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Unique Genomic Solutions

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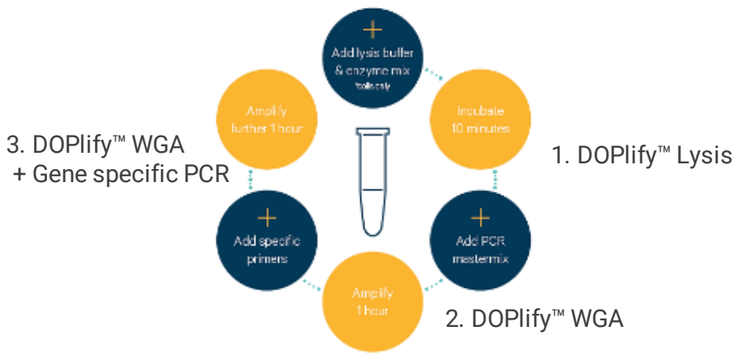
DOPlify™ A New Generation of Whole Genome Amplification

A combined PGD+PGS NGS solution for β-thalassemia and HLA-A typing

β-thalassemia screening (beta-globin; HBB) represents almost 15% of PGD cases, with a further 4.5% of cases combining human leukocyte antigen HLA-typing with HBB. There are more than 200 disease-causing HBB mutations described, so screening necessitates a pan-HBB mutation detection and HLA panel approach. The ability to combine β-thalassemia and HLA Preimplantation Genetic Diagnosis (PGD) with concurrent Preimplantation Genetic Screening (PGS) in one test maximizes the screening opportunity for a single embryo biopsy. RHS have developed a whole genome amplification (WGA) with DOPlify™ and Target Sequence Enrichment (TSE) protocol using gene-specific PCR that provides a novel comprehensive PGD + PGS solution.

Aim – To demonstrate that DOPlify™ with Target Sequence Enrichment achieves sensitive, accurate and economical combined PGS and PGD using low pass NGS.

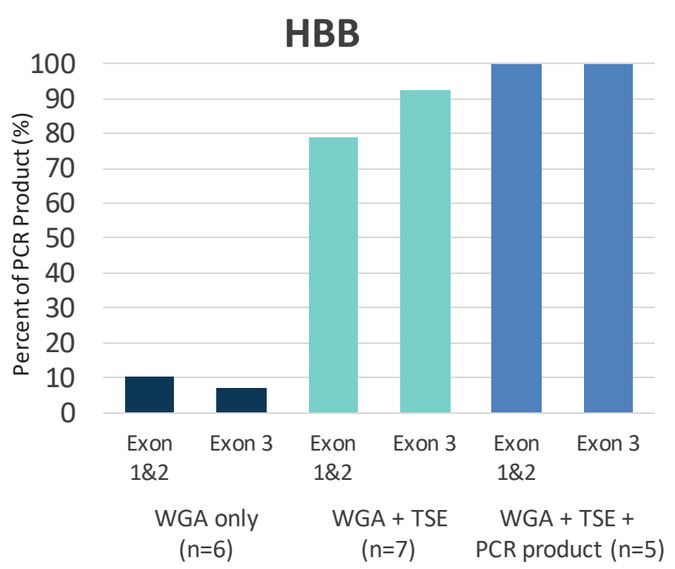
Methods – 5-cell aliquots were manually sorted from euploid female and male and an aneuploid cell line (48,XXY,+21; Coriell Institute). Cells were whole genome amplified using DOPlify™ with or without the addition of PGD primers (RHS and GenDx) for the target regions.

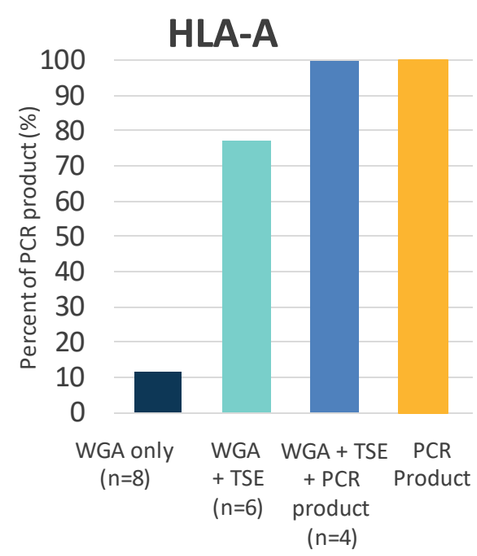


Following target enrichment, the targeted regions were then further amplified and the PCR product was seeded back into the target enriched DOPlify™ product to increase its concentration in the pool. Samples were then sequenced as detailed below.

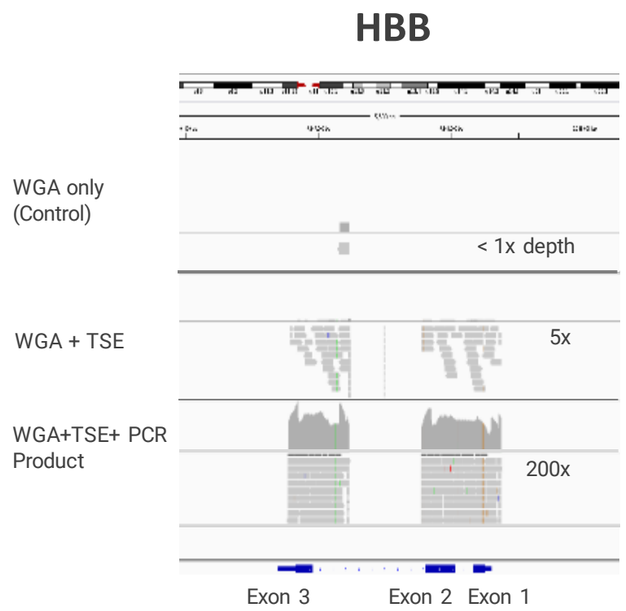
Results

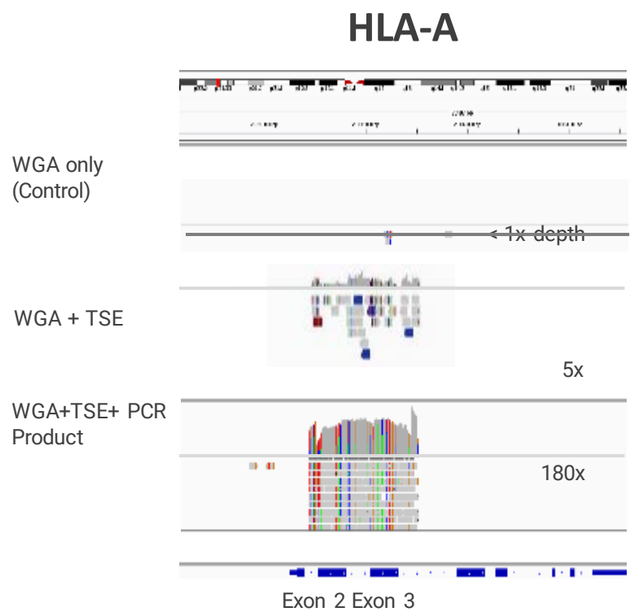
- ✓ Correct euploid and aneuploidy diagnoses (48,XXY,+21) were still achieved for all target sequence enriched and control samples (average > 500,000 reads mapped per sample).
- ✓ Breadth of PGD target regions sequenced (coverage) was 100%.





- ✓ Depth of coverage for PGD calling was > 60x, allowing confident PGD + PGS even with low pass NGS.





The NGS protocol used in this study is typical for low pass PGS and was not expected to yield the depth of reads required for PGD in the absence of enrichment. Targeted sequence enrichment provided the sequencing reads necessary for PGD without requiring costly deep sequencing of the entire genome.

Conclusions

DOPlify™ with target sequence enrichment and a low pass PGS NGS protocol;

- readily achieves reliable PGS and confident PGD results from a single indexed NGS sample in a 40 sample multiplex on a MiSeq sequencer and
- offers a scalable and economical PGD+PGS protocol

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