Preimplantation Genetic Diagnosis
Current status and future perspectives

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Introduction

Preimplantation genetic diagnosis (PGD) is the procedure for genetically analyzing embryos, after an in-vitro fertilization (IVF), in order to select the healthy ones for uterine transfer. PGD is a current option for couples with a risk of transmitting genetic disorders or to improve the chance of conception. The development of some embryo manipulation procedures are facilitating the utilization of PGD techniques (e.g.: ICSI, cryopreservation or vitrification, embryo biopsies…). Although the current methods for PGD, including PCR and FISH, have been widely used to effectively diagnose many genetic disorders, they have limitations in characterizing certain types of genetic conditions and cannot provide a genome-wide approach. Whole genome amplification (WGA) techniques have provided the adoption of microarrays for a genomic assessment in PGD/PGS. Nevertheless, advances in DNA sequencing are suggesting the possibility to finally introduce next-generation sequencing (NGS) technologies in the PGD/PGS programs in a near future.

Acronyms: PGD, preimplantation genetic diagnosis; PGS, preimplantation genetic screening; IVF, in vitro fertilitsation; ICSI, intracytoplasmic sperm injection; PCR, polymerase chain reaction; FISH, fluorescent in situ hybridization, WGA, whole genome amplification; NGS, next-generation sequencing; CNV, copy number variation; ADO, allele drop out; LOH, loss of heterozygosity; SNP, single nucleotide polymorphism.

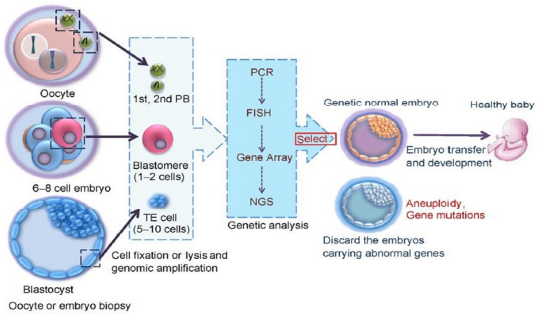
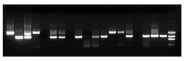


Figure 1. PGD procedures from three different embryo development stages. Yan, Li Ying et al. (2014)

Classical techniques

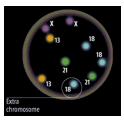
⊖ **Family-specific design → time consuming** ⊖ **Not *de novo*** ⊖ **Only targeted, not genomic analysis**

PCR



- ⊕ Detection of single-gene disorders
- ⊕ Aneuploidy screening and CNV (by qPCR)
- ⊕ Causal mutations (+ linkage markers)
- ⊕ Better outcome by multiplex PCR with WGA
- ⊖ Risk of errors by ADO or homologous recombination
- ⊖ Risk of contamination

FISH



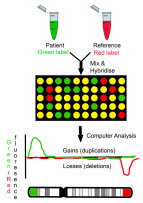
- ⊕ CNV and aneuploidy screening (few chromosomes)
- ⊕ Sex selection for X-linked diseases
- ⊖ Few coloured probes
- ⊖ Not for balanced rearrangements
- ⊖ Difficulties with multiple complex rearrangements
- ⊖ Not useful with single blastomeres

Microarrays

- ⊕ **Genome-wide analysis for aneuploidy screening and CNV**
- ⊕ **WGA allows PGD by microarrays**
- ⊕ **High resolution, rapid and simple procedure**
- ⊖ **Not mitochondrial**
- ⊖ **Not *de novo* base mutation**

aCGH

- ⊖ Risk of bias due to WGA artifacts (ADO, chimeric DNA molecules)



SNP-array

- ⊕ Genotype millions of known SNPs
- ⊕ Reduce risk of bias due to WGA artifacts
- ⊕ Detects LOH
- ⊕ Reconstruct haplotypes for carriers of single-gene or balanced rearrangements (by linked SNPs in the family)
- ⊖ Available genotype from parents and a close relative needed

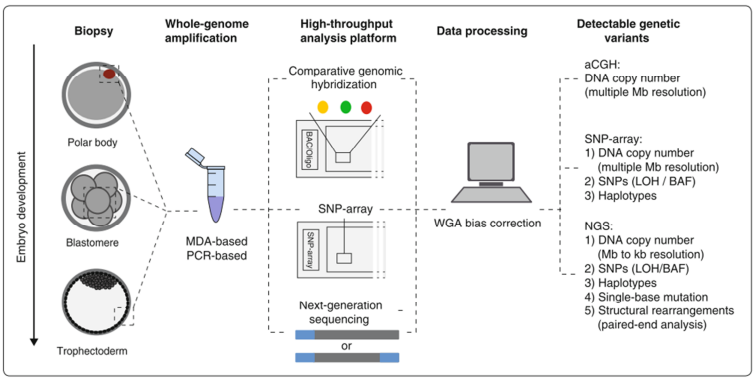


Figure 2. WGA is required before any genome-wide genetic analysis in PGD. There are still bias due to incomplete coverage, GC bias, chimeric DNA molecules, ADOs, preferential allelic amplifications and nucleotide copy errors. No single WGA method delivers an unbiased representation of a cell's genome or is best across all criteria and subsequent applications. Van der Aa, Niels et al. (2013)

Indications	PCR	FISH	aCGH	SNP-array	NGS
Sex selection (social or X-linked)	Yes	Yes*	Yes	Yes	Yes
Aneuploidy screening	Yes	Yes*; locus specific	Yes*; generic	Yes; generic	Yes; generic
DNA copy-number aberrations	Yes	Yes*; locus specific	Yes*; generic	Yes; generic	Yes; generic
Carriership of balanced chromosome rearrangements	No	No	No	Yes	Yes
Single-gene disorder	Yes*; family specific	No	No	Yes; generic	Yes; generic
De novo segmental copy-number aberrations	No	No	Yes; generic	Yes; generic	Yes; generic
De novo base mutations	No	No	No	No	Yes; generic
Mitochondrial mutations	Yes*; family specific	No	No	No	Yes

Table 1. Genetic conditions that can be diagnosed by each methodology. The current methodology in common practice is marked with an asterisk.

Next-Generation Sequencing

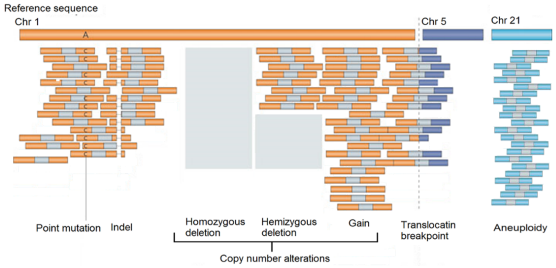


Figure 3. Types of genome alterations detectable by second-generation sequencing. Edited from Meverson et al. (2010). Nature Reviews Genetics.

Advantages

- Characterize entire genomes for full spectrum of genetic variants in a single experiment
- Any type of *de novo* mutation (including balanced rearrangements)
- Higher resolution, accuracy and reliability
- Interrogating almost every nucleotide amplified
- Digital precision (not relying on fluorescent intensities)
- Massive sequence data

Challenges

- **Reduction in sequencing costs**
- Improvements in WGA techniques to avoid bias by artifacts and providing reliable complete genome coverage
- Further validation studies for base calling and alignment accuracy
- Interpretation of the massive sequence data (e.g.: to distinguish background polymorphisms from disease-causing)
- Defining pertinent ethical guide-lines

Second-generation NGS methods

Template preparation (DNA, cDNA) → Massively parallel clonal amplification → Sequencing and alignment of short reads

Third-generation

Advances in 3G will offer:

- Higher throughput
- Longer read lengths
- Higher accuracy
- Less starting DNA
- Faster turnaround time
- Lower cost

Protocols favoring NGS

- Multiplex barcode sequencing (Bar-seq): Analyze multiple samples in a single run, even from different patients and for different analysis requirements.
- Small affordable instruments for low-throughput applications, requiring low-coverage and focused on target sequencing.
- Trophectoderm biopsy: Increases the number of cells and DNA. Reduces misdiagnosis rate (e.g.: by mosaicism) and number of samples to analyze.

Conclusions

Classical methods for PGD will be gradually replaced by the introducion of affordable tecniques that allow a thorough genome-wide analysis for almost every type of genetic alteration. Although NGS techniques are subject to constant ongoing refinements, the sequncing cost has drastically decreased and they show many advantages over PCR, FISH and microarray-base protocols, before its introduction in PGD some challenges must be overcome. Further advances in NGS technologies, coupled with improved embryo manipulation and DNA amplification methods, and the development of new affordable platforms and finding new ways to reduce costs, will favor the adoption of NGS in PGD/PGS programs in a near future.

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