Genome Medicine

REVIEW

Preimplantation genetic diagnosis guided by single-cell genomics

Niels Van der Aa[1], Masoud Zamani Esteki[1], Joris R Vermeesch[2] and Thierry Voet[1,3]*

Abstract

Preimplantation genetic diagnosis (PGD) aims to help couples with heritable genetic disorders to avoid the birth of diseased offspring or the recurrence of loss of conception. Following *in vitro* fertilization, one or a few cells are biopsied from each human preimplantation embryo for genetic testing, allowing diagnosis and selection of healthy embryos for uterine transfer. Although classical methods, including single-cell PCR and fluorescent *in situ* hybridization, enable PGD for many genetic disorders, they have limitations. They often require family-specific designs and can be labor intensive, resulting in long waiting lists. Furthermore, certain types of genetic anomalies are not easy to diagnose using these classical approaches, and healthy offspring carrying the parental mutant allele(s) can result. Recently, state-of-the-art methods for single-cell genomics have flourished, which may overcome the limitations associated with classical PGD, and these underpin the development of generic assays for PGD that enable selection of embryos not only for the familial genetic disorder in question, but also for various other genetic aberrations and traits at once. Here, we discuss the latest single-cell genomics methodologies based on DNA microarrays, single-nucleotide polymorphism arrays or next-generation sequence analysis. We focus on their strengths, their validation status, their weaknesses and the challenges for implementing them in PGD.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD), based on testing of oocytes or preimplantation stage embryos for genetic abnormalities following *in vitro* fertilization (IVF), was introduced over two decades ago by pioneering groups like the ones of Yury Verlinsky [1,2] and Alan Handyside [3]. Driven by technology, the use of PGD has grown immensely and increases annually, with more than 50,000 cycles performed worldwide, and more than 10,000 babies born thus far [4]. Specifically, PGD is offered to couples to avoid the transmission of heritable genetic disorders to their offspring or to increase their chances of a successful pregnancy. The main indications for PGD are Mendelian disorders, such as highly penetrant, often life-threatening autosomal dominant or recessive diseases; X-linked recessive diseases; and chromosomal copy-number aberrations that result from meiotic missegregation of a balanced chromosomal rearrangement present in a parent. The latter may in turn lead to recurrent miscarriage or severely disabled offspring resulting from segmental aneusomies [5,6]. A distinctive form of PGD, commonly referred to as preimplantation genetic diagnosis for aneuploidy screening (PGD-AS) or PGS, was tailored to help couples who have normal karyotypes but are burdened with fertility problems due to advanced maternal age, recurrent miscarriage, recurrent implantation failure or severe male factor infertility. PGS aims to detect *de novo* meiotic aneuploidies in IVF conceptions to increase the rate of successful pregnancy. Figure 1 summarizes the contribution of each of these indications to the number of PGD cycles over the past 10 years, as reported by the European Society of Human Reproduction and Embryology PGD consortium [6].

In brief, each PGD cycle starts with a hormonal ovarian stimulation followed by ultrasound-guided oocyte aspiration and IVF. Because embryos in the preimplantation stage consist of a very limited number of cells, diagnosis has to be performed by genetic testing of just a single or a few biopsied embryonic cells (Figure 2). The major approach for PGD today involves biopsy of one or two blastomeres from a 6- to 8-cell human cleavage-stage embryo on day 3 after IVF. The biopsied embryos are further cultured *in vitro,* and the biopsied cell is diagnosed by single-cell PCR or single-nucleus fluorescent *in situ* hybridization (FISH)-based assays targeted at the genetic risk alleles [5]. Table 1 gives an overview of

*Correspondence: Thierry.Voet@med.kuleuven.be
[1]Laboratory of Reproductive Genomics, Department of Human Genetics, KU Leuven, Leuven 3000, Belgium
Full list of author information is available at the end of the article

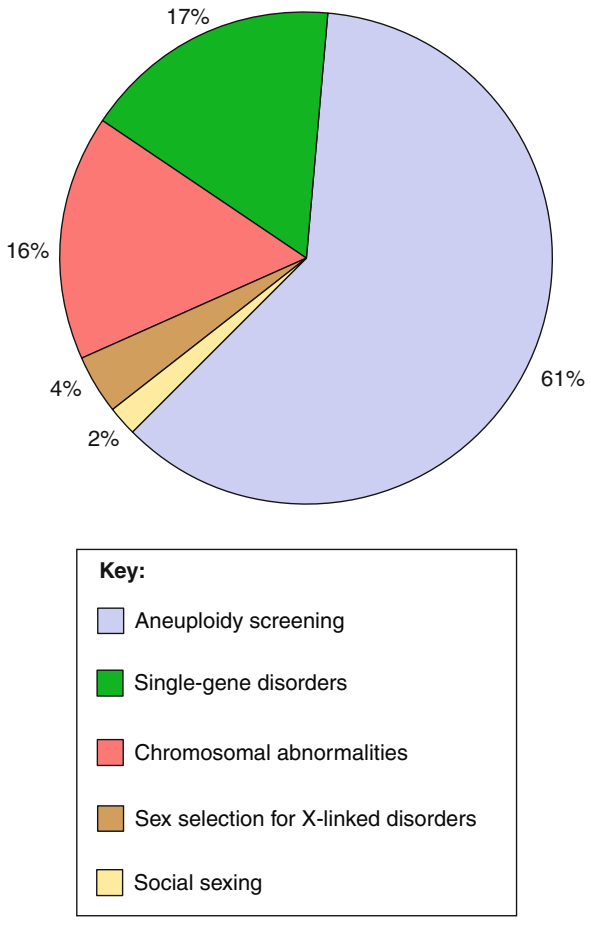

Figure 1. Indications for preimplantation genetic diagnosis. Numbers of PGD cycles reported to the European Society of Human Reproduction and Embryology PGD consortium during the first 10 years of data collection for different conditions [6]. 'Social sexing' refers to determination of the embryo's sex for social rather than medical reasons.

established and imminent methodologies (see below) and their capacity to detect various genetic anomalies.

Following diagnosis, one or more healthy embryos are selected for transfer to the uterus on day 4 or 5 of the same IVF-PGD cycle, before the embryo would naturally implant into the uterine wall. Hence, only a narrow, approximately 48 hour time frame is available for diagnosis after blastomere biopsy. The average successful pregnancy rate per embryo transferred following diagnosis reaches 30% [5]. Alternative time points for cell biopsy include the aspiration of the first and second polar body on day 0 (mature oocyte) or day 1 (zygote) after fertilization, and the biopsy of trophectoderm cells at the blastocyst stage, which requires embryo freezing and thawing following diagnosis (see below).

Here, we discuss the limitations of the classical approaches for PGD and how recent genome-wide methods for single-cell genomics can revolutionize PGD. Imminent methods will allow PGD to go far beyond what is currently feasible (Table 1, Figure 2).

Classical techniques for PGD and their limitations

Although established methods for PGD, including single-cell PCR and single-nucleus FISH, are very effective for diagnosis, these classical approaches do have limitations.

Single-cell PCR assays, performed either directly on the cell's DNA or after single-cell whole-genome amplification (WGA), are generally used to diagnose heritable single-gene disorders. In these assays, preferably the causal genetic mutation, along with a minimum of two linked polymorphic markers, are genotyped and phased [7]. This is needed to control for putative drop out of the mutant allele, which represents allele drop out (ADO) or the random failure of amplification of one allele at a locus. Consequently, these assays have to cope with several issues: identifying informative markers near the mutation; optimizing the multiplex format of the single-cell PCRs to minimize ADO; and avoiding misdiagnosis resulting from a possible disconnection of linkage between the mutation and a linked polymorphic marker allele caused by an intervening homologous recombination. Hence, these PCR methods require family-specific designs, resulting in time-consuming preparations, and can be performed only in specialized centers that have accumulated the staff required to design and pilot the diagnostic single-cell PCR tests. Recent state-of-the-art (single-cell) PCR designs also enable PGD for mitochondrial diseases [8-10], aneusomies [11] and aneuploidies [12] (Table 1).

FISH on single nuclei is mainly used to diagnose either heritable DNA copy-number aberrations or sex in cases of X-linked recessive Mendelian disorders with unknown causal mutation (Table 1). As with PCR, a major limitation is that FISH assays are also locus specific, often requiring a labor-intensive and family-specific design. Furthermore, not all couples are easily helped using the current FISH strategies. For instance, couples burdened with a complex chromosomal rearrangement in one of the partners are subfertile or are at risk of having disabled offspring because a high fraction of the gametes will be chromosomally unbalanced. Given the limited amount of differentially colored probes that can be applied in a single FISH round, the inherent degradation of DNA in subsequent FISH rounds and the limited time frame available for diagnosis (less than 48 hours after blastomere biopsy in cleavage stage), current FISH-based PGD protocols are often inadequate for supporting these couples. Furthermore, because current FISH-based PGD protocols only detect locus-specific copy-number states and do not analyze the breakpoints of the chromosomes involved in the rearrangement, the presence of a balanced

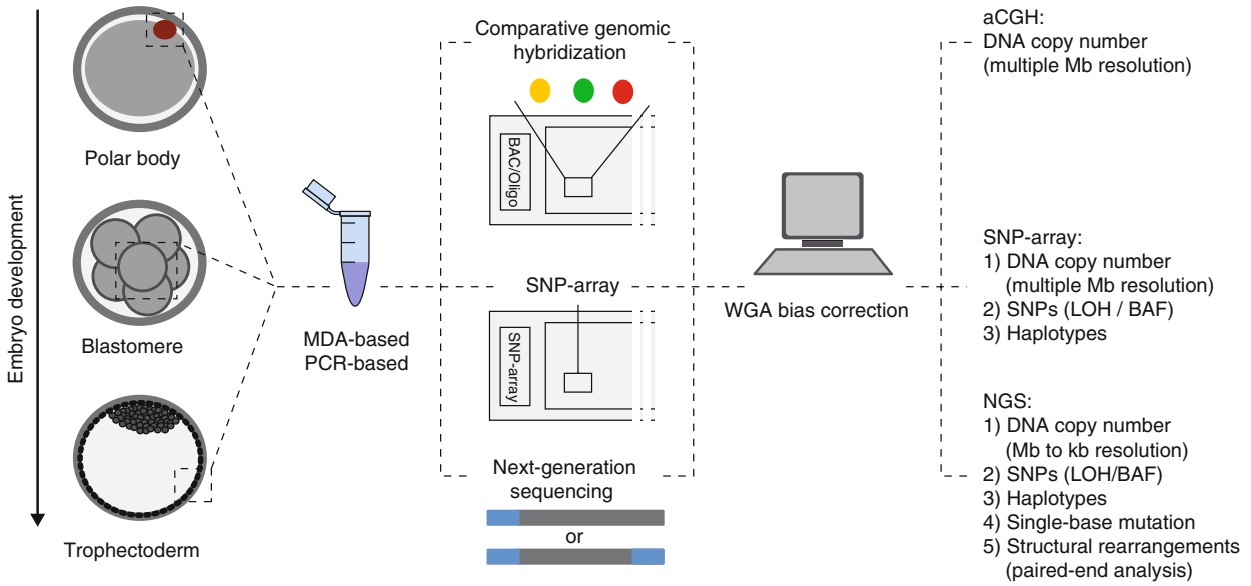

Figure 2. Single-cell genomics pipelines for PGD. After biopsy, the whole genome is amplified (WGA) and analyzed on a high-throughput platform by microarray comparative genomic hybridization and analysis, SNP-array analysis or massively parallel sequence analysis. All single-cell data need to be normalized and interpreted against a background of WGA error (such as GC bias). Every platform has its resolution limits and allows the detection of different types of genetic variants, as indicated on the right. aCGH, array comparative genomic hybridization; BAF, SNP B-allele fraction; LOH, loss of heterozygosity; MDA, multiple displacement amplification; NGS, next-generation sequencing; SNP, single-nucleotide polymorphism.

configuration of the rearranged chromosomes cannot be discriminated from the presence of a balanced set of normal chromosomes by ordinary FISH analysis of the blastomere's nucleus. Hence, current routine FISH methods can result in offspring that carries the same risk alleles as their parents. Last but not least, neither FISH nor PCR strategies are capable of detecting *de novo* abnormalities genome-wide.

Besides screening for heritable genetic aberrations, FISH-based PGS has been widely applied to increase the live birth rate for couples with normal karyotypes but infertility problems. The decreasing fecundity of older women associated with age-dependent aneuploidy in the oocyte, as well as the knowledge that the majority of chromosomal errors cause embryonic lethality, led to the hypothesis that selecting chromosomally normal embryos for uterine transfer would increase the live birth rate per embryo transferred. Although the hypothesis was conceptually sound, it is now apparent that there is no clinical benefit of applying FISH-based PGS to individual blastomeres biopsied from human embryos on day 3 following IVF [13-15]. This is because many human cleavage-stage embryos acquire *de novo* numerical chromosomal alterations during the first mitotic cell divisions following fertilization, implying that the DNA copy number of a chromosome assessed in the biopsied blastomere is not simply a reflection of the copy-number state of the same chromosome in all blastomeres remaining in the embryo [13]. It remains to be thoroughly investigated whether PGS using non-FISH genome-wide approaches might improve live birth rates per embryo transferred when the test is performed at another developmental stage, either earlier following polar body biopsy or later following trophectoderm biopsy [16].

High-resolution genome-wide approaches for genome analysis

Recently, state-of-the-art single-cell whole-genome approaches have been developed that could revolutionize PGD practice. Below we discuss these technologies and their potential application for PGD (Table 1 gives an overview). Following cell isolation, the genomes of the biopsied cells are commonly amplified to enable the genome-wide genetic test (Figure 2).

Single-cell isolation

Most PGD cycles are performed on one or two blastomeres biopsied from a day-3 human cleavage-stage embryo. The embryo is immobilized with a holding pipette, a hole is made in the zona pellucida using either

Table 1. Current and imminent technologies for preimplantation genetic diagnosis

Indications	PCR	FISH	aCGH	SNP array	NGS
Sex selection (social or X-linked disease)	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; generic

The genetic conditions that can be diagnosed by each methodology are indicated, with the current methodology in common practice marked with an asterisk.
aCGH, array comparative genomic hybridization; FISH, fluorescent *in situ* hybridization; NGS, next-generation sequencing; SNP, single-nucleotide polymorphism.

chemicals (for example, by applying acidic Tyrode's solution) or lasers [17] and one or more cells are then biopsied by aspiration. Recently, however, it has been suggested that biopsy of cells at other developmental stages may be less deleterious for the early embryo (Figure 2). Biopsy of the developmentally inert polar bodies at the stage of the mature oocyte or one-cell zygote just after fertilization might be less damaging to the developing embryo, but allows screening only for abnormalities present in the maternal genome [18-20]. Furthermore, advances in embryo culture and cryopreservation techniques allow the biopsy of a number of trophectoderm cells from a day 4 or 5 blastocyst for PGD, because embryo vitrification enables development to be halted for the time needed for analysis of the biopsy. Following diagnosis, suitable preimplantation end-stage embryos are thawed and transferred to the uterus at a receptive stage of the woman's natural, or hormone-supplemented, menstrual cycle [19,21-23].

Whole-genome amplification methods

Given that one diploid human cell contains only about 7 pg DNA and that modern genomics methodologies require hundreds of nanograms of input DNA, the genome(s) of the biopsied cell(s) must first be amplified thousands of times to allow genome-wide analysis. Given that the product of the WGA will ultimately produce the signals on the genomics platform for interpretation and diagnosis (Figure 2), it is crucial to understand the working and the various imperfections of the different WGA methods [24]. Recent WGA protocols are based on multiple displacement amplification (MDA), PCR or a combination thereof.

In MDA-based protocols, primers first randomly anneal to a denatured single-cell DNA template. Subsequently, a DNA polymerase with strong strand displacement capacity copies the genome many times in an isothermal reaction at 30°C [25]. When the 3' end of an extending fragment reaches the 5' end of a neighboring primed chain, it will displace this neighboring strand, liberating single-stranded DNA for new primer annealing and DNA synthesis [25]. The most commonly used enzyme for MDA is the bacteriophage ϕ29 DNA polymerase, which can generate nucleotide chains over 10 kb in size and has a strong proof-reading capacity, ensuring faithful nucleotide copying during WGA [26]. MDA WGA products usually cover the majority of the human genome and seem to be a preferred method for single-nucleotide polymorphism (SNP) genotyping [27,28] or base-mutation detection [29,30]. However, single-cell copy-number profiles following MDA can be significantly distorted [27,28], ADOs do occur, and a large number of chimeric DNA-amplification products that distort the cell's original genomic architecture are created during WGA [28,31].

PCR WGA methods can be based on linker adaptor PCR (LA-PCR), in which specific adaptors are ligated to fragmented or restriction digested single-cell template DNA prior to PCR amplification. Commercial forms of LA-PCR are available from Sigma-Aldrich (GenomePlex), Rubicon Genomics (ThruPLEX) and Silicon BioSystems (Ampli1) and rely on efficient fragmentation of a cell's genome, proficient adaptor ligation and subsequent PCR. Alternatively, primer extension pre-amplification (PEP)-PCR [32] and degenerate oligonucleotide-primed (DOP)-PCR [33] methods have been developed, as well as WGA methods that combine features of MDA- and PCR-based amplification. PicoPlex [34] (Rubicon Genomics; marketed as SurePlex by BlueGnome) uses MDA-based pre-amplification of the single-cell DNA template followed by PCR-based amplification of the new templates. The latest method, multiple annealing and looping based amplification cycles (MALBAC) [35], initiates with multiple rounds of displacement amplification using a specific primer design to form looped pre-amplification products of a cell's template DNA, which are then amplified exponentially by PCR. In general, the PCR-based WGA products deliver a more accurate copy-number profile

[27,28] and can also be used for SNP genotyping and base-mutation detection [35].

Importantly, however, no WGA method delivers an unbiased representation of a cell's genome. The breadth of genomic coverage, GC bias, chimeric DNA molecules, ADOs, preferential allelic amplifications and nucleotide copy errors can vary significantly between different WGA approaches, making some WGA methods better suited than others for specific genetic tests [27,28,35]. MDA and PicoPlex are currently the predominant methods used for PGD; however, a comprehensive study of all (dis)advantages of the various WGA methods for detecting the different classes of genetic variants in single cells using genome-wide platforms is needed.

Single-cell array comparative genomic hybridization for preimplantation genetic diagnosis

Single-cell array comparative genomic hybridization (aCGH) offers the ability to screen all 24 chromosomes of a biopsied blastomere simultaneously for aneuploidy and at high resolution for segmental DNA copy-number aberrations, thereby surpassing FISH, which is limited to a few loci at a time (Table 1). In general, aCGH is based on the hybridization of a fluorescently labeled test DNA sample against a differentially labeled reference DNA sample with known karyotype onto DNA microarrays (Figure 2). The latter can contain thousands to millions of DNA spots (bacterial artificial chromosomes or oligonucleotides), each probing for the abundance of short unique genomic loci in the hybridized samples. Following analysis of the hybridization signals, numerical chromosomal aberrations can be discovered in the test sample. DNA copy-number aberrations as small as about 2.5 Mb have been detected in a single cell following PCR-based WGA and aCGH analysis [36,37].

Although single-cell aCGH has several advantages over FISH, it also comes with its own set of difficulties. The major challenge in copy-number profiling a single cell by aCGH remains a coalescence of artifacts in the WGA product. Not only ADO and preferential allelic amplifications, but also chimeric DNA molecules may bias the fluorescent signal on the probe in favor of one or the other allele. In addition, locus-specific amplification yields can depend on genomic features such as the richness of the locus in guanine and cytosine bases. Furthermore, the cell-cycle stage of the isolated cell can complicate the analysis as cells in S phase can have copy numbers of 2, 3 or 4 for a diploid locus, leading to false structural DNA-imbalance discoveries [38]. In human cleavage-stage embryos, multiple blastomeres may reside in S phase at the time of biopsy [39]. As a consequence of this cocktail of WGA artifacts and possibly also biologically determined noise, the standard deviation of fluorescent intensity ratio signals on probes interrogating consecutive domains in the genome will be significantly higher than that found in a similar analysis of a non-amplified DNA test sample extracted from many cells. This affects the sensitivity and specificity of algorithms for detecting DNA copy-number changes in the single-cell WGA product. WGA biases over longer distances may be misinterpreted as genuine copy-number changes. To enable reliable detection of copy-number variants in single-cell genomes, custom data analyses have been developed [36,37,40-44] (Figure 2).

Following the shortening of the aCGH protocol to less than 24 hours and the development of sophisticated algorithms that interpret the single-cell WGA probe signals, aCGH methods can now be used for PGD [36,44,45]. Alfarawati *et al.* [44] reported the first live births after PGD for DNA copy-number aberrations resulting from the inheritance of an unbalanced configuration of chromosomes involved in a balanced reciprocal translocation in one of the parents. Using 24Sure aCGH-based chromosome screening (BlueGnome) of single blastomeres biopsied from human cleavage-stage embryos after IVF (with an approximately 90% success rate), they were able to select multiple embryos for transfer to the woman's uterus because the biopsied cell had a balanced chromosome configuration following aCGH [44]. Some of the transferred embryos subsequently resulted in healthy babies. Fiorentino *et al.* [36] applied single-blastomere aCGH for PGD to 24 couples carrying 18 different balanced translocations. Of 200 embryos analyzed, 93.5% were successfully diagnosed. A clinical pregnancy rate of 70.6% per embryo transfer was achieved, leading to three successful deliveries and another nine ongoing pregnancies [36]. Vanneste *et al.* [45] applied aCGH to blastomeres biopsied from day-3 embryos derived from a couple of whom the male partner had a karyotype 46,XY,ins(3;2)(p23;q23q14.2),t(6;14)(p12.2;q13). Embryos that were normal or balanced for both the insertion and translocation could be identified, but no live birth was obtained following transfer [45]. Building on these successful validations of single-cell aCGH for PGD, the technology is mature for routine application in PGD. Furthermore, extensive studies are ongoing to validate the clinical utility of aCGH for PGS on polar-body biopsies [16,46] and trophectoderm biopsies [16].

Single-cell SNP-array analyses, a route to a generic method for PGD

Standard SNP-array platforms allow genotyping of hundreds of thousands to millions of SNPs known in the population at once in a DNA sample. Furthermore, the signals for each SNP allele can be exploited for the computation of DNA copy-number states (Figure 2). Like aCGH, SNP arrays thus offer a genome-wide method to

screen for DNA copy-number aberrations, but in addition allow integration of single-cell DNA copy-number calls with their SNP genotypes. This property can be exploited to differentiate a genuine DNA imbalance from likely WGA artifacts [39,47-49], which is a notable advantage over aCGH platforms [24]. For instance, a real deletion of a diploid locus will demonstrate loss of heterozygosity (LOH), whereas the locus delineated by a false deletion due to, for example, inefficient amplification of the region will still show signals for both alleles. In addition, ADO is random and not expected to act on each heterozygous SNP across a large region. Furthermore, SNP arrays allow detection of copy-neutral loss of heterozygosity, indicating uniparental isodisomy in single cells, and determination of the parental origin of DNA anomalies [49,50].

The number of SNPs called and the accuracy of the SNP genotype and copy number differ significantly between different WGA methods. Treff *et al*. [27] tested two MDA-based WGA methods, QIAgen's REPLI-g and GE Healthcare's GenomiPhi, as well as one PCR-based WGA method (GenomePlex) on cultured cells. The MDA-based REPLI-g WGA method allowed the most SNPs to be genotyped and attained the highest genotype accuracy. On the other hand, single-cell SNP copy numbers were most accurate after the PCR-based GenomePlex WGA method.SNP-array platforms and customized computational analyses have been evaluated for diagnosing copy-number aberrations in individual blastomeres, and time-consuming protocols have been reduced to 24 hours [47]. Various groups have shown that hybridizing single-blastomere WGA products on high-density SNP arrays allows reliable calling of chromosome aneuploidies using DNA copy-number and genotype information [39,47,51,52]. For instance, van Uum e*t al.* [50] detected successfully unbalanced translocations encompassing segments down to 5 Mb in size.

Importantly, single-cell SNP genotypes can also be used to reconstruct the haplotypes of the entire cell's genome and thus, in theory, to perform PGD. Haplotypes carrying a disease allele can be inferred from a single-blastomere SNP genotype, on condition that the SNP genotypes from the parents and a close relative, typically a sibling, are available. If the latter is afflicted with a disease of which the locus is mapped and the parental risk allele(s) can be defined, single-blastomere haplotyping in turn allows tracking of the inheritance of the genetic (non-)risk allele(s) transmitted by the parents in the biopsied embryos. Hence, single-cell genome-wide SNP-haplotyping methods provide a stepping stone to a generic alternative for PGD for indications currently diagnosed by either PCR or FISH. In an elegant approach called karyomapping, Handyside *et al.* [53] phased single-cell SNP genotypes for which parental and sibling genotypes were available. To minimize errors due to ADO, karyomapping considers only the informative SNPs that are heterozygous in a cell's genotype following SNP-array analysis. The method was able to pinpoint recombination sites between parental homologous chromosomes and inherited DNA mutations could be correctly inferred from the reconstructed patchwork of haplotype blocks, despite the fact that the causal mutation is not genotyped directly in the cell. They further tested the methodology on a family in which both parents were carriers of a deletion of the nucleotides encoding the codon Phe508 in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Following conventional PGD, five preimplantation embryos that were not selected for transfer were used to evaluate karyomapping. The original PGD result could be replicated by interpreting the haplotype blocks inferred in the embryo biopsies. In addition, karyomapping allowed the detection of chromosome aneuploidies and their parental origin.

Single-cell genome sequencing, a route to novel forms of PGD

With the introduction of second-generation sequencing technologies, also known as next-generation sequencing (NGS), it became feasible to characterize entire genomes for the full spectrum of genetic variants in a single experiment. In the context of PGD, this may not only enable a generic method for all possible PGD indications, but also to diagnose each embryo individually for any class of *de novo* DNA mutation.

For sequencing a cell, a single-cell WGA product is shattered into smaller DNA molecules from which a library of DNA templates for massively parallel sequencing is generated. The resulting short sequence reads, typically up to 100 bases, from one end or both ends of each DNA molecule, termed single-end or paired-end sequencing, respectively, are mapped to the human reference genome for detecting genetic variants using computational methods.

NGS has several important advantages over DNA microarrays that can improve the resolution, accuracy and reliability of variant calls [24]. First, by NGS one can interrogate almost every nucleotide amplified by the WGA method, whereas microarrays examine only certain loci of a single-cell WGA product defined by the physical positions of the probes. Whereas SNP arrays can probe for only a limited number of SNPs known in the population, sequencing allows *de novo* discovery of the full spectrum of DNA mutations genome-wide. Second, instead of relying on probe-specific fluorescent intensities, single-cell WGA products can now be characterized at unprecedented digital precision in terms of genomic breadth and depth, with one digital unit

representing a mapped sequence read. Third, paired-end sequencing and mapping can unveil the linkage between both ends of each interrogated linear DNA molecule of a single-cell WGA product. This allows discovery of signatures for all types of structural variants in a genome, from various intrachromosomal to interchromosomal rearrangements, simply by interpreting read pairs that map discordantly to the reference genome.

Despite these advantages, the interpretation of single-cell sequencing data remains complicated [24]. Uneven amplification of one locus compared with another still has to be discriminated from genuine submicroscopic copy-number variants. Similarly, chimeric DNA molecules created by WGA have to be distinguished from genuine structural variants in the cell following paired-end sequence analysis. And nucleotide WGA copying errors may be falsely interpreted as true nucleotide changes in the single-cell genome. Nevertheless, several groups have demonstrated the efficacy of single-cell NGS to detect different classes of mutation.

Navin and colleagues [54,55] showed that low-coverage single-end sequencing after PCR-based WGA enabled profiling a cell's DNA copy-number landscape at a resolution unattainable with even the highest-resolution array approaches available [37,56]. They developed a methodology using focal read depth analysis, in which the number of single-end reads mapping uniquely to specific bins across the human reference genome is counted and transformed to a copy-number state per bin. Similar approaches were applied by others using single-cell MDA- or PCR-based WGA products [35,57,58]. Furthermore, approaches for detecting structural variation in a solitary cell by paired-end sequencing of single-cell MDA- or PCR-based WGA products were developed [28].

To investigate subclonal single-nucleotide mutations in cancer cells, Xu *et al.* [30] and Hou *et al.* [29] captured all DNA molecules encompassing exons, representing just over 1% of a human genome, from a sequencing library derived from a single tumor cell following MDA WGA. However, data from a minimum of three cells were required to deliver reliable nucleotide variant calls because of WGA and sequencing errors [30]. Zong *et al.* [35] required data from multiple MALBAC-amplified single cells to detect reliable nucleotide variants. In contrast to diploid or multiploid cells [29,30,35,59], WGA products of single haploid cells can be used for *de novo* mutation detection [58]. In these cases WGA nucleotide copy errors can be discriminated from true base variants in the cell, because no heterozygous base variants are expected for unique loci in a haploid cell, offering interesting routes to profiling polar bodies.

Single-blastomere sequencing for clinical practice has not yet been reported. Nevertheless, NGS of single blastomeres biopsied from human embryos following IVF allows the detection of not only inherited, but also *de novo* submicroscopic DNA copy-number aberrations, and even to fine-map an inherited interchromosomal rearrangement by identifying the discordantly mapping read-pairs spanning the translocation breakpoint [28]. These data hint that single-blastomere sequencing for PGD applications may soon become reality. Furthermore, two research groups recently reported the validation of NGS methods applied on multi-cell trophectoderm biopsies for PGD of IVF embryos. Yin *et al.* [60] demonstrated that low-coverage single-end genome sequencing of WGA products derived from trophectoderm biopsies can be used to detect DNA copy-number aberrations. Treff *et al.* [61] used targeted deep sequencing of PCR amplicons obtained from pools of five lymphocytes and from multi-cell trophectoderm biopsies not subjected to WGA. The amplicons encompassed nucleotide substitutions or indels anticipated through inheritance. Their NGS results proved to be fully concordant with the traditional PGD [61].

Conclusions and future perspectives

Single-cell genomics is remodeling PGD. Rapid single-cell aCGH- and SNP-array protocols enable the detection of inherited or even *de novo* DNA copy-number aberrations encompassing a few megabases simultaneously across all 24 chromosomes. These genome-wide methods are likely to gradually replace locus- and family-specific FISH-based PGD approaches [36,44,50,62], offering a generic and standard approach for couples burdened with a balanced (complex) translocation or other type of intra- or interchromosomal rearrangement. Furthermore, single-cell SNP genotypes can be phased using the genotypes of the parents and a close relative, and thus allow genome-wide tracing of the inheritance of parental haplotypes that bear a disease-causing non-mitochondrial genetic variant [53,63]. Here, a mutation - such as a point mutation, indel, microdeletion or microduplication - inherited by an embryo is not genotyped directly in a blastomere of that embryo, but is rather imputed from linked SNPs embedded in the same inferred nuclear haplotype block. Additionally, in cases in which PGD is performed for balanced chromosomal rearrangements present in one of the parents, single-cell haplotyping can discriminate the inheritance of a balanced rearrangement from a balanced normal chromosome configuration. Last but not least, it may allow the discovery of the presence and parental origin of copy-neutral uniparental disomies, as well as of, for example, meiotic trisomies. Although single-cell genome-wide haplotyping technology is not routine in PGD practice yet, large-scale validation studies are ongoing.

In the near future, genome sequencing of single blastomeres biopsied from cleavage-stage embryos or

trophectoderm biopsies from human blastocysts may become routine practice in PGD [60,61]. Currently, single-cell genome sequencing already allows detection of inherited and *de novo* copy-number aberrations at a resolution and accuracy far beyond that attainable with microarray analyses. In addition, genotypes computed from single-cell sequences may allow not only the imputation of inherited risk variants by inferring SNP haplotypes, as for single-cell SNP-array data, but also genotyping of the inherited causal mutation directly. Single-cell genome sequencing furthermore holds the potential to detect mitochondrial DNA mutations and in fact any type of *de novo* mutation genome-wide.

Challenges such as the cost and duration of high-resolution genome-wide analyses will soon be overcome. From various ongoing large-scale human genome sequencing projects, our knowledge will increase to sift deleterious *de novo* mutations from neutral ones, and thus the interpretation of detected (*de novo*) genetic variants in the context of PGD will become clearer. Hence, time is pressing to define pertinent ethical guidelines for human embryo selection following genome-wide analysis as the technology will allow selection of embryos not only for highly penetrant Mendelian diseases, but also for various Mendelian traits at once, as well as for a combination of susceptibility factors for complex diseases.

Abbreviations

aCGH, array comparative genomic hybridization; ADO, allele drop out; FISH, fluorescent *in situ* hybridization; IVF, *in vitro* fertilization; LOH, loss of heterozygosity; MALBAC, multiple annealing and looping based amplification cycles; MDA, multiple displacement amplification; NGS, next-generation sequencing; PCR, polymerase chain reaction; PGD, preimplantation genetic diagnosis; PGS, preimplantation genetic diagnosis for aneuploidy screening; SNP, single-nucleotide polymorphism; WGA, whole genome amplification.

Competing interests

TV, MZE and JRV are co-inventors on a submitted patent application PCT/EP2011/060211 (Methods for haplotyping single cells - KU Leuven).

Acknowledgements

This work was supported by the EU FP7-PEOPLE-2012-IAPP grant SARM [No. 324509]; Research Foundation Flanders (FWO) [FWO-G.A093.11]; KU Leuven SymBioSys [PFV/10/016]. NVdA is a PhD student supported by the Research Foundation Flanders (FWO [1.1.H.28.12]).

Author details

[1]Laboratory of Reproductive Genomics, Department of Human Genetics, KU Leuven, Leuven 3000, Belgium. [2]Laboratory of Cytogenetics and Genome Research, Department of Human Genetics, KU Leuven, Leuven 3000, Belgium. [3]Single-cell Genomics Centre, Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK.

Published: 19 August 2013

References

1. Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, Strom CM: **Analysis of the first polar body: preconception genetic diagnosis.** *Hum Reprod* 1990, **5:**826-829.
2. Strom CM, Verlinsky Y, Milayeva S, Evsikov S, Cieslak J, Lifchez A, Valle J, Moise J, Ginsberg N, Applebaum M: **Preconception genetic diagnosis of cystic fibrosis.** *Lancet* 1990, **336:**306-307.
3. Handyside AH, Kontogianni EH, Hardy K, Winston RM: **Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification.** *Nature* 1990, **344:**768-770.
4. Simpson JL: **Preimplantation genetic diagnosis at 20 years.** *Prenat Diagn* 2010, **30:**682-695.
5. Goossens V, Traeger-Synodinos J, Coonen E, De Rycke M, Moutou C, Pehlivan T, Derks-Smeets IA, Harton G: **ESHRE PGD Consortium data collection XI: cycles from January to December 2008 with pregnancy follow-up to October 2009.** *Hum Reprod* 2012, **27:**1887-1911.
6. Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, SenGupta SB, Pehlivan Budak T, Renwick P, De Rycke M, Geraedts JP, Harton G: **The ESHRE PGD Consortium: 10 years of data collection.** *Hum Reprod Update* 2012, **18:**234-247.
7. Harton GL, De Rycke M, Fiorentino F, Moutou C, SenGupta S, Traeger-Synodinos J, Harper JC: **ESHRE PGD consortium best practice guidelines for amplification-based PGD.** *Hum Reprod* 2011, **26:**33-40.
8. Steffann J, Frydman N, Gigarel N, Burlet P, Ray PF, Fanchin R, Feyereisen E, Kerbrat V, Tachdjian G, Bonnefont JP, Frydman R, Munnich A: **Analysis of mtDNA variant segregation during early human embryonic development: a tool for successful NARP preimplantation diagnosis.** *J Med Genet* 2006, **43:**244-247.
9. Sallevelt SC, Dreesen JC, Drusedau M, Spierts S, Coonen E, van Tienen FH, van Golde RJ, de Coo IF, Geraedts JP, de Die-Smulders CE, Smeets HJ: **Preimplantation genetic diagnosis in mitochondrial DNA disorders: challenge and success.** *J Med Genet* 2013, **50:**125-132.
10. Unsal E, Aktas Y, Uner O, Baltac IA, Ozcan S, Turhan F, Baltaci V: **Successful application of preimplantation genetic diagnosis for Leigh syndrome.** *Fertil Steril* 2008, **90:**2017.e11-2017.e13.
11. Fiorentino F, Kokkali G, Biricik A, Stavrou D, Ismailoglu B, De Palma R, Arizzi L, Harton G, Sessa M, Pantos K: **Polymerase chain reaction-based detection of chromosomal imbalances on embryos: the evolution of preimplantation genetic diagnosis for chromosomal translocations.** *Fertil Steril* 2010, **94:**2001-2011.
12. Treff NR, Scott RT Jr: **Four-hour quantitative real-time polymerase chain reaction-based comprehensive chromosome screening and accumulating evidence of accuracy, safety, predictive value, and clinical efficacy.** *Fertil Steril* 2013, **99:**1049-1053.
13. Vanneste E, Voet T, Melotte C, Debrock S, Sermon K, Staessen C, Liebaers I, Fryns JP, D'Hooghe T, Vermeesch JR: **What next for preimplantation genetic screening? High mitotic chromosome instability rate provides the biological basis for the low success rate.** *Hum Reprod* 2009, **24:**2679-2682.
14. Mastenbroek S, Twisk M, van der Veen F, Repping S: **Preimplantation genetic screening: a systematic review and meta-analysis of RCTs.** *Hum Reprod Update* 2011, **17:**454-466.
15. Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NE, Arts EG, de Vries JW, Bossuyt PM, Buys CH, Heineman MJ, Repping S, van der Veen F: **In vitro fertilization with preimplantation genetic screening.** *New Engl J Med* 2007, **357:**9-17.
16. Harper J, Coonen E, De Rycke M, Fiorentino F, Geraedts J, Goossens V, Harton G, Moutou C, Pehlivan Budak T, Renwick P, Sengupta S, Traeger-Synodinos J, Vesela K: **What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium steering committee.** *Hum Reprod* 2010, **25:**821-823.
17. Boada M, Carrera M, De La Iglesia C, Sandalinas M, Barri PN, Veiga A: **Successful use of a laser for human embryo biopsy in preimplantation genetic diagnosis: report of two cases.** *J Assist Reprod Genet* 1998, **15:**302-307.
18. Durban M, Benet J, Boada M, Fernandez E, Calafell JM, Lailla JM, Sanchez-Garcia JF, Pujol A, Egozcue J, Navarro J: **PGD in female carriers of balanced Robertsonian and reciprocal translocations by first polar body analysis.** *Hum Reprod Update* 2001, **7:**591-602.
19. Xu K, Montag M: **New perspectives on embryo biopsy: not how, but when and why?** *Sem Reproductive Med* 2012, **30:**259-266.
20. Kuliev A, Rechitsky S: **Polar body-based preimplantation genetic diagnosis for Mendelian disorders.** *Mol Hum Reprod* 2011, **17:**275-285.
21. McArthur SJ, Leigh D, Marshall JT, Gee AJ, De Boer KA, Jansen RP: **Blastocyst trophectoderm biopsy and preimplantation genetic diagnosis for familial monogenic disorders and chromosomal translocations.** *Pren Diagn* 2008, **28:**434-442.
22. Zhu D, Zhang J, Cao S, Heng BC, Huang M, Ling X, Duan T, Tong GQ: **Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and**

implantation rates compared with fresh blastocyst transfer cycles - time for a new embryo transfer strategy? *Fertil Steril* 2011, **95:**1691-1695.
23. Chang LJ, Huang CC, Tsai YY, Hung CC, Fang MY, Lin YC, Su YN, Chen SU, Yang YS: **Blastocyst biopsy and vitrification are effective for preimplantation genetic diagnosis of monogenic diseases.** *Hum Reprod* 2013, **28:**1435-1444.
24. Kumar P, Zamani Esteki M, Van der Aa N, Voet T: **How to analyse a single blastomere? Application of whole-genome technologies: micro-arrays and next generation sequencing** In *Textbook of Human Reproductive Genetics.* Edited by Sermon K, Viville S. Cambridge: Cambridge University Press; in press
25. Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, Sun Z, Zong Q, Du Y, Du J, Driscoll M, Song W, Kingsmore SF, Egholm M, Lasken RS: **Comprehensive human genome amplification using multiple displacement amplification.** *Proc Natl Acad Sci U S A* 2002, **99:**5261-5266.
26. Spits C, Le Caignec C, De Rycke M, Van Haute L, Van Steirteghem A, Liebaers I, Sermon K: **Optimization and evaluation of single-cell whole-genome multiple displacement amplification.** *Hum Mutat* 2006, **27:**496-503.
27. Treff NR, Su J, Tao X, Northrop LE, Scott RT Jr: **Single-cell whole-genome amplification technique impacts the accuracy of SNP microarray-based genotyping and copy number analyses.** *Mol Hum Reprod* 2011,**17:**335-343.
28. Voet T, Kumar P, Van Loo P, Cooke SL, Marshall J, Lin ML, Zamani Esteki M, Van der Aa N, Mateiu L, McBride DJ, Bignell GR, McLaren S, Teague J, Butler A, Raine K, Stebbings LA, Quail MA, D'Hooghe T, Moreau Y, Futreal PA, Stratton MR, Vermeesch JR, Campbell PJ: **Single-cell paired-end genome sequencing reveals structural variation per cell cycle.** *Nucleic Acids Res* 2013, **41:**6119-6138.
29. Hou Y, Song L, Zhu P, Zhang B, Tao Y, Xu X, Li F, Wu K, Liang J, Shao D, Wu H, Ye X, Ye C, Wu R, Jian M, Chen Y, Xie W, Zhang R, Chen L, Liu X, Yao X, Zheng H, Yu C, Li Q, Gong Z, Mao M, Yang X, Yang L, Li J, Wang W, *et al.*: **Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm.** *Cell* 2012, **148:**873-885.
30. Xu X, Hou Y, Yin X, Bao L, Tang A, Song L, Li F, Tsang S, Wu K, Wu H, He W, Zeng L, Xing M, Wu R, Jiang H, Liu X, Cao D, Guo G, Hu X, Gui Y, Li Z, Xie W, Sun X, Shi M, Cai Z, Wang B, Zhong M, Li J, Lu Z, Gu N, *et al.*: **Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor.** *Cell* 2012, **148:**886-895.
31. Lasken RS, Stockwell TB: **Mechanism of chimera formation during the Multiple Displacement Amplification reaction.** *BMC Biotechnol* 2007, **7:**19.
32. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N: **Whole genome amplification from a single cell: implications for genetic analysis.** *Proc Natl Acad Sci U S A* 1992, **89:**5847-5851.
33. Cheung VG, Nelson SF: **Whole genome amplification using a degenerate oligonucleotide primer allows hundreds of genotypes to be performed on less than one nanogram of genomic DNA.** *Proc Natl Acad Sci U S A* 1996, **93:**14676-14679.
34. Langmore JP: **Rubicon Genomics, Inc.** *Pharmacogenomics* 2002, **3:**557-560.
35. Zong C, Lu S, Chapman AR, Xie XS: **Genome-wide detection of single-nucleotide and copy-number variations of a single human cell.** *Science* 2012, **338:**1622-1626.
36. Fiorentino F, Spizzichino L, Bono S, Biricik A, Kokkali G, Rienzi L, Ubaldi FM, Iammarrone E, Gordon A, Pantos K: **PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization.** *Hum Reprod* 2011, **26:**1925-1935.
37. Geigl JB, Obenauf AC, Waldispuehl-Geigl J, Hoffmann EM, Auer M, Hormann M, Fischer M, Trajanoski Z, Schenk MA, Baumbusch LO, Speicher MR: **Identification of small gains and losses in single cells after whole genome amplification on tiling oligo arrays.** *Nucleic Acids Res* 2009, **37:**e105.
38. Van der Aa N, Cheng J, Mateiu L, Esteki MZ, Kumar P, Dimitriadou E, Vanneste E, Moreau Y, Vermeesch JR, Voet T: **Genome-wide copy number profiling of single cells in S-phase reveals DNA-replication domains.** *Nucleic Acids Res* 2013, **41:**e66.
39. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, D'Hooghe T, Moreau Y, Vermeesch JR: **Chromosome instability is common in human cleavage-stage embryos.** *Nat Med* 2009, **15:**577-583.
40. Le Caignec C, Spits C, Sermon K, De Rycke M, Thienpont B, Debrock S, Staessen C, Moreau Y, Fryns JP, Van Steirteghem A, Liebaers I, Vermeesch JR: **Single-cell chromosomal imbalances detection by array CGH.** *Nucleic Acids Res* 2006, **34:**e68.
41. Fiegler H, Geigl JB, Langer S, Rigler D, Porter K, Unger K, Carter NP, Speicher MR: **High resolution array-CGH analysis of single cells.** *Nucleic Acids Res* 2007, **35:**e15.
42. Cheng J, Vanneste E, Konings P, Voet T, Vermeesch JR, Moreau Y: **Single-cell copy number variation detection.** *Genome Biol* 2011, **12:**R80.
43. Konings P, Vanneste E, Jackmaert S, Ampe M, Verbeke G, Moreau Y, Vermeesch JR, Voet T: **Microarray analysis of copy number variation in single cells.** *Nat Protoc* 2012, **7:**281-310.
44. Alfarawati S, Fragouli E, Colls P, Wells D: **First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis.** *Hum Reprod* 2011, **26:**1560-1574.
45. Vanneste E, Melotte C, Voet T, Robberecht C, Debrock S, Pexsters A, Staessen C, Tomassetti C, Legius E, D'Hooghe T, Vermeesch JR: **PGD for a complex chromosomal rearrangement by array comparative genomic hybridization.** *Hum Reprod* 2011, **26:**941-949.
46. Geraedts J, Montag M, Magli MC, Repping S, Handyside A, Staessen C, Harper J, Schmutzler A, Collins J, Goossens V, van der Ven H, Vesela K, Gianaroli L: **Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results.** *Hum Reprod* 2011, **26:**3173-3180.
47. Johnson DS, Gemelos G, Baner J, Ryan A, Cinnioglu C, Banjevic M, Ross R, Alper M, Barrett B, Frederick J, Johnson DS, Gemelos G, Baner J, Ryan A, Cinnioglu C, Banjevic M, Ross R, Alper M, Barrett B, Frederick J: **Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol.** *Hum Reprod* 2010, **25:**1066-1075.
48. Iwamoto K, Bundo M, Ueda J, Nakano Y, Ukai W, Hashimoto E, Saito T, Kato T: **Detection of chromosomal structural alterations in single cells by SNP arrays: a systematic survey of amplification bias and optimized workflow.** *PLoS One* 2007, **2:**e1306.
49. Voet T, Vanneste E, Van der Aa N, Melotte C, Jackmaert S, Vandendael T, Declercq M, Debrock S, Fryns JP, Moreau Y, D'Hooghe T, Vermeesch JR: **Breakage-fusion-bridge cycles leading to inv dup del occur in human cleavage stage embryos.** *Hum Mutat* 2011, **32:**783-793.
50. van Uum CM, Stevens SJ, Dreesen JC, Drusedau M, Smeets HJ, Hollanders-Crombach B, Die-Smulders CE, Geraedts JP, Engelen JJ, Coonen E: **SNP array-based copy number and genotype analyses for preimplantation genetic diagnosis of human unbalanced translocations.** *Eur J Hum Genet* 2012, **20:**938-944.
51. Treff NR, Levy B, Su J, Northrop LE, Tao X, Scott RT Jr: **SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH.** *Mol Hum Reprod* 2010, **16:**583-589.
52. Treff NR, Su J, Tao X, Levy B, Scott RT Jr: **Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays.** *Fertil Steril* 2010, **94:**2017-2021.
53. Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara N, Shaw MA, Griffin DK: **Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes.** *J Med Genet* 2010, **47:**651-658.
54. Baslan T, Kendall J, Rodgers L, Cox H, Riggs M, Stepansky A, Troge J, Ravi K, Esposito D, Lakshmi B, Wigler M, Navin N, Hicks J: **Genome-wide copy number analysis of single cells.** *Nat Protoc* 2012, **7:**1024-1041.
55. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D, Muthuswamy L, Krasnitz A, McCombie WR, Hicks J, Wigler M: **Tumour evolution inferred by single-cell sequencing.** *Nature* 2011, **472:**90-94.
56. Bi W, Breman A, Shaw CA, Stankiewicz P, Gambin T, Lu X, Cheung SW, Jackson LG, Lupski JR, Van den Veyver IB, Beaudet AL: **Detection of >/=1Mb microdeletions and microduplications in a single cell using custom oligonucleotide arrays.** *Prenat Diagn* 2012, **32:**10-20.
57. Evrony GD, Cai X, Lee E, Hills LB, Elhosary PC, Lehmann HS, Parker JJ, Atabay KD, Gilmore EC, Poduri A, Park PJ, Walsh CA: **Single-neuron sequencing analysis of l1 retrotransposition and somatic mutation in the human brain.** *Cell* 2012, **151:**483-496.
58. Wang J, Fan HC, Behr B, Quake SR: **Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm.** *Cell* 2012, **150:**402-412.
59. Gundry M, Li W, Maqbool SB, Vijg J: **Direct, genome-wide assessment of DNA mutations in single cells.** *Nucleic Acids Res* 2012, **40:**2032-2040.
60. Yin X, Tan K, Vajta G, Jiang H, Tan Y, Zhang C, Chen F, Chen S, Zhang C, Pan X, Gong C, Li X, Lin C, Gao Y, Liang Y, Yi X, Mu F, Zhao L, Peng H, Xiong B, Zhang S, Cheng D, Lu G, Zhang X, Lin G, Wang W: **Massively parallel sequencing for chromosomal abnormality testing in trophectoderm cells of human blastocysts.** *Biol Reprod* 2013, **88:**69.

61. Treff NR, Fedick A, Tao X, Devkota B, Taylor D, Scott RT Jr: **Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease.** *Fertil Steril* 2013, **99:**1377-1384.
62. Treff NR, Northrop LE, Kasabwala K, Su J, Levy B, Scott RT Jr: **Single nucleotide polymorphism microarray-based concurrent screening of 24-chromosome aneuploidy and unbalanced translocations in preimplantation human embryos.** *Fertil Steril* 2011, **95:** 1606-1612.e2.
63. Altarescu G, Zeevi DA, Zeligson S, Perlberg S, Eldar-Geva T, Margalioth EJ, Levy-Lahad E, Renbaum P: **Familial haplotyping and embryo analysis for Preimplantation Genetic Diagnosis (PGD) using DNA microarrays: a proof of principle study.** *J Assist Reprod Genet* 2013. doi:10.1007/s10815-013-0044-8.

doi:10.1186/gm475
Cite this article as: Van der Aa N, *et al*.: **Preimplantation genetic diagnosis guided by single-cell genomics.** *Genome Medicine* 2013, **5**:71.