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# Recent advances in preimplantation genetic diagnosis and screening

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**Abstract** Preimplantation genetic diagnosis/screening (PGD/PGS) aims to help couples lower the risks of transmitting genetic defects to their offspring, implantation failure, and/or miscarriage during in vitro fertilization (IVF) cycles. However, it is still being debated with regard to the practicality and diagnostic accuracy of PGD/PGS due to the concern of invasive biopsy and the potential mosaicism of embryos. Recently, several non-invasive and high-throughput assays have been developed to help overcome the challenges encountered in the conventional invasive biopsy and low-throughput analysis in PGD/PGS. In this mini-review, we will summarize the recent progresses of these new methods for PGD/PGS and discuss their potential applications in IVF clinics.

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**Capsule** PGD/PGS has become a routine clinical procedure in many IVF clinics worldwide. New techniques have been quickly adopted as embryo selection strategies in hopes of improving live birth outcomes in human ARTs.

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## Introduction

Preimplantation genetic diagnosis (PGD) is a procedure in infertility clinics whereby cells from oocytes or in vitro fertilized (IVF) embryos are examined for molecular anomalies such as chromosomal abnormalities, mutations in the genomic DNAe, and human leucocyte antigen (HLA) matching [1]. The first PGD/PGS detection of X-chromosome linked diseases was by Handyside et al. in 1990 [2, 3]. Preimplantation genetic screening (PGS) utilizes the methods in PGD to screen IVF embryos for their potential success in uterine implantation to achieve a high pregnancy rate. PGD/PGS are particularly relevant in cases of male infertility, advanced maternal age, and recurrent miscarriage [4]. In addition, PGD/PGS reduces the risk of conceiving a child with genetic disorders and thus lowering the rates of elective pregnancy termination [5, 6]. However, conventional PGD/PGS procedures require the invasive removal of the cells from preimplantation embryos, which may intervene with embryonic development. In this review, we summarize recent technologies in PGD/PGS and highlight the new non-invasive biopsy technologies including time-lapse technology, blastocoele fluid (BF) sampling, and cell-free nucleic-acid collection, as well as new breakthroughs in diagnosis methods. We also discuss the recent and potential clinical application of these new technologies in IVF clinic.

## Recent perspectives on sampling approaches

In PGD/PGS, there are three major biopsy methods: blastocyst biopsy, blastomere biopsy, and polar body (PB) biopsy. Blastocyst biopsy has been more widely used than PB biopsy and blastomere biopsy, especially in the past 5 years, due to its low misdiagnosis rate [7, 8] and cost-effectiveness [9, 10]. Compared to the conventional biopsy methods, the newly developed non-invasive sampling methods have many advantages with regard to the ethical, legal, and economic issues.

### BF sampling

BF is the liquid substance within the blastocyst cavity sealed in by the TE epithelium. Palini et al. showed, for the first time, the presence of genomic DNA in the BF by whole-genome amplification (WGA), quantitative PCR (qPCR), and analyzed multicopy genes such as TSPY1 and TBC1D3. This implies the possibility of using this method in sex-linked disorders screening. [11, 12]. Gianaroli et al. used aCGH to evaluate BF biopsies for ploidy prediction in comparison with the conventional biopsy methods, i.e., blastocyst, blastomere, and PB biopsy, and concluded that BF biopsy was comparable to conventional biopsy materials for chromosomal analysis [13]. Nevertheless, discordance of ploidy prediction was observed between BF and blastocyst biopsy possibly due to the embryo quality [14], suggesting that more experiments and clinical trials should be performed before BF sampling is ready for PGD/PGS.

### Non-invasive technology—cell-free nucleic-acid collection in culture medium and time-lapse technology

Inspired by BF sampling, Assou et al. collected cell-free nucleic acids released from embryos in the culture medium and successfully determined the embryo sex by PCR [15]. Similarly, DNA isolated from media culturing  $\alpha$ -thalassemias<sup>-SEA</sup> carrier embryos had significantly higher diagnosis efficiency compared to blastomere biopsy [16]. However, this method still needs to be further validated by eliminating potential contamination and providing more robust evidences in PGD/PGS.

Another non-invasive technology, time-lapse imaging, was developed to select the best embryos for single-embryo transfer (SET) by correlating cellular morphology and morphokinetic parameters [17, 18]. Combining time-lapse imaging with comparative genomic hybridization (CGH), Chawla et al. found that some significant morphokinetic parameters can be used as markers to predict aneuploidies [19]. Meanwhile, different assessment assays and statistical models were applied to refine the morphokinetic variables and increase the chance of predicting a top-quality blastocyst [20]. However, considering the potential technical and statistical

error in time-lapse imaging, one should be cautious in over-interpreting the results [21–24].

## Advanced techniques of genetic analysis in PGD/PGS

After biopsy, PGD/PGS can be performed at either DNA or chromosome level. Polymerase chain reaction (PCR) is the first technique in PGD and had been developed to detect many genetic abnormalities such as single-gene mutations [3, 25–27], chromosomal imbalances [28], and mitochondrial mutations [29]. Likewise, fluorescence in-situ hybridization (FISH) had been performed to screen aneuploidy and chromosomal translocation for many years [30–36]. However, these two methods become obsolete due to their limitations, e.g., incapability of detecting de-novo genetic mutations, contamination, and sensitivity issues that lead to the false positive or negative. New diagnosis methods, such as array-comparative genomic hybridization (aCGH), single-nucleotide polymorphism (SNP) microarray, multiplex quantitative PCR (qPCR), karyomapping, and next generation screening (NGS) are developed to improve clinical efficiency and outcomes [37–39]. We will elaborate these new methods in detail below.

### Multiplex qPCR

Recently, PCR has been adapted for chromosome copy number analysis [40]. This technique requires a high-order multiplex reaction after pre-amplification is conducted to amplify at least two sequences on each arm of each chromosome for rapid quantification and comparison of each product across the genome within 4–6 h [41, 42]. This qPCR technology has been investigated in PGS and shown improvement in implantation and live birth rates in IVF cycles [41]. It is reliable in determining aneuploidy, but not ideal for detecting structural chromosomal aberrations or uniparental disomy [43].

### Microarray-based methods

Microarray-based CGH has higher resolution, throughput, and speed than conventional CGH [44, 45] and has been successfully adopted in the field of PGD/PGS [46]. With the differential labelled DNA hybridized onto the microarray which contains BAC DNA probes or long oligonucleotides [47], aCGH can assess chromosomal abnormalities such as copy number and unbalanced translocations [48]. It is compatible with sampling methods, i.e., blastocyst biopsy [49–53], blastomere biopsy [37, 46, 48, 52–54], and PB biopsy [54, 55], as well as BF sampling [56]. Most importantly, two independent clinical studies were conducted almost at the same time and both achieved healthy offsprings after PGD by aCGH [48, 57].

SNP array was initially designed for genome-wide association studies (GWAS) before its first application in PGD/PGS in 2011 [58]. With millions of probes covering the whole genome, the SNP array has relatively high resolution in detecting single-gene disorders, balanced or imbalanced translocation [59–61], as well as aneuploidy including triploidy and uniparental disomy [61]. Li et al. utilized a dense SNP microarray that detected approximately 300,000 genetic markers which made them capable of identifying all parental translocation imbalances in embryos [61]. These two array platform-based methods could strongly increase the reliability and stability in PGS, but they still need to be optimized for their future applications.

To expand and improve the diagnosis spectrum of the well-established aCGH and SNP array methods, Handyside et al. first proposed the term “karyomap,” which, in contrast to “karyotype,” identifies the offspring’s SNP genotypes from four possible inherited haplotypes in all the chromosomes and thus reveals potential chromosomal abnormalities, CNVs, and single-gene mutations [62]. In the study of two families carrying cystic fibrosis transmembrane receptor (*CFTR*) mutations, Handyside et al. reported that karyomapping has the power to determine *CFTR* mutation and even distinguish monosomy and uniparental disomy [62]. Karyomapping is compatible with many different biopsy methods such as blastocyst biopsy [62, 63] and blastomere biopsy [62, 64, 65]. It was successfully adapted to a 24-h clinical timeframe in PGD of Marfan syndrome [64] and was performed to screen embryos of the parent with a de-novo mutation related to tuberous sclerosis [66]. The technical improvement resulted in the success of live births in several reports via aCGH, SNP array, and DNA fingerprinting [63–66].

### Next generation sequencing (NGS)

NGS is based on ultra-high throughput parallel DNA sequencing that achieves genome-scale sequencing within days, or even within 24 h. It can detect genetic mutations at single-nucleotide level, with the capacity of detecting aneuploidy such as triploidy and uniparental disomy. Many studies also showed that NGS has become an efficient and robust technology for PGD/PGS [67–69]. For example, one study reported 24 healthy births after NGS testing [70] and another one resulting in a healthy birth to the parents carrying Robertsonian translocation after NGS-based PGD [71]. It has been tested following different biopsy methods in PGD/PGS, e.g., blastocyst biopsy [70, 72], blastomere biopsy [55, 69, 73], and PB biopsy [74]. We expect that it will soon be combined with the new non-invasive sampling methods in the near future. With advent of the third generation sequencing that generates longer, and more accurate single reads in even faster mode, NGS will bring new excitement and revolutionize the clinical application of PGD/PGS.

### The application of single-cell genomics in PGD/PGS

Recently, we have witnessed fast development of single-cell genomics which offers higher resolution for PGD/PGS with limited sample size. Single-cell genomics consists of two major steps: whole-genome amplification (WGA) and high-throughput analysis. WGA aims to amplify DNA from a single cell and generate sufficient template for either microarray or NGS assays. Many WGA methods were developed based on the principles of PCR, including primer extension PCR (PEP) [75], degenerate oligonucleotide primed PCR (DOP-PCR) [76] and multiple displacement amplification (MDA) which employs  $\phi$ 29 DNA polymerase instead of *Taq* DNA polymerase to increase enrichment of genes and reduce PCR bias [77]. However, these WGA methods were limited by the technical obstacle of non-linear amplification. To solve this issue, a new approach called multiple annealing and looping-based amplification cycles (MALBAC) was recently introduced. This approach achieves quasi-linear amplification by initiating the reaction with random primers evenly binding to the template [78]. Thus, MALBAC showed a significant higher coverage of genome than that of the prevailing MDA [78]. Furthermore, the same group successfully examined the crossovers and aneuploidy in single sperm cells by MALBAC [79]. Similarly, Hou et al. also reported MALBAC-based sequencing could simultaneously detect maternal aneuploidy and monogenic disorders of oocytes and polar bodies with higher consistency and resolution than MDA-based aCGH [77]. Though it is not totally free of amplification bias [80], MALBAC-based sequencing is widely used in single-cell genomics tailored for PGD/PGS [81, 82] and shown to be highly sensitive, specific, and reproducible [77–79, 83].

Single-cell genomics has been applied to comprehensively study the genome and transcriptome of individual cells to select an optimal embryo in IVF. Since Tang et al.’s seminal work [84], single-cell RNA sequencing (RNA-seq) has been developed, by many groups including our labs [82, 84–87], to study the transcriptional regulation of IVF embryos at the single-cell level. Combined with exome sequencing of parental genotype, we showed that single-cell RNA-seq was capable of uncovering monoallelic expression patterns and screening for single-nucleotide variants (SNVs), which would be useful for future PGS/PGD [87]. More recently, Dey et al. reported the method of conducting genome DNA and mRNA sequencing simultaneously in single cells and discovered the transcriptional variation related to copy number variations (CNVs) [88]. The so-called gDNA-mRNA sequencing (DR-seq) showed consistent results with genomic DNA sequencing by MALBAC-seq and RNA-seq via Cell Expression by Linear amplification and sequencing (CEL-seq). Meanwhile, Macaulay et al. developed another new approach called genome and transcriptome sequencing (G&T-seq) which can detect both genetic alteration and subsequent

perturbation in transcriptional regulation [89]. Together, these new techniques offer promising tools for detecting the genetic variants and elucidating the regulatory mechanisms in preimplantation embryos, therefore, improving the quality of PGD/PGS. The successful use of NGS-based PGD/PGS in IVF clinic is reported in 2014 in China after MALBAC-based NGS PGD/PGS [90]. Yan et al. reported that an NGS-based PGD/PGS procedure simultaneously detected a single-gene disorder and aneuploidy by low coverage whole-genome sequencing for euploidy validation and targeted single allele deep-sequencing of amplicons [91].

## Summary

Over the years, PGD/PGS has been increasingly performed in IVF clinic, which helps thousands of patients give healthy births. With the fast development in biotechnologies, new methods will reduce adverse impact on embryo development and increase the accuracy and efficiency of PGD/PGS. As discussed, blastocyst biopsy method is significantly improved and considered as the best choice among the three conventional biopsy methods, i.e., blastocyst, blastomere, and PB biopsy. Meanwhile, several new non-invasive sampling methods were introduced into the field, including time-lapse imaging technology, BF sampling, and cell-free nucleic-acid collection. However, these methods inevitably have limitations despite their advantages and would require further examination and validation for their clinical use. On the other hand, state-of-the-art technologies including aCGH, SNP array, and NGS analyzing genetic materials have revolutionized PGS/PGD in the post-genomic era. Because of the quick emergence of new technologies, there are still very limited randomized controlled trials (RCTs) to evaluate the clinical efficacy such as implantation and pregnancy rates [4]. So the level of evidence should be further thoroughly assessed before the application of these new technologies. Nevertheless, we anticipate that the combination of non-invasive sampling and powerful genomic analysis will bring PGD/PGS to higher levels in the near future.

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