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Unique Genomic Solutions

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DOPlify® A new generation of whole genome amplification

DOPlify®, Target Sequence Enrichment and Allele Drop Out – is there a benefit?

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 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 monogenic disease detection (PGT-M) is an advantage of DOPlify® and RHS' Target Sequence Enrichment (TSE) protocol.

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 (DOPlify® PGT-A with RHS' TSE patented approach).

Methods - Thirty 5-cell aliquots were manually sorted from cell line GM14090 containing a 2bp heterozygous BRCA1 mutation and aneuploid cell line GM04965 48,XXY,+21 (Coriell Institute). 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 2bp 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.

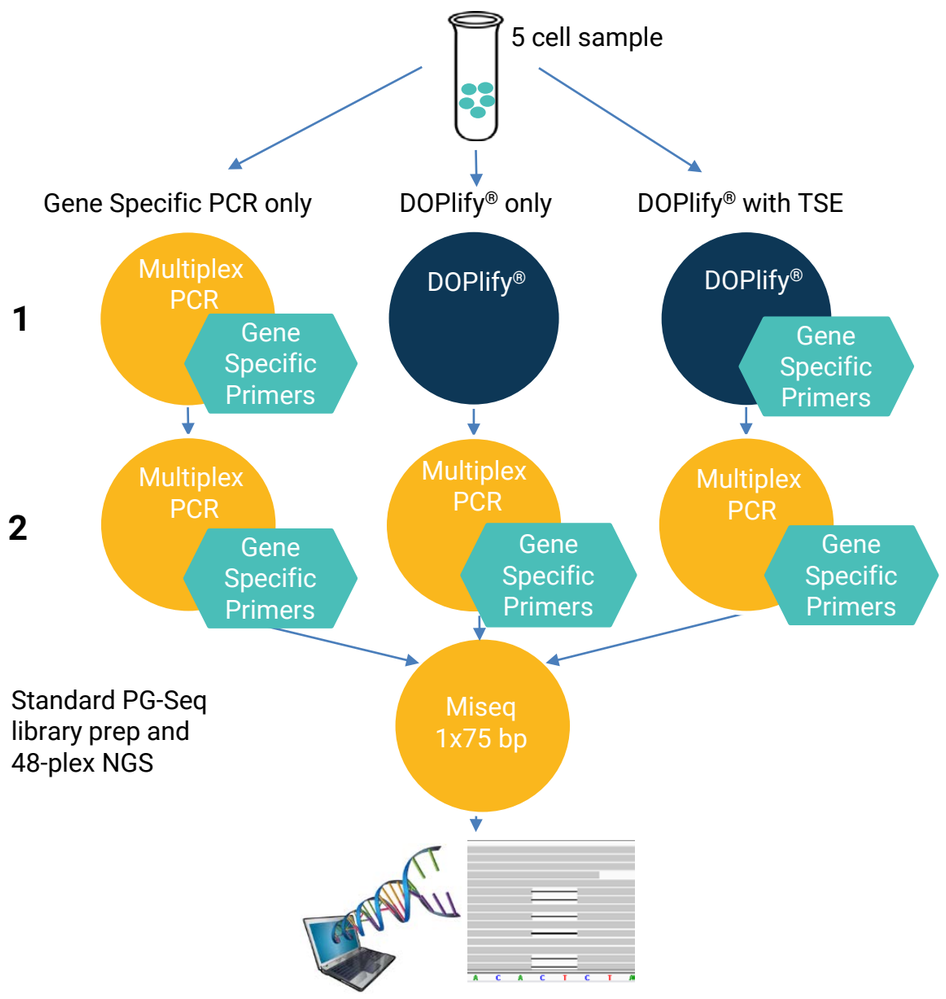

Figure 1. Flow diagram of the three different PCR methodologies tested.

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Results

- ✓ The DOPlify® with TSE protocol showed no allele drop out in all three regions amplified by the mutation specific primers and linked markers.
- ✓ Gene Specific PCR after DOPlify® 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® Only	DOPlify® with TSE
PGT-A	X	✓	✓
PGT-M	✓	X	✓

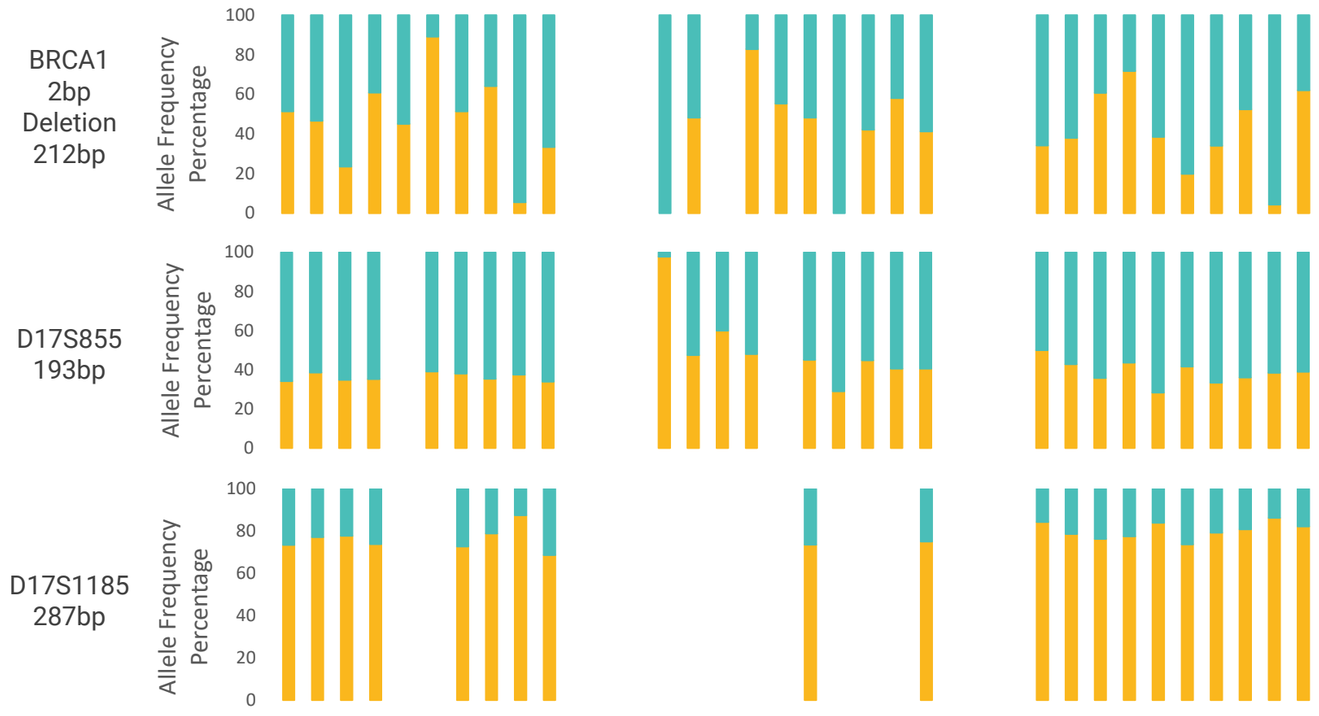

Figure 2. Allele frequency percentages for three target regions analysed with three different PCR methodologies (n=10 per method). Single colour columns indicate that one allele dropped out. No column indicates samples excluded due to read depth <10x.

- ✓ The average read depth across the 2bp 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 2bp deletion	129 ± 38	138 ± 12	120 ± 13
D17S855	113 ± 40	66 ± 44	69 ± 12
D17S1185	77 ± 49	14 ± 36	28 ± 8

- ✓ Correct aneuploidy results were obtained for all BRCA1 and GM04965 cells amplified with the WGA with TSE protocol. See Figures 3 and 4 for examples.

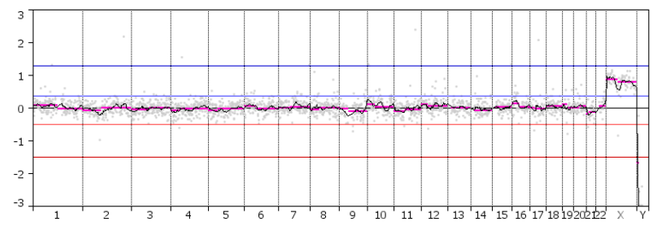

Figure 3. PGT-A result for GM14090 (46,XX)

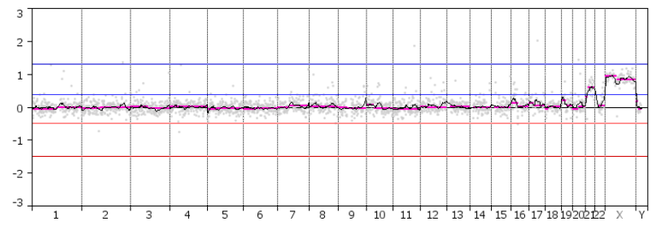

Figure 4. PGT-A result for GM04965 (48,XXY,+21)

Conclusions

DOPlify® with Target Sequence Enrichment achieves:

- complete coverage of multiplexed target gene sequences during whole genome amplification; and
- a sensitive and reliable PGT-M result while also providing accurate PGT-A information from a single embryo biopsy

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