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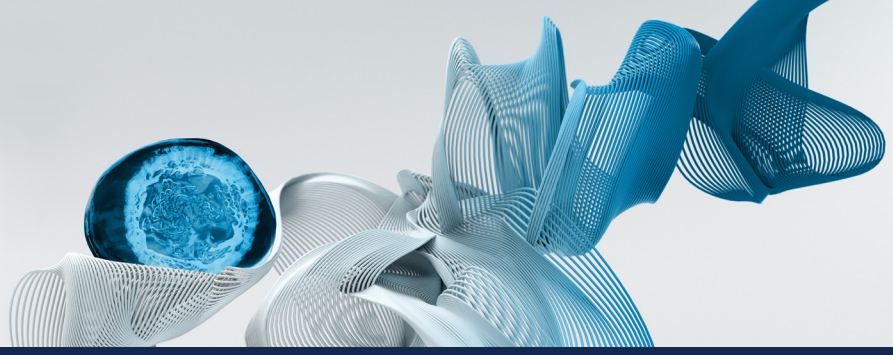

Product Note

Preimplantation Genetic Testing Protocol

Cells explored. Answers revealed.

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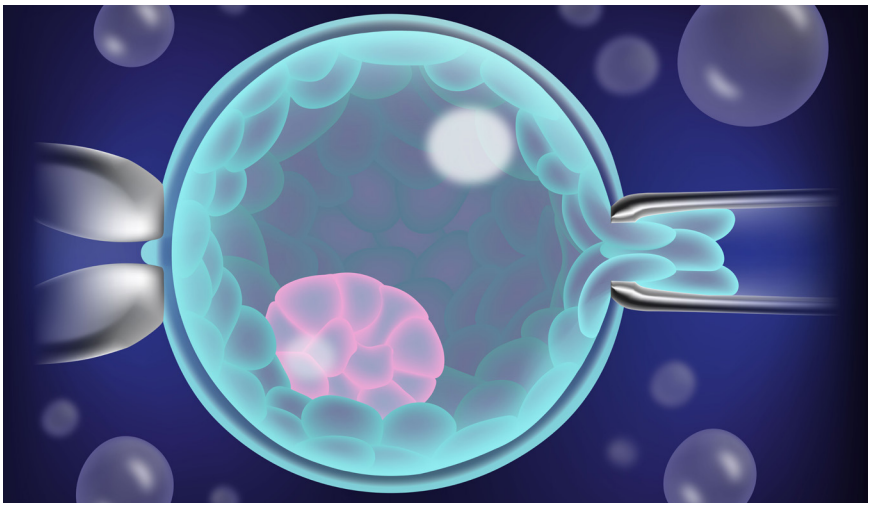
A Fully Integrated Workflow Based on ResolveDNA WGA for Complete Analysis of Human Embryos for Aneuploidy and Monogenic Disease (PGT-A + M)



BioSkryb Genomics has developed a workflow to enable single-cell whole genome amplification and sequencing analysis. The newly-developed BioSkryb preimplantation genetic testing (PGT) workflow solves a significant workflow challenge by allowing the analysis of gross chromosomal aneuploidy errors (PGT-A) simultaneously with comprehensive monogenic genetic disorder single nucleotide variation analysis (PGT-M).

This fully-integrated workflow (Figure 1) highlights the steps; from embryo biopsy at the IVF center through whole genome amplification (WGA), library preparation, sequencing and analysis. The industry-leading BioSkryb Genomics chemistry (Figure 2) gives laboratories the capability to combine PGT-A and PGT-M in a single PGT workflow from the biopsy of a single embryo. This allows for whole genome and/or specific panels for known inherited mutations to be leveraged. Due to the unprecedented completeness of genome coverage by PTA, there is no need for splitting samples, multiple workflows or any other downstream changes to your normal PGT workflow. The workflow makes this possible from a 4-6 cell embryo biopsy down to a down to single blastomere.

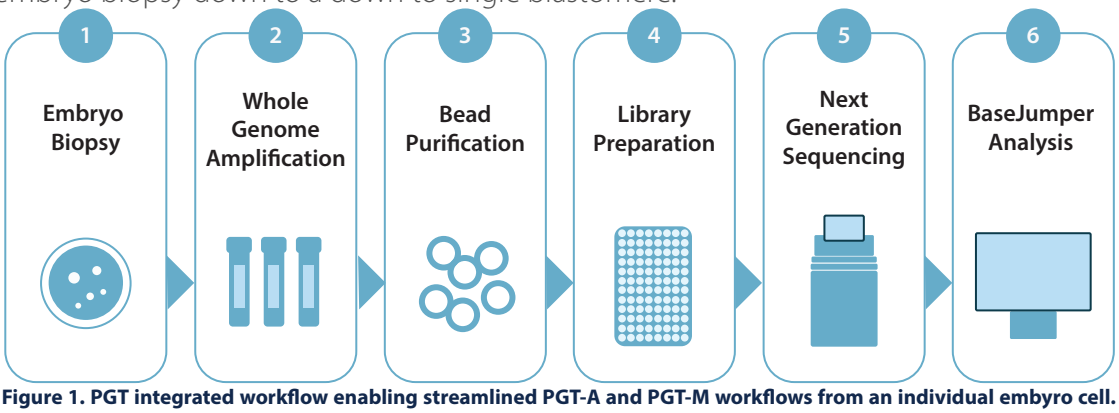


Figure 1. PGT integrated workflow enabling streamlined PGT-A and PGT-M workflows from an individual embryo cell. The easy-to-use ResolveDNA workflow allows for simultaneous analysis of PGT-A and PGT-M from a single embryo biopsy sample taken during IVF.

Introduction

Preimplantation genetic testing (PGT) has been used in the assisted reproduction technology (ART) space for over 30 years[1] as a means to allow for embryo selection during in vitro fertilization (IVF) cycles. Classes of PGT as a technology can be broken down into various tests depending on the rationale, including:

- **Aneuploidy (PGT-A)**: In PGT-A, each sample is tested for chromosome gains (trisomy) and losses (monosomy) of all or part of each chromosome. Effective tests in this space are able to capture chromosome errors, such as trisomy 21 (Down syndrome), trisomy 16 (leading to miscarriage), and/or others facilitating implantation failure and/or pregnancy loss. PGT-A has been shown to improve outcomes during IVF cycles by eliminating aneuploid embryos not destined to result in a normal live birth[2].
- **Monogenic Disease (PGT-M)**: In PGT-M, each sample is tested for specific inherited monogenic mutations that lead to disease in humans[6]. Known carriers of a monogenic disease aim to selectively transfer embryos that have been tested for a specific genetic (monogenic) disease such as cystic fibrosis (CF) or Huntington disease (HD)[4].
- **Structural Chromosome Rearrangements (PGT-SR)**: In PGT-SR, samples are tested for known structural chromosome rearrangement or chromosome translocation. People with structural chromosome rearrangements typically have no signs or symptoms. However, when they go through meiosis, errors during chromosome alignment cause abnormal gametes with unbalanced chromosomes to be created. PGT-SR allows for selective transfer of normal/balanced embryos and higher chances of delivery following PGT-SR[5].

All forms of PGT detailed above generally require a biopsy of *4-6 cells* from the growing embryo followed by genome amplification to create enough DNA to analyze for PGT. These methods include such approaches as multiple displacement amplification (MDA) (Vitrolife), PicoPlex™ (Takara), and MALBEC™ (Yikon). Here we detail a new technology, Primary Template-directed Amplification-PTA (ResolveDNA™). The ResolveDNA™ PTA platform allows for unprecedented enrichment of cellular genomes, including best-in-class robustness on individual cells[6].

ResolveDNA™ is built upon the foundation of Primary Template-directed Amplification (PTA)[4] (Figure 2). By limiting product amplification bias and error propagation, PTA has enabled highly accurate whole-genome and targeted analysis of a single cell.

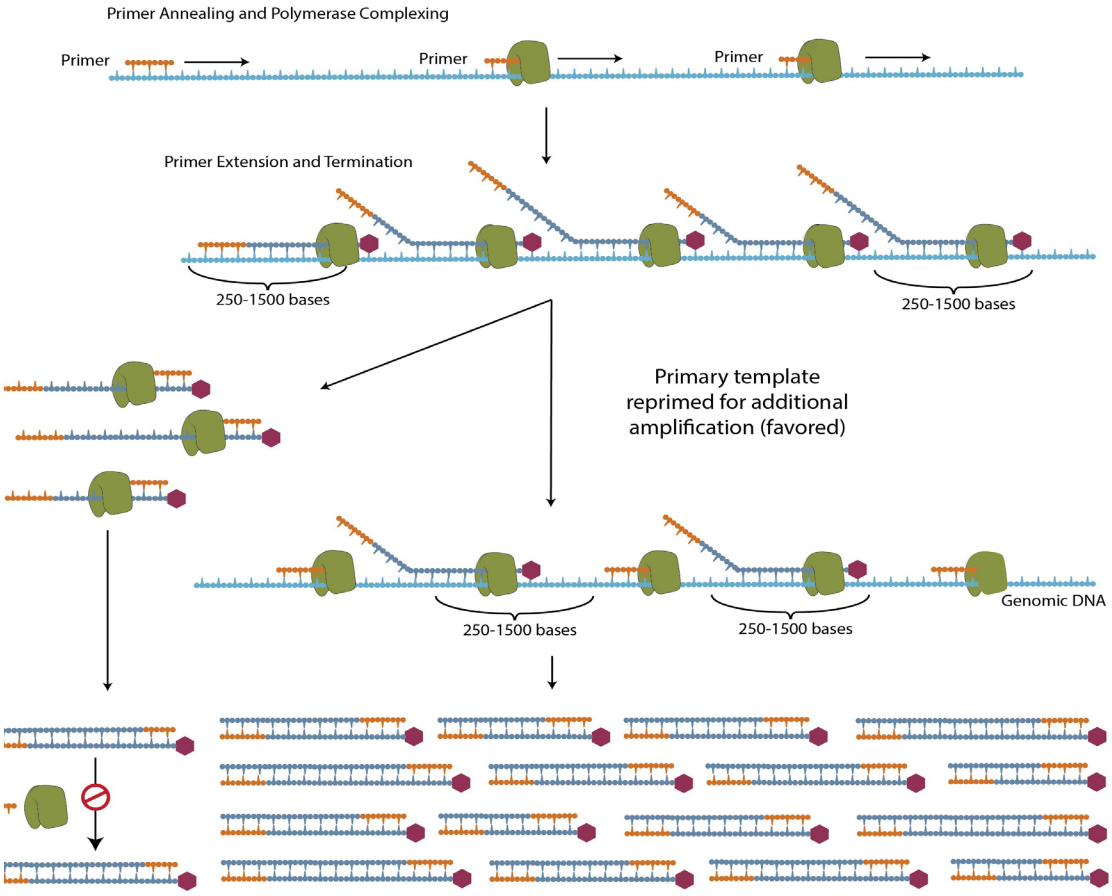


Figure 2. The principle of PTA. ResolveDNA provides unbiased amplification by utilizing random priming combined with amplicon termination to produce a true representation of original sample template. A novel approach employing proprietary nucleotides prevents the production of long amplicons, which are kinetically unfavored to be re-copied during the amplification reaction. By limiting the size of the produced amplicon, primers are re-directed to the primary template.

Initial Validation of PTA for PGT

In collaboration with Praxis Genomics (6115 Peachtree Dunwoody Road, Suite 220, Atlanta, GA), 42 embryo samples donated for research at a local IVF center were biopsied and the cells placed in 0.2 ml Eppendorf tubes in 1XPBS and shipped on dry ice for testing. Following receipt in the lab, the samples were processed through the ResolveDNA™ workflow using the initial biopsy tube, followed by library preparation and sequencing on Illumina platforms (Praxis).

Due to the unprecedented genomic representation of the ResolveDNA™ PGT workflow, analysis of each individual embryo sample can be extended beyond conventional PGT-A/PGT-M/PGT-SR to delve deeper into the genetics and potentially genomics of embryos assessed during an IVF cycle.

DNA assessment following ResolveDNA™

Yield and Genomic Coverage

Cells from control and embryo samples were taken through the ResolveDNA workflow as outlined in the first part of Figure 1. Yields for all specimens were as expected for control cells, NTC (No Template Control) negative samples, and embryo samples. At this point, each sample went through library preparation and whole genome sequencing. Following sequencing, we report overall average depth of the genome to evaluate ability to enable multiple classes of PGT. Well over 95% of the genome was covered across all 42 embryo samples and the target of 20X depth for variant detection was achieved for the vast majority of the samples (Figure 3). The majority of embryos tested had greater than 15X depth across over 96% of the genome.

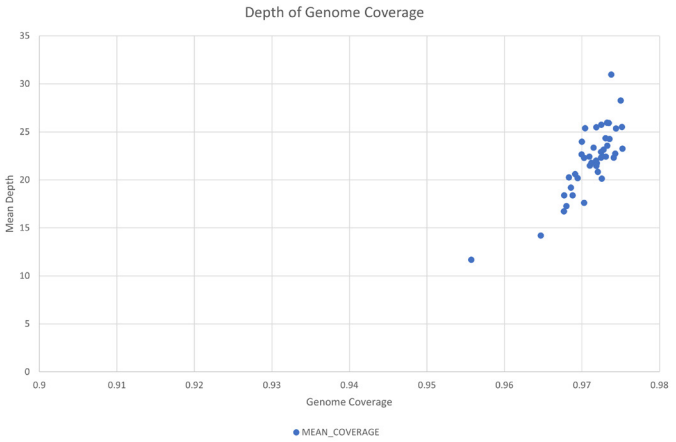


Figure 3: Genome coverage summary for ResolveDNA performance on embryo samples. Genome coverage (x-axis) and average genome depth (y-axis) shown. Roughly 78Gbp were generated for 20x depth.

[PGT-M] Single Nucleotide Variation

Current technology for PGT-M requires case-specific workflows in the laboratory that require multiple workflows/platforms (i.e. NGS + PCR or SNP array + PCR)[4]. More importantly, current technology generally requires splitting of the sample sometime during WGA to aid in creating enough coverage to allow for CNV analysis (PGT-A) while also deeply sequencing in and around the gene(s) of interest for PGT-M. In addition to the robust, wide genome coverage required for assessment of copy number variation, the ResolveDNA PGT Workflow allows for an industry-leading ability to report changes in alleles across the genome. Table 1 highlights the genomic representation observed using the ResolveDNA workflow, while Figure 4 demonstrates the recovery of both alleles.

Genome Coverage	Sensitivity	Precision	Allelic Balance (@20x)	Allelic Balance (@40x)
96%	93.5%	99.2%	88.1%	98.1%

Table 1. Performance characteristics for ResolveDNA™ workflow Cells isolated from NA12878 (HG001) were leveraged to characterize workflow performance. Values represent averages across all replicates in an internal study. Additional sequencing was performed to measure allelic balance up to 40x mean depth. Allelic balance is a summary of all heterozygous loci that were called as heterozygous in our pipeline.

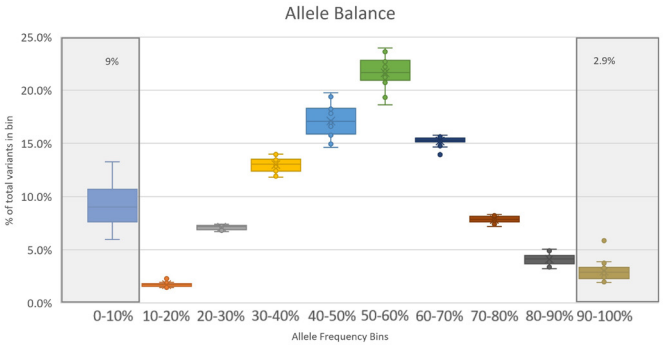


Figure 4: Allelic balance of embryo samples through the workflow. Allele ratio bins for the embryos sequenced to 20x were compared from genome-wide variants confirmed to be heterozygous. Allele drop out is for variants <10% or >90% and the median value of variants in those bins across all samples are shown.

To better understand the genomic coverage, embryos were assessed for the uniformity and depth of coverage in 5 example genes that are frequently tested for using PGT-M including BRCA1 (Breast Cancer), CFTR (Cystic Fibrosis), DMD (Duchenne Muscular Dystrophy) and HTT (Huntington Disease). In these specific genes, we assessed coverage of the genome that would allow for analysis of typical mutations in each gene along with single nucleotide polymorphisms (SNPs) to add assurance (ADO, contamination, PA, etc.) to their testing (Table 2)[7].

Gene	Coverage (%)	Coverage > 4x (%)	Mean Depth	Uniformity of Coverage
BRCA1	100	100	32.1	36.43
CFTR	100	99.98	25.6	28.98
DMD	99.98	99.26	17.8	33.99
HTT	98.36	94.76	23.5	29.29

Table 2. Performance of typical genes involved in PGT-M testing. Gene results are summarized across all coding regions of a gene. Depth of 4 provides power to call heterozygous variants. Uniformity of coverage is the percent of gene regions where depth is within 20% of the mean.

PGT-A (Copy Number Variation Detection)

For PGT-A, copy number variation (CNV) is typically limited to assessment of chromosome changes no smaller than 7-10 MB gains/losses. Therefore any gain/loss smaller than this window will not be called or reported. Analysis of CNV relies on wide genome coverage and can be affected by sequencing parameters (single end vs. paired end, number of sequencing cycles, etc.). To evaluate the performance of the ResolveDNA PGT-A workflow, we assessed individual embryo biopsy samples with known aneuploidy using ResolveDNA chemistry and an optimized, 1Mb bin CNV calling algorithm (Figure 5). The ResolveDNA PGT-A workflow characterizes known aneuploidy with a resolution of 5-7 Mb, consistent with industry standard.

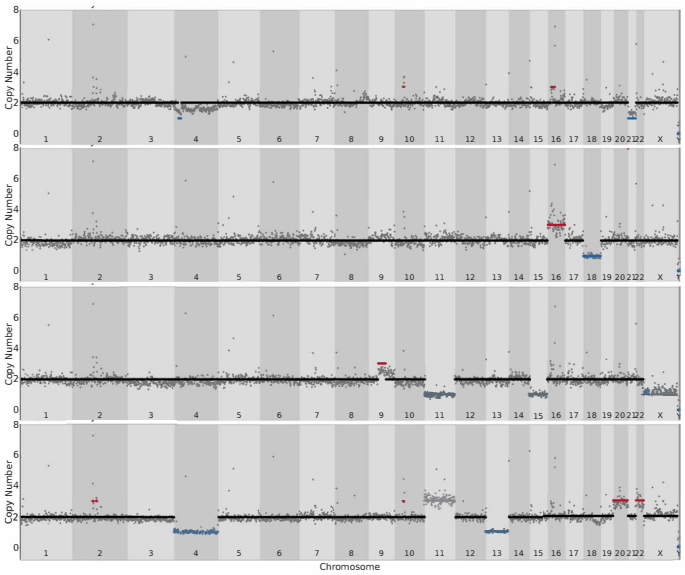


Figure 5: Examples of output from PGT-A copy number variation (CNV) calling from ResolveDNA PGT workflow. Known aneuploid embryos screened at 1Mb bin size on 5 million reads. Individual biopsy samples containing full chromosome gain/loss and segmental gain/loss are faithfully identified with high accuracy and resolution (~5-7 Mb).

Conclusions

Preimplantation genetic testing has been in use now for over 30 years and has become part of the conversation during most ART consults today. To date, no one system has been able to overcome the basic needs of all types of PGT to allow for truly universal PGT from a single biopsy. Here we reviewed the results of our initial validation of ResolveDNA in human embryos in collaboration with Praxis Genomics[8]. Together we were able to show unprecedented completeness of coverage and genome depth on biopsied empbryos. This allowed for testing embryos for PGT-A, PGT-M and PGT-SR from a single biopsy without the need for additional platforms, software, amplification or sample splitting. PTA offers a unique approach to whole genome amplification that should revolutionize the field of PGT going forward as laboratories take advantage of the improvements this platform offers.

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