PerkinElmer®
For the Better

DOPlify® Kit - A new generation of whole genome amplification

Combined multiplex target enrichment and CNV detection using a single NGS index

Combining Whole Genome Amplification (WGA) along with gene-specific primers in a single PCR reaction to simultaneously allow copy number variation (CNV) detection and high resolution DNA analysis from a single cell or low-DNA template sample is an advantage of the DOPlify® kit and the Target Sequence Enrichment (TSE) protocol.

Aim – The aim of this study was to demonstrate a novel multiplex sequence enrichment protocol using the DOPlify® kit for combined detection of a 2bp heterozygous deletion and CNV detection using a single sequencing index and low-pass Next Generation Sequencing (NGS).

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 WGA using the DOPlify® kit and TSE protocol (PerkinElmer). The enrichment included primer sets for BRCA1; intragenic marker, D17S855; and flanking marker, D17S1185. Enrichment of the targeted regions was determined using semi-quantitative PCR and also NGS. Gene-specific PCR products further amplified from the WGA+TSE products were pooled 1:10 with the WGA+TSE DNA prior to library preparation. (Figure 1). NGS was performed using a standard MiSeq® instrument, 1x75bp protocol (Illumina); n=48 samples. Breadth of coverage, allelic frequency and read depth of the 2bp heterozygous deletion and the linked markers were measured. Allele dropout (ADO) rate for the TSE protocol was compared to gene-specific PCR amplification alone or gene-specific PCR amplification using the WGA for template.

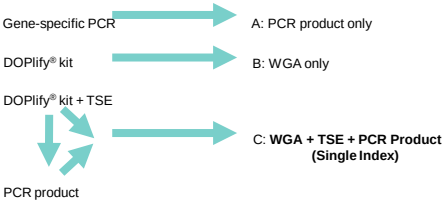

Figure 1. Sample format

The cost to performed the DOPlify® kit TSE workflow was US$100 per sample using low pass sequencing.

Results

- ✓ 2bp heterozygous BRCA1 deletion was evident with an allelic frequency of 43% (Figure 2 and 3).

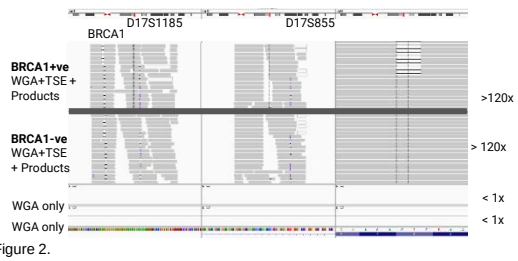

Figure 2.

- ✓ Breadth of coverage was 100% with TSE compared to < 10% with WGA only and no enrichment using low pass NGS.
- ✓ The DOPlify® kit with TSE protocol showed no allele drop out in all three regions amplified by the mutation specific primers and linked markers (Figure 3).
- ✓ Gene-specific PCR after DOPlify® kit only (no TSE) showed higher ADO rates for all three regions, suggesting it is unsuitable for combined DNA analysis.
- ✓ The gene-specific PCR only protocol showed no ADO however this approach does not allow for CNV assessment.

	Gene-specific PCR Only	DOPlify® kit only with no TSE	DOPlify® kit with TSE
Genome wide CNV	X	✓	✓
High resolution DNA analysis	✓	X	✓

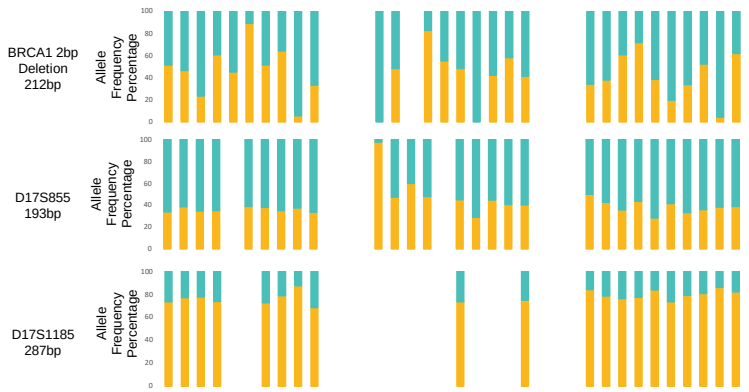

Figure 3. Allele frequency percentages for three target regions analysed with three different PCR methodologies (n=10 per method). Single colour columns indicate no amplification of one allele. No column indicates samples excluded due to read depth <10x.

- ✓ Correct CNV results were obtained for all 5-cell samples amplified with the DOPlify® Kit TSE protocol (see example Figures 4)

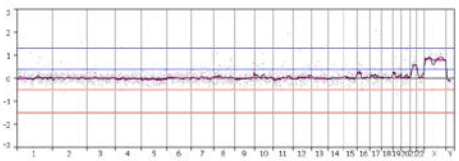

Figure 4. CNV result for GM04965 (48,XXY,+21)

- ✓ Successful evaluation of a multiplex of 36 primer sets demonstrated the utility of the workflow, and the possibility of expanding the primer panel size. An upper primer limit has not yet been determined.

Conclusions

The DOPlify® kit with Target Sequence Enrichment and a low pass NGS protocol readily achieves ;

- complete coverage for high resolution DNA analysis using a multiplex primer panel combined with
- copy number detection from a low-DNA template sample.

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