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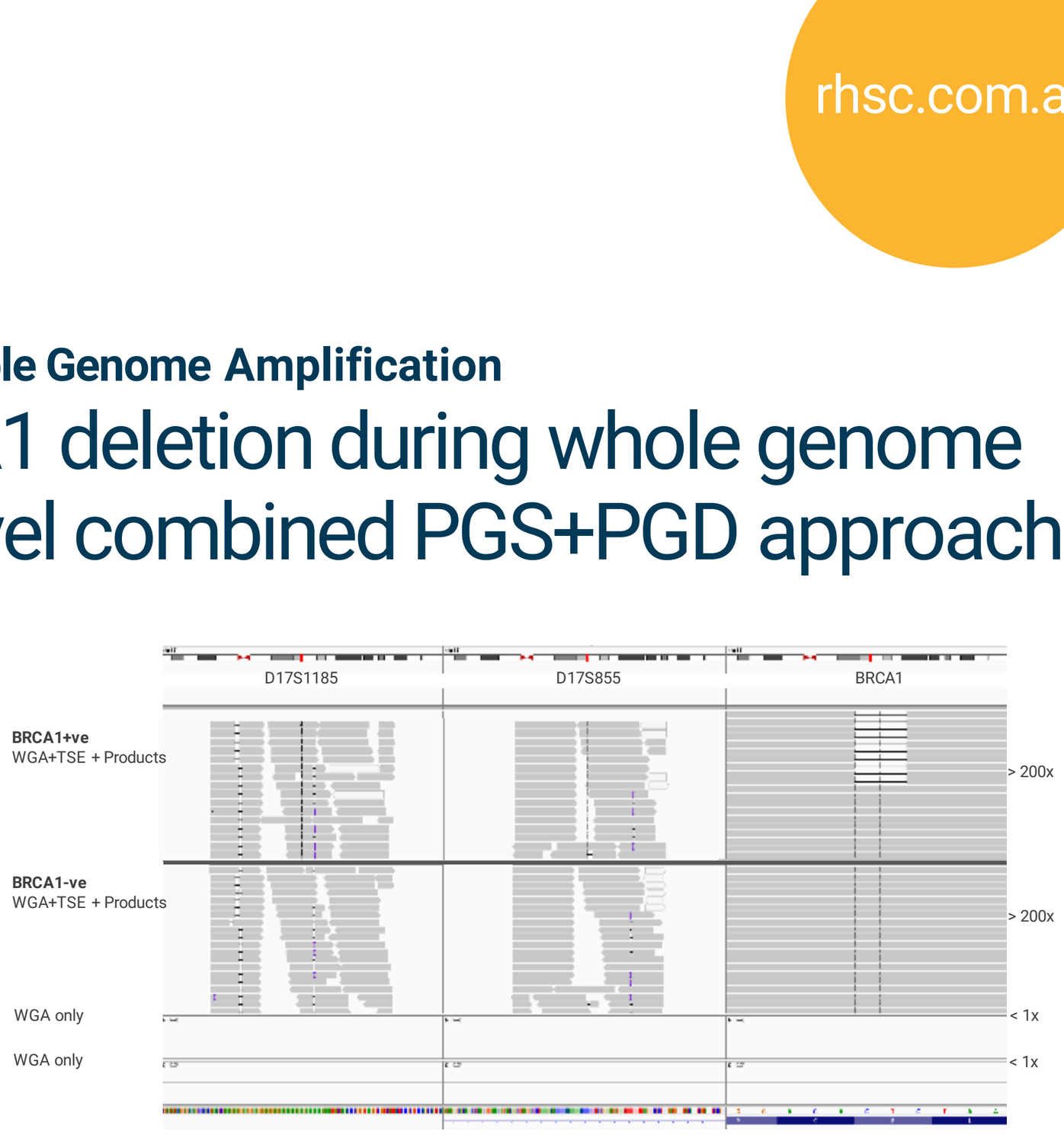
DOPlify™ A New Generation of Whole Genome Amplification

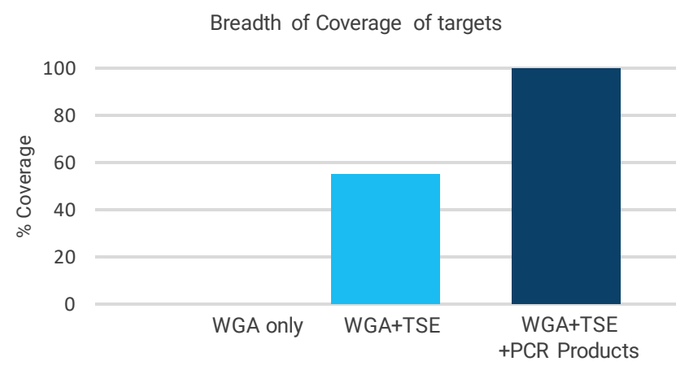
Enrichment of a BRCA1 deletion during whole genome amplification for a novel combined PGS+PGD approach

Breast cancer risk can be increased by mutations in the BRCA1 gene. It is possible to perform preimplantation genetic diagnosis (PGD) to pre-screen IVF embryos and remove the risk factor from future generations. RHS have developed a novel method for concurrent PGD and aneuploidy screening for preimplantation genetic screening (PGS) from a single embryo biopsy.

Aim – To demonstrate combined PGD and PGS using DOPlify™ and next generation sequencing (NGS) for detection of a 2bp clinically-relevant BRCA1 deletion.

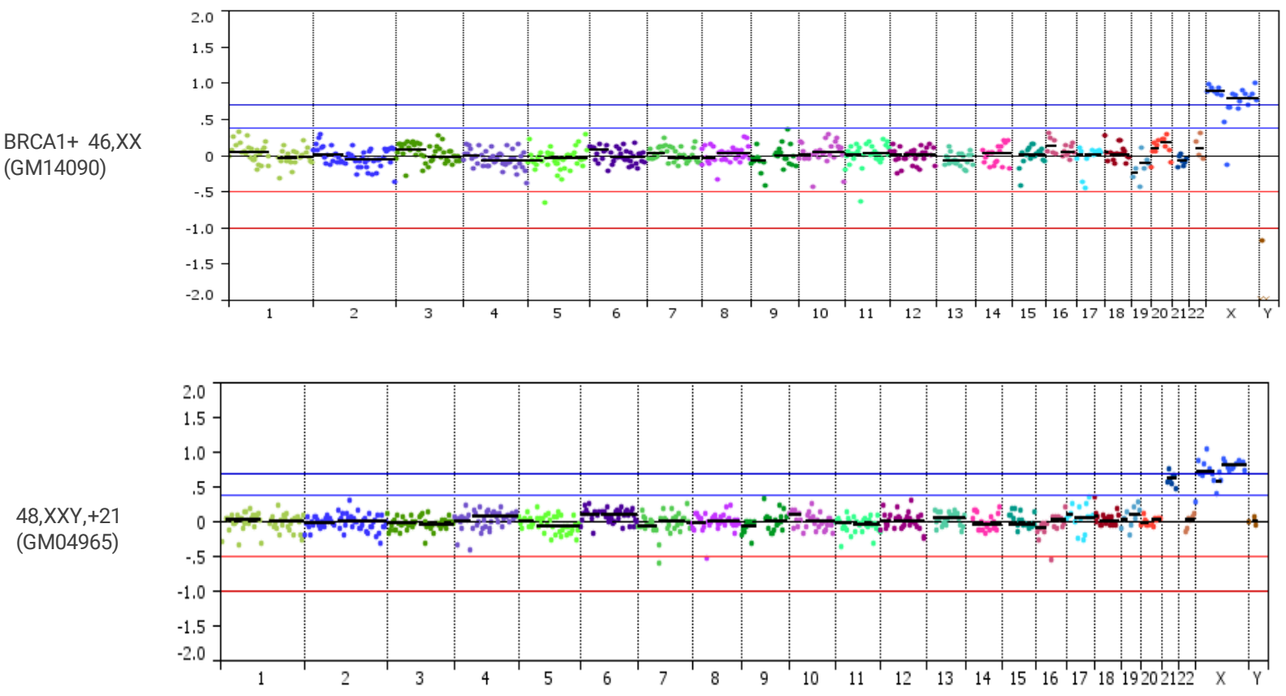
Methods – 5-cell aliquots from a BRCA1 mutation positive (GM14090) cell line and a control 48,XXY,+21 (GM04965) cell line (Coriell Institute) were subjected to whole genome amplification (WGA) using the DOPlify™ Targeted Sequence Enrichment (TSE) protocol (RHS Ltd). The enrichment included primer sets for BRCA1; intragenic marker, D17S855; and flanking marker, D17S1185. Enrichment of the targeted region was determined using semi-quantitative PCR and also NGS. Gene specific PCR products further amplified from the WGA+TSE products were pooled with the WGA+TSE DNA prior to library preparation and NGS using a standard MiSeq, 2x75bp protocol (Illumina); n=40 samples).





✓ Breadth of coverage was 100% with TSE compared to 0% with WGA only and no enrichment using low pass NGS.

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



Results

✓ Enrichment of the BRCA1 targeted region and the linked markers for PGD was achieved.

✓ Depth of coverage for PGD calling was > 60x, sufficient for confident diagnosis even with economical low pass NGS.

✓ 2bp heterozygous BRCA1 deletion was evident with an allelic frequency of 43%.

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 MiSeq run and
- offers a scalable and economical PGD+PGS protocol

Kimberly Warren, Bree Hodgson, Melinda Jasper

RHS Ltd, 40-46 West Thebarton Road, Thebarton South Australia 5031, Australia | **rhsc.com.au**