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Combined PGD and PGS for β-Thalassemia and HLA using Targeted Sequence Enrichment

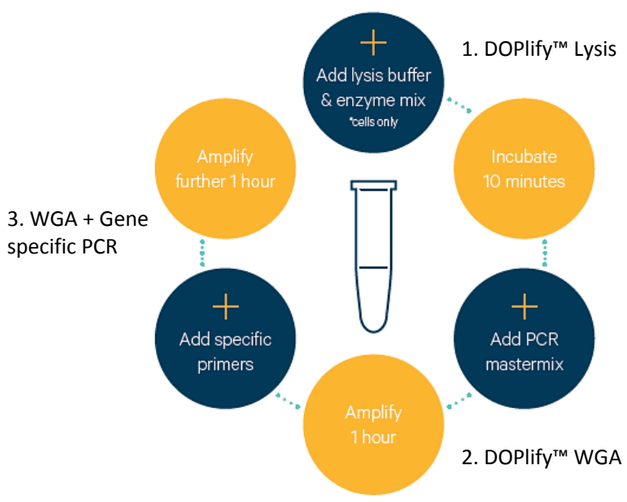
β-Thalassemia is caused by mutations within the beta globin (HBB) gene involved in haemoglobin production, with more than 200 disease causing mutations described so far.

ESHRE data suggests that β-Thalassemia screening represents almost 15% of PGD cases, with a further 4.5% of cases combining β-Thalassemia with HLA-typing. The ability to combine PGD with PGS presents an opportunity to transfer euploid embryos free of the disease causing mutation, removing the disease from the family lineage.

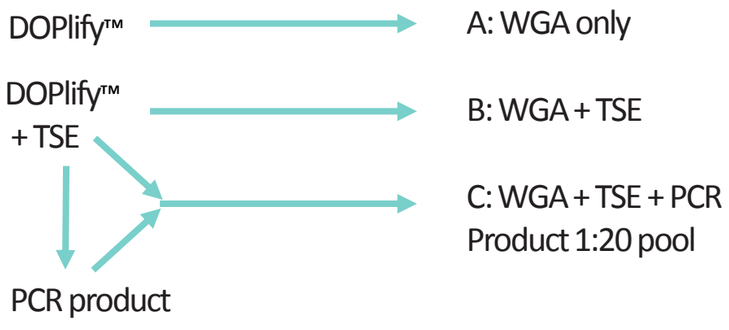
RHS has developed a novel combined approach using whole genome amplification (WGA) with DOPlify™ and gene-specific PCR for Target Sequence Enrichment (TSE). This approach readily allows PGD and PGS using an economical low pass, multiplexed NGS protocol.

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 for the target regions.



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



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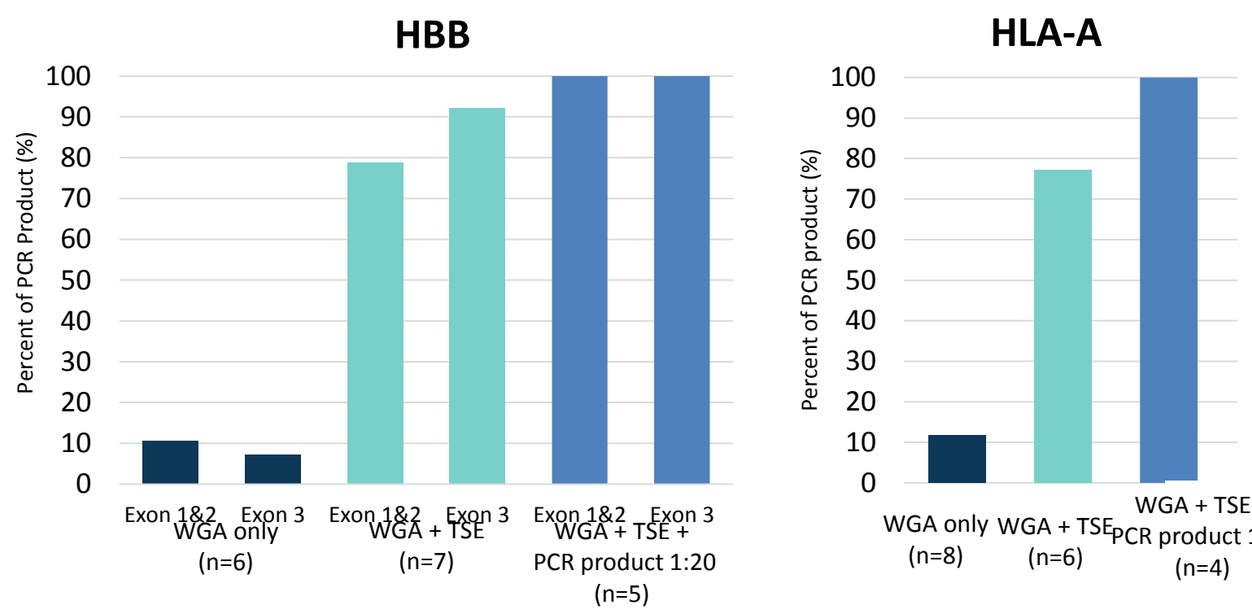
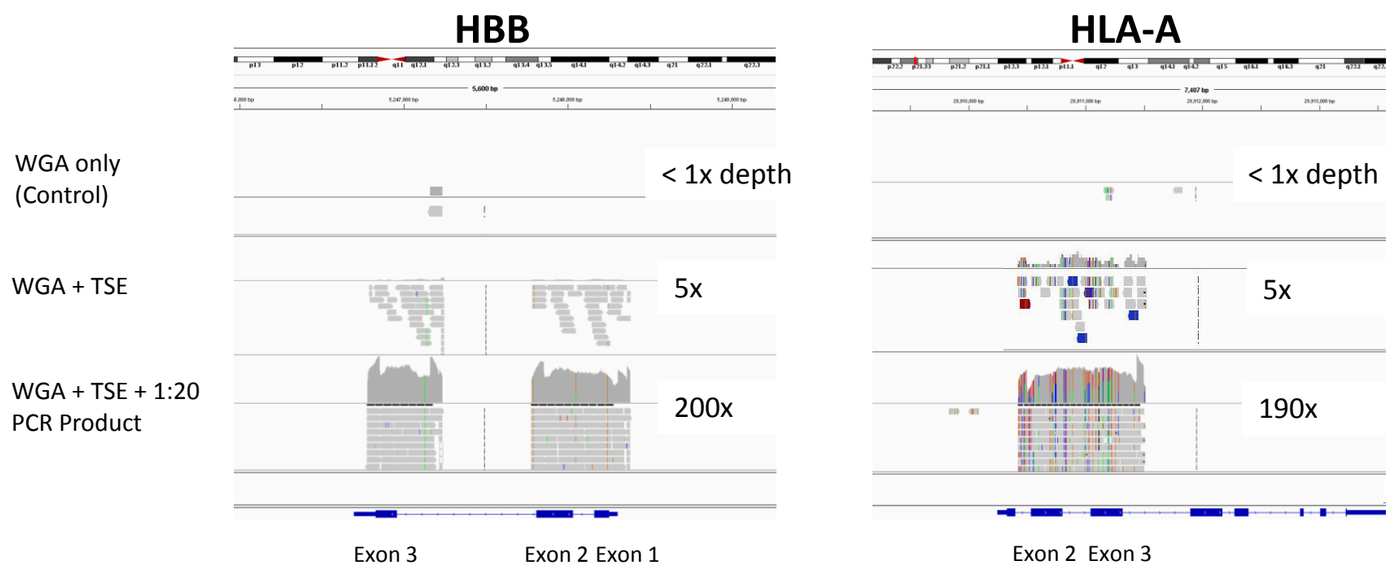
Results

✓ Correct euploid and aneuploidy diagnoses (48,XXY,+21) were achieved for all target sequence enriched and control samples (average > 500,000 reads mapped per sample).

✓ Breadth of PGD target region sequenced (coverage) is 100%



✓ Depth of coverage for PGD calling is >60x, even with low pass NGS



The NGS protocol used was 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 provides the NGS reads necessary for PGD without requiring deep sequencing of the rest of the genome, as is evident in the IGV screenshots above.

Conclusions

A low pass PGS NGS protocol using DOPlify™ with target sequence enrichment;

- 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

DOPlify™ A New Generation of Whole Genome Amplification