DOPlify® A new generation of whole genome amplification

Accurate combined preimplantation genetic testing with DOPlify® WGA and target sequence enrichment

Allele Drop Out (ADO), or failed PCR amplification of one or both alleles, presents a significant risk of misdiagnosis or the return of a “no result” for Preimplantation Genetic Testing for monogenic disease (PGT-M) cycles. Although a PGT-M result may indicate that an embryo is unaffected by a monogenic disease, standard PGT-M methods are unable to assess aneuploidy and up to 50% of these transferred embryos may be aneuploid[1]. Combining Whole Genome Amplification (WGA) along with gene-specific primers in a single PCR reaction to allow both aneuploidy detection (PGT-A) and PGT-M is an advantage of the DOPlify® kit and Target Sequence Enrichment (TSE) protocol (patent pending).

Aim – To compare ADO rates across three different approaches; Gene Specific PCR only (current PGT-M methodology), DOPlify® WGA only (current PGT-A methodology) and DOPlify® WGA with TSE.

Methods - Thirty 5-cell aliquots were manually sorted from the euploid cell line GM14090 containing a 2 bp heterozygous BRCA1 mutation and aneuploid cell line GM04965 48,XXY,+21 (Coriell Institute for Medical Research). Cells were amplified using three different PCR methodologies as shown in the flow diagram below (Figure 1). Gene specific primers included one BRCA1 primer set designed to specifically amplify the region on chromosome 17 containing the deletion site in GM14090 (212bp) and another two primer sets to amplify BRCA1 linked markers (D17S855; 193bp and D17S1185; 287bp). Presence, allelic frequency and read depth of the 2 bp heterozygous deletion and the linked markers were measured to determine ADO rates. Samples with read depth less than 10x were excluded from analysis, as would be done clinically.

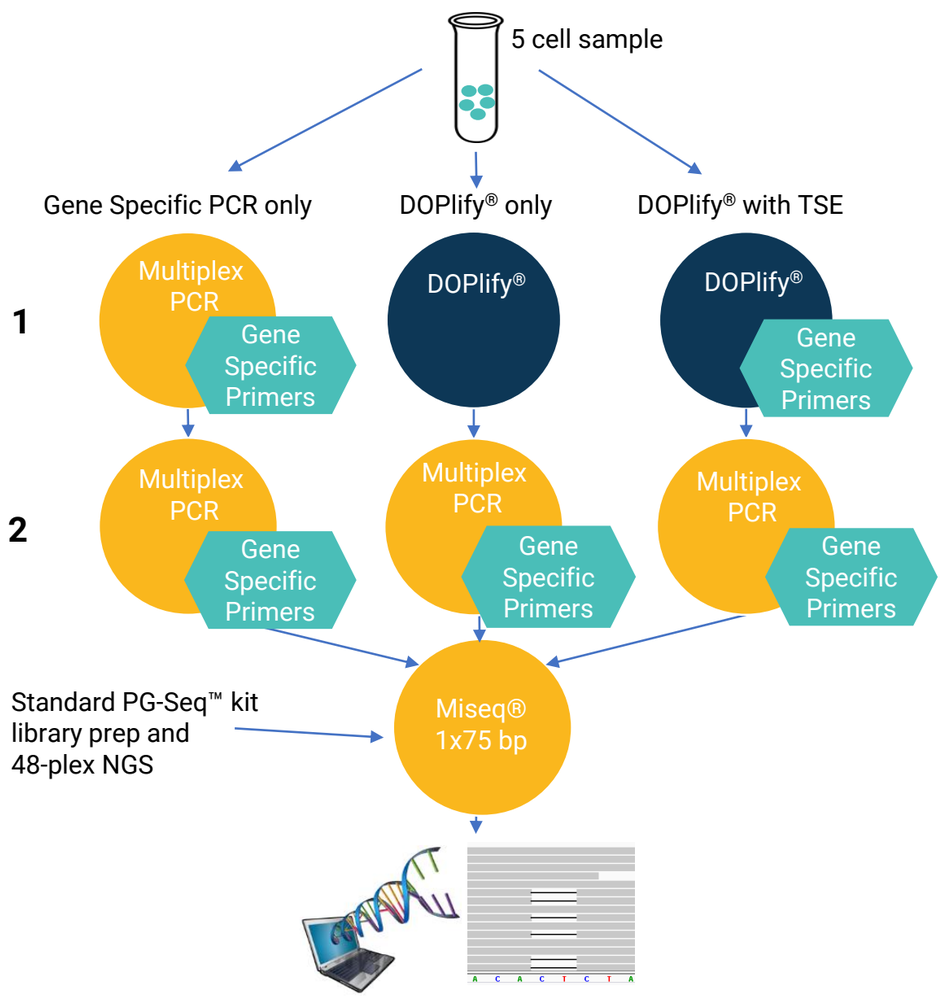

Figure 1. Flow diagram of the three different PCR methodologies tested.

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Results

- ✓ DOPlify® WGA with TSE protocol showed no ADO in all three regions amplified by the mutation specific and linked marker primers.
- ✓ Gene Specific PCR after DOPlify® WGA only (no TSE) showed higher ADO rates for all three regions, suggesting it is unsuitable for combined PGT-M and PGT-A.
- ✓ The Gene Specific PCR only protocol showed no ADO however this approach also does not allow for PGT-A assessment.

	Gene Specific PCR Only	DOPlify® kit Only	DOPlify® kit with TSE
PGT-A	X	✓	✓
PGT-M	✓	X	✓

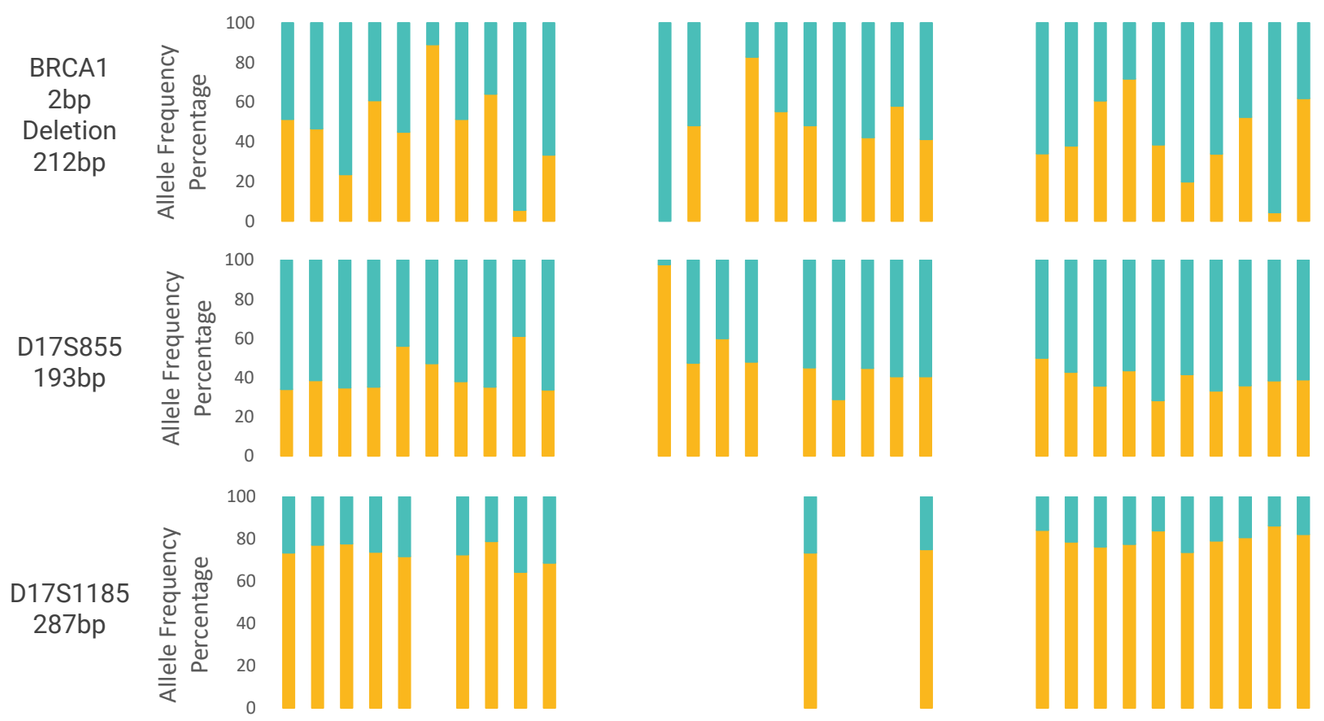

Figure 2. Allele frequency percentages for three target regions analysed with three different PCR methodologies (n=10 per method). Single colour columns indicate the amplification of one allele only. No column indicates samples excluded due to read depth <10x.

- ✓ The average read depth across the 2 bp deletion site was >100 for each approach. The D17S855 and D17S1185 linked marker primer sets were less efficient in the multiplex PCR, which may be improved with further optimisation and deeper sequencing.

Read Depth	Gene Specific PCR Only	DOPlify® Only	DOPlify® with TSE
BRCA1 2 bp deletion	142 ± 4	137 ± 12	119 ± 13
D17S855	112 ± 19	72 ± 40	96 ± 11
D17S1185	98 ± 34	64.5 ± 74	28 ± 8

- ✓ Correct aneuploidy results were obtained for all GM04965 and GM14090 cells amplified with the DOPlify® WGA with TSE protocol (see examples Figures 3 and 4).

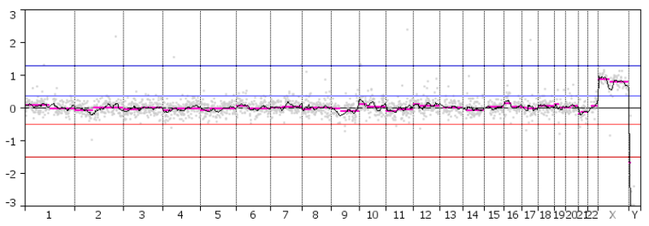

Figure 3. PGT-A result for GM14090 (46,XX)

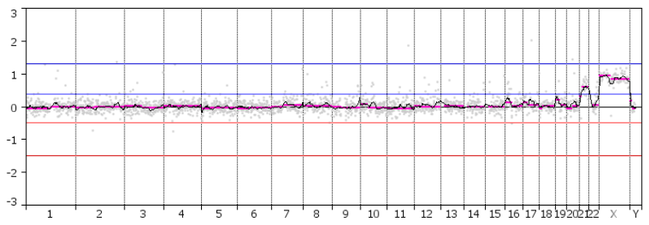

Figure 4. PGT-A result for GM04965 (48,XXY,+21)

Conclusions

DOPlify® WGA with Target Sequence Enrichment achieves:

- complete coverage of multiplexed target gene sequences during whole genome amplification; and
- a sensitive and reliable PGT-M result, showing no ADO in any of the three regions while also providing accurate PGT-A information from a single embryo biopsy

Reference: [1] Goldman et al. J Genet Couns 2017;25:1327-37