PG-Seq™ A novel complete NGS solution

Combined β-thalassemia mutation detection and PGT-A using a novel PCR barcoding approach for Ion Torrent™ NGS

Thalassemia was the 4th most frequent monogenic disorder screened using Preimplantation Genetic Testing (PGT-M) according to 10 years of ESHRE data collection. The ability to combine β-thalassemia-specific PCR primers and Whole Genome Amplification (WGA) for concurrent PGT-M and PGT for aneuploidy (PGT-A) together in a single PCR reaction represents a unique approach to maximise the screening opportunity for a single embryo biopsy. DOPlify® WGA provides a flexible technology to amplify whole genomes and clinically relevant sequences using the Target Sequence Enrichment (TSE) protocol and also a unique mechanism to incorporate specific sequences needed for Next Generation Sequencing (NGS) sample preparation, offering a streamlined solution for combined PGT-M and PGT-A.

Aim – To develop a novel approach that allows amplification and PCR barcoding of low template samples for combined PGT-A and PGT-M for β-thalassemia mutation testing in a single tube for Ion Torrent™ NGS.

Methods – 5-cell samples were manually sorted from aneuploid cell lines (Coriell Institute for Medical Research) and prepared for sequencing using a two-stage, single tube protocol. DNA was amplified using standard DOPlify® kit reagents with a modified primer (PerkinElmer). Subsequently, publicly available Ion Torrent™ (Thermo Fisher) NGS adapter sequences and barcodes were incorporated utilising a second PCR step within the same PCR tube. Incorporation of the adapter sequences (at both the 5' and 3' ends of the amplified DNA) was quantified using qPCR (Kapa Biosystems) with adapter sequence-specific primers (PerkinElmer), providing a library viability score.

A number of different barcoding methods were trialled. WGA DNA yield, DNA fragment size range and library viability were compared for each method. The barcoded samples were pooled and sequenced using Ion Torrent™ instrument sequencing. The sequencing data was bioinformatically aligned to hg19, sequencing metrics collated and the data analysed to determine sample ploidy status.

Results

- ✓ PCR incorporation of the Ion Torrent™ barcodes was successfully achieved using several methods.
- ✓ The most efficient & versatile method (Method 3) was selected for further evaluation based on the differences observed for DNA yield, fragment size and library viability (Table 1).

	Method 1	Method 2	Method 3
WGA DNA yield (ng/µl)	+	+++	++++
Size range (bp)	300-800	280-680	300-700
Library viability	+	+++	++

Table 1. Summary of WGA PCR DNA characteristics for select methods of PCR barcoding.

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Results (continued)

- ✓ Whole chromosome aneuploidy results from 5-cell samples were concordant with the expected karyotypes of the 47,XY,+13, 47,XY,+15 and 48,XXY,+21 cell lines.
- ✓ Detection of sub-chromosomal duplications and deletions using 5-cell samples, including 7-32 Mb aberrations were also concordant with the expected karyotype of the cell line (Figure 1 and 2).

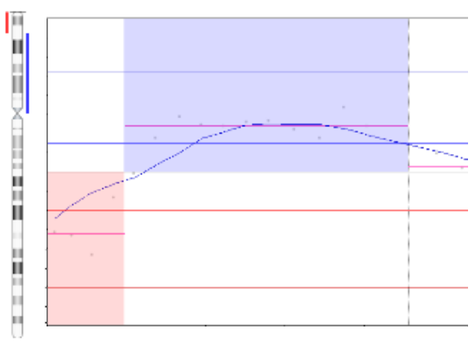


Figure 1. A 5-cell sample result, 7 Mb deletion and 32 Mb duplication on chromosome 8 (GM14485).

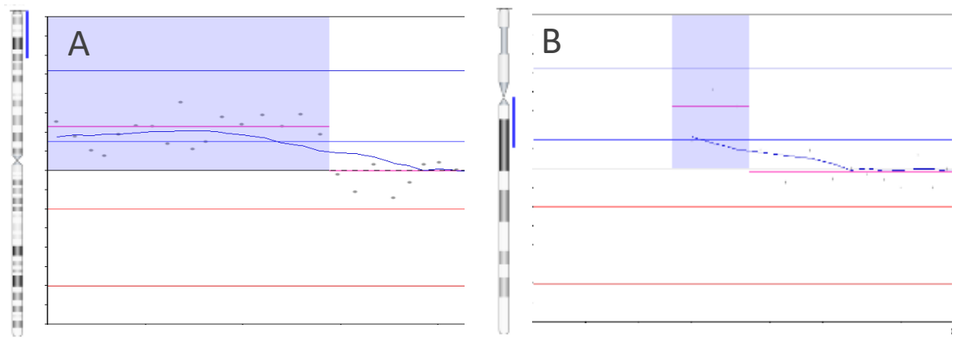


Figure 2. A 5-cell sample result, A. 31 Mb duplication on chromosome 3 and B. 7 Mb duplication on chromosome 21 (GM09552).

- ✓ The average mtDNA content was 0.65-1%, which is comparable to the mtDNA content of the human genome of 0.7%.
- ✓ Target Sequence Enrichment using the patent pending approach was achieved using Haemoglobin Subunit Beta (HBB) specific PCR primers for β-thalassemia mutation detection (Figure 3 and 4).

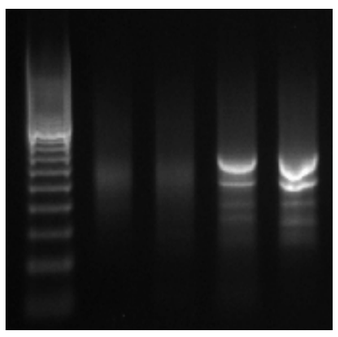


Figure 3. Enrichment of targeted DNA during WGA with TSE following the addition of HBB specific primers compared to WGA only. Multiplex PCR products for HBB. Lane 1 DNA marker, lane 2-3 WGA only and lane 4-5 WGA with TSE.

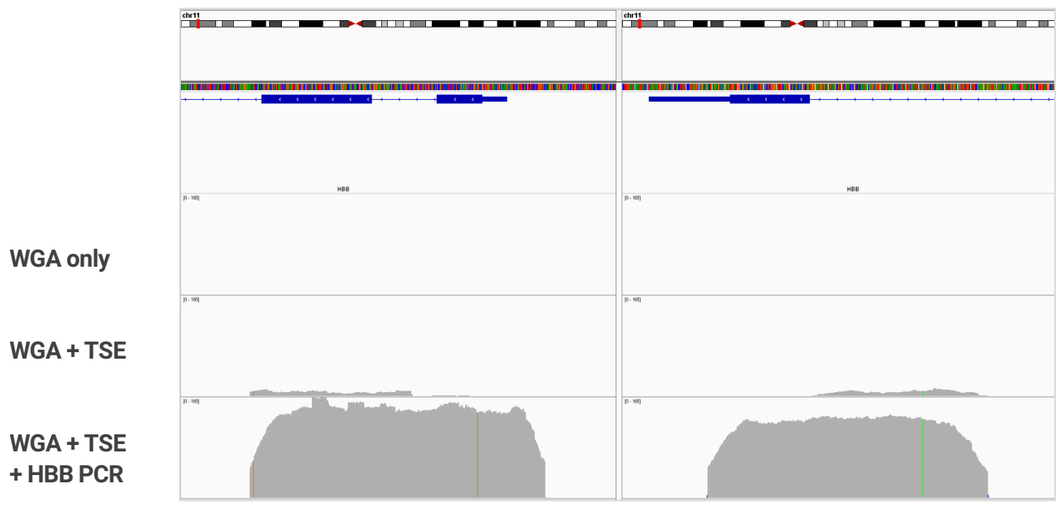
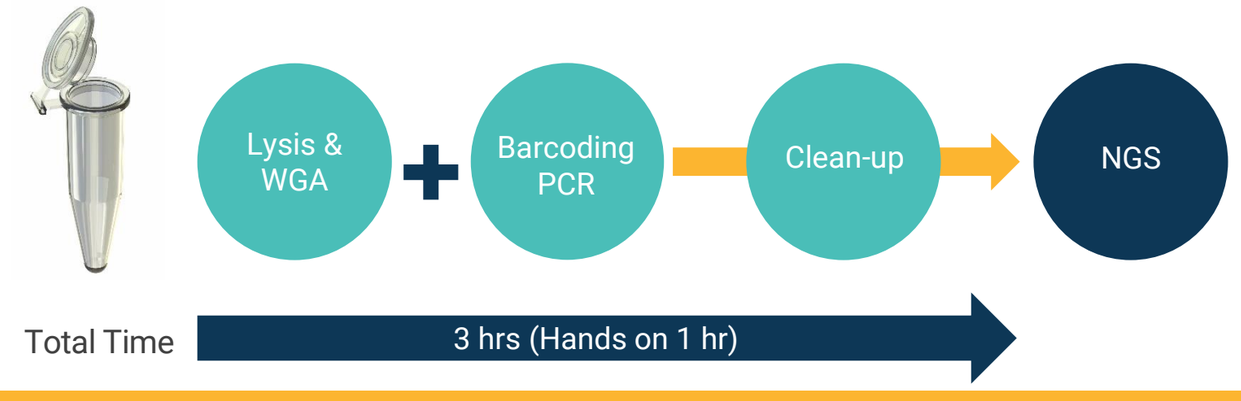


Figure 4. Integrative Genomics Viewer (IGV) screenshot of HBB for DOPlify® WGA only sample (x0 reads) and enrichment of target DNA during DOPlify® WGA with TSE (x13 reads) or following combined DOPlify® WGA + TSE + HBB multiplex PCR (1:10 dilution) (x165 reads).

- ✓ The most time efficient protocol produced amplified, sequencing ready samples within 3 hours including clean-up time, with a hands on time of approximately 1 hour.



Conclusions

Leveraging the unique characteristics of the DOPlify® kit reagents, this novel method provides a single tube amplification and barcoding protocol for Ion Torrent™ NGS to allow:

- rapid, scalable and economical sequencing for PGT-A; and
- the incorporation of the Target Sequence Enrichment protocol for combined PGT-M and PGT-A from a single embryo biopsy