A novel NGS solution for combined PGT-M and PGT-A: Analysis for β-thalassemia

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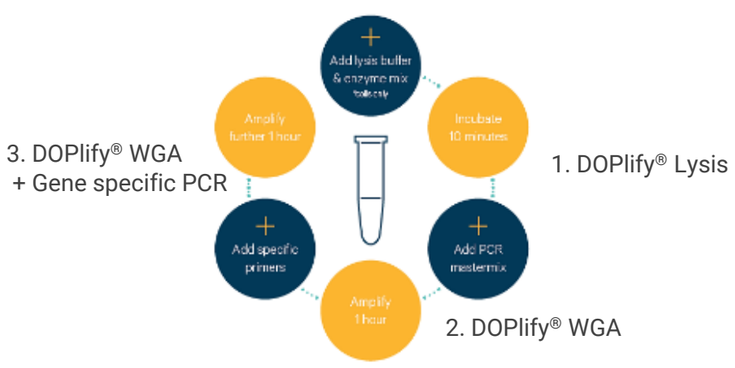
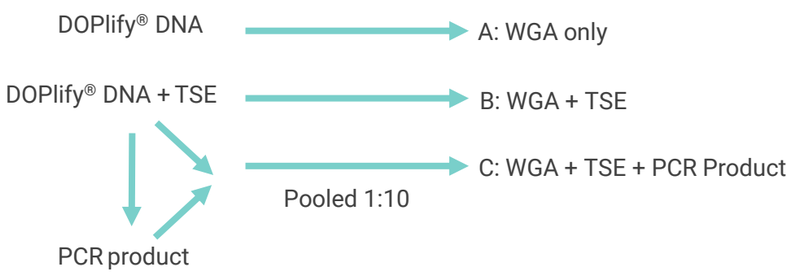
Thalassemia, caused by mutations within genes involved in haemoglobin production, including the haemoglobin subunit beta gene (*HBB*), has become one of the most prevalent blood disorders around the world and is a frequent target for Preimplantation Genetic Testing for Monogenic disorders (PGT-M). ESHRE data suggests that β-Thalassemia screening represents almost 15% of PGT-M cases, with a further 4.5% of cases combining β-Thalassemia with HLA-typing. The ability to combine aneuploidy detection (PGT-A) and PGT-M, by adding gene-specific PCR primers to a Whole Genome Amplification (WGA), maximises the screening opportunity for a single embryo biopsy.

Aim – To demonstrate the application of a novel Target Sequence Enrichment (TSE) protocol using the DOPlify® kit for combined PGT-M for β-thalassemia and PGT-A by Next Generation Sequencing (NGS).

Methods – 5-cell aliquots were manually sorted from euploid female and male, and an aneuploid cell line (48,XXY,+21; Coriell Institute for Medical Research) modelling Day 5 embryo biopsy. Cells were whole genome amplified using the DOPlify® kit (PerkinElmer) with or without the addition of PGT-M primers for *HBB* (in-house design; PerkinElmer) and *HLA-A* (GenDx) for the target regions.

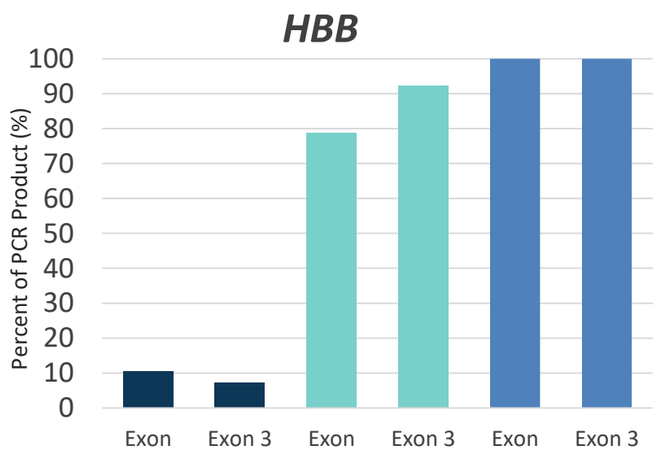


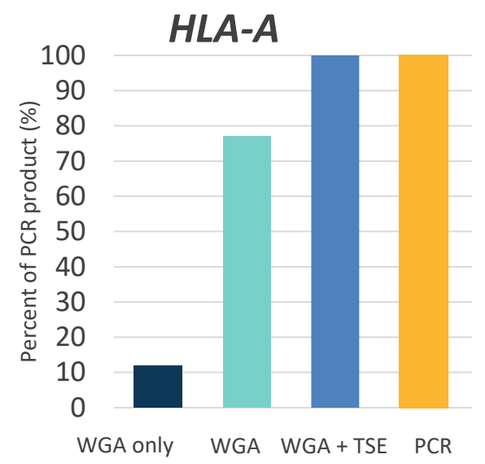
Following target enrichment, the targeted regions were then further amplified and the PCR product was pooled back into the target enriched DOPlify® WGA+TSE DNA to increase its concentration in the pool as detailed below. Samples were then sequenced according to the standard PG-Seq™ kit (PerkinElmer) protocol for MiSeq® instrument (Illumina) sequencing.



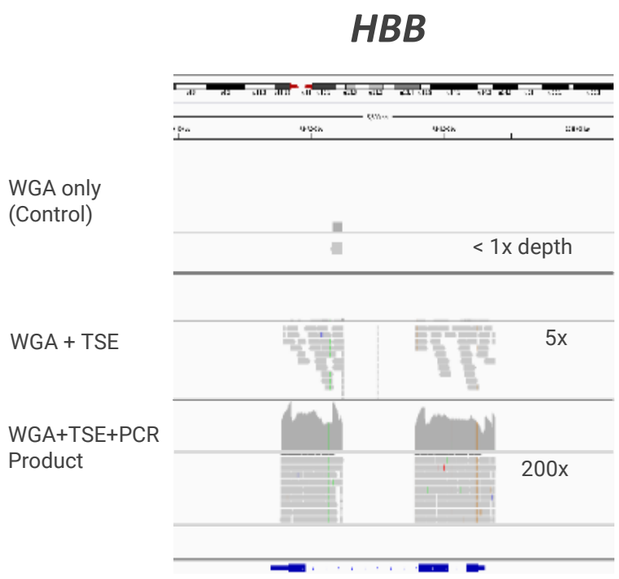
Results

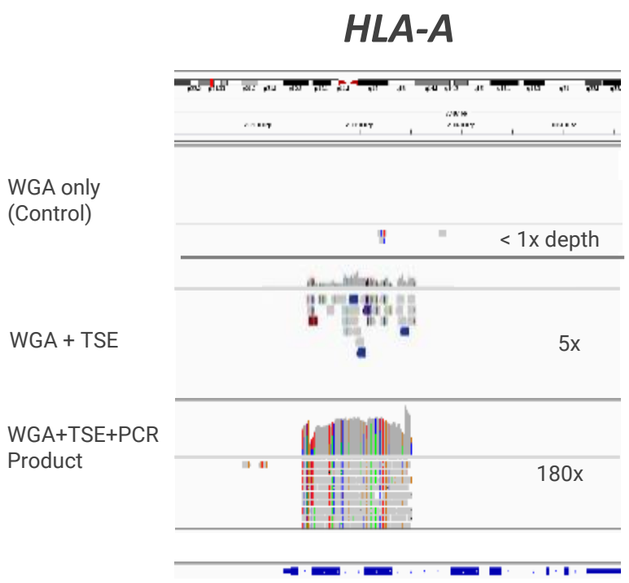
- ✓ Correct euploid and aneuploidy diagnoses (48,XXY,+21) were still achieved for all target sequence enriched and control samples (average > 500,000 reads mapped per sample).
- ✓ As expected, ≤10% breadth of coverage was achieved for *HBB* or *HLA* with no enrichment. In contrast, WGA+TSE achieved ~ 80% breadth of coverage, while further enrichment achieved 100% breadth of coverage of PGT-M target regions sequenced.





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





The PG-Seq™ kit NGS protocol used in this study is typical for low pass PGT-A and was not expected to yield the depth of reads required for PGT-M in the absence of enrichment. Target sequence enrichment provided the sequencing reads necessary for PGT-M without requiring costly deep sequencing of the entire genome.

Conclusions

DOPlify® kit with target sequence enrichment and a low pass NGS protocol;

- readily achieves reliable PGT-A and confident PGT-M results from a single indexed NGS sample in a 48 sample multiplex and,
- provides a unique opportunity to develop a multiplex panel targeting the most common disorders tested by PGT-M.