is more likely to be present in the embryos. In 2013, Capalbo et al. [11] showed that mosaicism can be avoided or reduced if the biopsy is done on blastocysts instead of blastomeres. In another study by Scott et al. [9], biopsy performed on the blastomere at the cleavage stage had more damaging effects on the embryos. The results from studies by Jones et al. [12] and Capalbo et al. [13] both show that less genetic material is obtained from biopsy of the embryo at the cleavage stage. The reasons are due to the higher efficiency with trophectoderm biopsy. The likelihood for an euploid embryos to reach the blastocyst stage is higher than that of an abnormal embryo. This decreases the number of biopsies that have to be performed in order to obtain a normal euploid embryo from the mix that will be ultimately used for intrauterine transfer. The positive predictive value is therefore increased when trophectoderm biopsy is used. Lee et al. [14] in a randomized controlled trial and Dahdouh et al. [15] in a meta-analysis both demonstrated the better effectiveness of trophectoderm biopsy.

2.2 Polar bodies

Treff et al. [16], in 2016, extracted oocytes from mouse models and subjected them to in-vitro maturation after which, they separated the polar bodies from the mature oocytes. Wide Genome Amplification followed by NGS were performed on the oocytes, polar bodies and blastocysts to check for aneuploidy. They showed that NGS can be applied to gametes and embryos for the chromosome evaluation as long as the genome is known. Furthermore, Yan et al. [17] performed an analysis on both trophectoderm and polar bodies biopsies using NGS and linkage analysis for a single gene disorder PGD, the female patient was a carrier of an X-linked chromosome recessive disorder; X-linked hypohidrotic ectodermal dysplasia and successful live birth was achieved. Thus, showing that NGS can be used for analyzing human polar bodies.

2.3 Blastocoele fluid and culture medium

Recently, non-invasive or low-invasive approaches for chromosomal analysis in PGT have been tried. Several studies mentioned the existence of DNA material including chromosome, genomic and mitochondrial DNA in the blastocoele fluid and culture medium. Xu et al. [18] conducted a study using NGS to analyze fluid from the culture medium of human embryo from day 3 to day 5 and validated their results by comparing it with the comprehensive chromosome sequencing analysis obtained by the corresponding Day 5 whole embryo. Their results showed that non-invasive chromosome screening had a specificity of 84% and a sensitivity of 88.2% with a positive and negative predictive value of 78.9% and 91.3% respectively. Furthermore, using non-invasive chromosome screening only, they also carried out the first live birth in a patient with a balanced translocation t(14;15)(q22;q24) and repeated the procedure on six more patients which resulted in five healthy chromosomally normal offspring and one ongoing pregnancy. Zhang et al. [19] on the other hand, did single gene PGD using NGS on the DNA found in blastocoele fluid, which is routinely removed from the embryo prior to vitrification to prevent damage by ice crystal formation, and compared the results with the corresponding blastomere embryo. They tested ten genes namely TCIRG1, SCN5A, RHO, EXTL1, SLC4A1, VWF, HSF4, NPC1, PTCH1, and EPS8L3. They obtained a gene level amplification efficiency of 84% which is lower compared to WGA for a single cell which is 90~95%. There is still room for improvements in the chromosomal analysis and gene level analysis for both fluid culture medium analysis and blastocoele fluid analysis. The source of the DNA should be explained and the results from the blastocoele fluid analysis should be validated. Their non-invasiveness gives these two methods a great potential to make PGT even safer and more routinely used in clinics.

3. Frequent abnormalities which can be detected

3.1 Structural abnormalities: Translocation (reciprocal, Robertsonian), inversion, deletion, duplication insertion, ring, marker

Chromosomal aberrations are often responsible for infertility and can be associated with spontaneous abortions and fetal malformations. One of the most common ones is translocation which is a breakage and transfer of the broken part to another location on the same chromosome or most frequently on another chromosome. Two types of translocations can be identified in the process of PGD. They are Robertsonian translocation and reciprocal translocation. Robertsonian translocation involves the breakage at or near the centromere while reciprocal translocation involves the exchange of materials between non-homologous chromosomes. Inversions, whereby part of the chromosome is reversed end to end, and insertions, where part of one chromosome is deleted from its normal location and inserted into another chromosome, are also often detected during PGD. Up till now, aCGH remains the gold standard to detect those chromosome aberrations in clinics. Recently, newer methods have emerged to challenge aCGH more specifically, NGS.

In 2013, Yin et al. [20] showed that NGS can be used to detect chromosomal abnormalities. They analyzed the trophectoderm of patients with translocations and inversions using both NGS and SNP array. They also validated their results using qPCR and found that the sensitivity and specificity to detect copy number variance (CNV) was 95% and 96% respectively. They also analyzed for aneuploidies and found that NGS sensitivity and specificity were

both 100%. In 2015, Bono et al. [21] conducted a blinded retrospective study to validate the use of a semiconductor NGS for the detection of reciprocal translocations. They used cells from the blastocyst stage and embryo cleavage cells from patients previously identified with reciprocal translocations for their analyses and compared the comprehensive chromosome analysis results with that obtained from aCGH. They successfully showed that semiconductor NGS is a robust method of detection for unbalanced reciprocal translocations with a specificity of 99.75% and sensitivity of 100% at the chromosome identification level. As for the identification of chromosomally normal embryo, both their specificity and sensitivity were 100%. The smallest detectable chromosomal segment was 5MB. Other investigators have also further validated NGS for not only reciprocal translocation but also robertsonian translocations, insertions and inversions. Deleye et al. [22] in 2015, published a paper in the journal of fertility and sterility that compared Illumina and ion torrent (life technologies) NGS to aCGH. They used trophectoderm biopsies at day 5/6 to analyze for numerical and structural chromosome aberrations. The results from NGS were concordant with that of aCGH. The specificity and sensitivity of NGS were both 100%. They reported a smallest detectable chromosomal abnormality of 4.5MB and also that NGS provided an increased signal to noise ratio for lengths up to 3MB. But at lengths lesser than 3MB, the WGA representation bias affects the results. Following these promising results, in 2016, Zhang et al. [23] was the first to apply CNV-seq NGS in clinics to identify chromosomally normal embryos for intrauterine transfer without the assistance of aCGH to validate the embryos biopsies results for patients previously established as having Robertsonian and reciprocal translocations. Due to the shift of trend during the study, they started with cleavage stage biopsy and ultimately changed to blastocyst biopsy. After having been identified as normal/balanced euploid, one or two frozen embryos were transferred. They observed an implantation rate of 80%, a 20-week gestation clinical pregnancy rate of 60% and a live birth rate of 70%. They demonstrated that CNV-seq NGS has a high resolution which can detect unbalances near the terminal parts of the p and q arms, and can detect mosaicism down to 20%. Also, they suggested that NGS is a reliable and accurate method that has great clinical applications.

For the purpose of PGD, clinicians usually stop at differentiating normal/balanced from chromosomally abnormal/unbalanced aneuploid embryos. But many of those embryos may be carriers of the chromosomal defect and babies from these embryos may in turn have infertility issues and pass on the defect to future generations. Efforts were made to go a step further so as to identify completely chromosomally normal embryos from carrier embryos. In 2016, Hu et al. [24] published a paper in which they used 8 patients with reciprocal translocations. Peripheral blood samples from these patients were used for chromosomal microdissection and PCR amplification followed by NGS to obtain the precise location of the breakpoints. Using this information, they modulated breakpoint specific primers. Trophectoderm biopsies were taken from the embryos of these patients and PGD was done using NGS. The embryos identified as balanced/euploid embryo were further analyzed using linkage analysis with informative SNP and junction spanning PCR analysis to separate the normal embryo from the carrier embryos. For patients who did not have completely normal embryos, the carrier embryos were still transferred. Single or double frozen embryo transfer was done in 8 patients resulting in 6 live births (5 balanced translocation carriers and one normal karyotype) with karyotypes corresponding to the PGD. The specificity and sensitivity, and its use in other types of chromosomal aberrations is yet to be tested.

Furthermore, small deletion and duplication can cause chromosome disease syndromes associated with copy number variance which can lead to severe diseases. With the use of NGS, we can now accurately detect duplication and deletions as small as 1-2Mb. Fan J et al. [25] validated their results using 3 genomic DNA samples with these specific copy number variances and they also used 5 peripheral blood samples from patients with these diseases. They were able to accurately detect a 4.98 Mb 5q35.2-qter deletion associated with Sotos syndrome and a 6.66 Mb 7p22.1-pter deletion associated with 7p terminal deletion syndrome. Moreover, Gui B et al. [26] was able to successfully detect euploid Charcot-Marie-Tooth disease type A for embryo transfer after NGS-based method using linkage analysis. These results show that NGS can detect very small copy number variance and can help couples conceive a disease free embryo.

Although there have not yet been any publications on PGD using NGS for ring and marker structural chromosome abnormality, according to the studies mentioned above, NGS has the efficiency and potential to detect them. Thus, further studies are needed to validate the use of NGS for ring and marker chromosome.

3.2 Aneuploidy

When the number of chromosome in a cell deviates from the normal chromosomal composition, it is called aneuploidy. It can be due to the addition of a single chromosome (trisomy) or the addition of a pair of chromosomes (tetrasomy) or the loss of a single chromosome (monosomy) or the loss of a pair of chromosomes (nullisomy). Incorrect number of chromosomes in an embryo often leads to implantation failure, miscarriage and fetal malformations. With the development of preimplantation genetic, 24 chromosome screening for aneuploidy is very common now. Fiorentino et al. [27,28] designed a 2 phase study for the development, validation and application of NGS for 24 chromosome aneuploidy screening. During the first phase, they evaluated samples with known abnormal cytogenetic karyotype from cultured amniotic fluid and products of conception, and they re-evaluated previously tested aCGH blastomeres with their NGS-based protocol and any conflicting results were reevaluated using QF-PCR. They showed that NGS had a 99.98% specificity and a 100% sensitivity to detect aneuploidy according to chromosome copy number assignment. NGS specificity and sensitivity to detect aneuploidy for 24-chromosome diagnosis were both 100%. The positive and negative predictive value of NGS for 24 chromosome aneuploidy screening were both 100%. In the second phase of their study, they analyzed blastocysts prior to embryo transfer using both NGS and aCGH, and conflicting results were reevaluated using QF-PCR. Their results were similar to the first phase and they demonstrated the clinical application of NGS with a clinical pregnancy rate per embryo transfer of 63.8% and an on-going implantation rate of 62%. For Kung et al. [29] previous blastocyst and cleavage stage embryos which were tested with aCGH were reanalyzed using NGS. They found that NGS specificity and sensitivity were both 100% and that there was more than 99% concordance between NGS and aCGH. These results were also confirmed by Zheng et al. [30] using VeriSeq PGS kit (Illumina) with a smallest detection size of 1.8MB and Sachdeva et al. [31] using Ion torrent Personal Genome Machine NGS. After the validation of NGS, its clinical applications in PGS cycles were also evaluated. There is a new targeted next generation sequencing (tNGS) platform which has recently undergone preclinical validation for comprehensive chromosome screening by Zimmerman et al. [32]. They tested the new tNGS platform on both commercially available cell line and discarded

blastocyst and found the consistency with conventional karyotyping for the cell lines to be 99.2% and 98.7% for real-time PCR for the blastocyst. Yang et al. [33] first re-validated NGS by showing specificity, sensitivity, positive and negative predictive value of NGS to be 100% by reanalyzing embryos which were previously tested with aCGH. Next, they performed a randomized controlled trial with one group using only NGS and the other group using only aCGH. Their results revealed no significant difference in clinical pregnancy, ongoing pregnancy and miscarriage rates between the two groups. Now, NGS is applied in the evaluation of different PGS indication like AMA, RPL and RIF [34].

3.3 Mosaicism

Mosaicism in an embryo is defined as having two or more different cell lines with different chromosomal number or structure in one embryo caused by errors in chromosomal segregation during mitosis. At the beginning, when FISH was used for PGS, about 30% of embryos were found to be mosaic. With recent technologies, new methods for comprehensive chromosome screening (CCS) like aCGH, qPCR and SNP array can detect both euploid and aneuploid embryos but are unable to detect mosaicism. However high-resolution NGS has the ability to detect euploid and aneuploid embryos as well as mosaicism. Maxwell et al. [35] internally validated NGS protocols to detect mosaicism. They compared the results from previous euploid embryos which were tested using aCGH and miscarried, to the results of the same embryos using NGS. They found that 31.6% were mosaic. Both Maxwell et al. [35] and Munne et al. [36] showed that 9% of all transferred blastocysts leading to ongoing pregnancies were mosaic.

Munne et al. [37] propose that there should be a third and intermediary group for mosaic embryo. According to the Controversies in Preconception, Preimplantation and Prenatal Genetic Diagnosis (COGEN) and Preimplantation Genetic Diagnosis International Society (PGDIS) blastocyst biopsies are now categorized into 3 groups for implantation: euploid embryo (<20% aneuploidy cells), aneuploid embryo (>80% aneuploidy cells) and euploid-aneuploid mosaics (20%-80% aneuploidy). Although the latter often miscarries and implants less frequently, about 40% of mosaic embryo can lead to live birth [38,39]. Munne et al. [36] retested and analyzed the results using high-resolution NGS from previous PGS cycle that used aCGH and divided them into two groups: mosaic group and euploid group. They found that even though the euploid group (71%) had a higher implantation rate than the mosaic group (53%), the difference was insignificant. They also showed that the mosaic embryos do miscarry and implant less, however they discovered that complex mosaic embryos have a significantly lower implantation rate (10%) compared to other mosaic embryos. They proposed that embryos with 20%-40% mosaicism should be selected first instead of embryos with >40% mosaicism in accordance with COGEN guidelines. Since complex mosaic embryos had a 10% implantation rate, they proposed that the latter could be used in patients with advanced maternal age and poor reproductive history. PGDIS proposed guideline suggest that monosomic embryos should be prioritized over trisomic embryos, though Munne et al. [36] presented that trisomic, monosomic or segmental mosaics had similar implantation rates. Munne et al. [36] also suggested more research is needed to validate the other recommendations made by COGEN and PGDIS regarding which mosaic embryo should be transferred according to the particular chromosomes involved. Caution should be taken when proposing the transfer of mosaic embryo, if for the first cycle

only one mosaic embryo is found, then the patient should perform a second cycle and if the second cycle is also unsuccessful, then the patient should consider the transfer of a mosaic embryo. The patients should be offered different options along with the associated risk and advantages so that an informed decision can be made.

3.4 Triploidy

Triploidy is a rare chromosomal disorder which affects 2-3% of human pregnancies. It is defined as a polyploid with three sets of chromosomes. Triploidy in humans are 69XXX, 69XXY, and 69XYY. According to its etiology, it is usually classified into two groups: 1. Digyny which is when the additional haploid is maternal, 2. Diandry which is when the additional haploid is paternal. It is associated with multiple abnormalities, partial hydatidiform mole and miscarriages. Maxwell et al. [35] internally validated NGS protocols which was able to detect different triploidies (69 XYY and 69 XXY) which aCGH could not. But Xu et al. [40] found that MALBAC-NGS was not able to detect triploidy compared to SNP array. Recently, Marin et al. [41] validated triploidy detection in blastocyst using tNGS. They were able to accurately detect triploidy in their control samples as well as in trophoctoderm biopsies and found that triploidy during in vitro fertilization is a rare occurrence and is mainly linked to maternal origin.

3.5 Single gene disorder

Single gene disorder, also known as monogenic disease, is when a single mutation in a specific gene leads to a hereditary disease which can occur early during childhood or have a late onset. Usually these disorders are debilitating, lowers patients' quality of life and sometimes may even be associated with a shorter life span. With the use of PGD, we can now select embryo which are free from these mutations. In fact, in 1992, the first baby free from cystic fibrosis was born after PGD and since then PGD has been increasingly used to detect monogenic disorders [42]. NGS-based PGD for monogenic diseases was first validated by Treff et al. [43] They showed that the genotype obtained with NGS was 100% consistent with Taqman allelic discrimination test as well as their reference laboratory genotypes for CF: cystic fibrosis and WWS: Wlaker-Warburg syndrome. Then Well et al. [44] showed that low-pass whole genome amplification (WGA) and linkage polymorphism before NGS can detect cystic fibrosis mutation. Together with Fragouli et al. [45], they both showed that the whole mitochondrial genome can be analyzed using NGS. Furthermore, live births were achieved after the use of NGS. Yan et al. [17] described two couples, one with a family history of autosomal dominant disorder, hereditary multiple exostoses (HME), who had trophoctoderm biopsies and the other female patient was an X-linked chromosome recessive disorder carrier; X-linked hypohidrotic ectodermal dysplasia and had a trophoctoderm and polar bodies biopsies. Both couples underwent PGD using NGS and linkage analysis. The results were confirmed by Sanger sequencing, CGH array and STR analyses. The two couples successfully gave birth to healthy babies. Chen et al. [46] also published an NGS-based SNP haplotyping method for Hb H disease. They ran the same samples in both Gap-PCR and NGS-based SNP haplotyping, the latter was able to obtain results for all the samples while Gap-PCR was only able to diagnose one sample. Chen et al. [46] also achieved a live birth.

All the NGS based method used for monogenic diseases use linkage analysis to obtain their results and require prior analysis of the parents lymphocytes and other reference laboratory samples to acquire a locus-specific product.

Although this process may require more time and can increase the cost of PGD, single gene disorder screening and comprehensive chromosome screening for aneuploidies can be performed on a single chip and for several samples at one time, thereby reducing its cost.

4. Embryo transfer

After NGS analysis, which can take a minimum of 24 hours, either fresh embryo or frozen embryo transfer can be performed. Most clinics perform frozen embryo transfer because NGS is more cost effective when multiple samples are loaded on a single chip and waiting for the appropriate number of samples requires the vitrification of the samples. A randomized controlled trial performed by Coated et al. [47] compared fresh embryo transfer cycles to frozen embryo transfer cycles. They found a significant increase in ongoing pregnancies and live birth rate per intended treatment as well as per embryo transfer in the frozen group. Their results also showed that for double embryo transfer, the live birth rate was significantly increased in the frozen embryo transfer group. Frozen embryo transfer has the advantage of both being cost effective and patients are able to achieve their targeted embryo transfer strategy. Thus preventing OHSS and transferring the embryo into a uterus with the best environment for implantation.

5. Limitation and efficiency

NGS has the ability to detect aneuploidies, partial or segmental aneuploidies, chromosomal aberrations including imbalanced translocations, inversion, deletion, duplication insertion and mosaicism, triploidy, single gene disorder and even mitochondrial disorders and its detection capability is more precise and accurate than aCGH with the smallest detection size of 1.8MB [48, 30]. It is the only technology which can accurately detect mosaicism. Comprehensive chromosomal screening for all 24 chromosomes together with single gene disorder detection can be achieved at the same time in a single run using NGS and the analysis of multiple samples with different indication can be done on a single chip which reduces the cost [49]. NGS is highly automated and has a high-throughput which further reduces the cost. The time for analysis has been shortened and can now be used in fresh cycles, however for cost saving to allow it to run to its full capacity, frozen cycles are more commonly performed.

One of the limitations of NGS is that it cannot detect triplet repeat disorder such as fragile X syndrome. For single gene disorder detection, the phenomenon of allele drop out may occur and may lead to erroneous results. Therefore, linkage analysis needs to be performed together with NGS to achieve positive results [50].

6. Conclusion

With preimplantation genetic testing (PGT) becoming more and more common in clinic nowadays and with the gradual shift in technology towards next generation sequencing (NGS) which has the ability to detect numerical, structural and genetic abnormalities at a low cost, highly automated, high-throughput and improves efficiency and accuracy.

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Conflict of interests

The authors declare that there is no conflict of interest

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