Molecular Inversion Probe based *BRCA1 and BRCA2* re-sequencing in a clinical setting

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Introduction

Mutations in *BRCA1* and *BRCA2* confer high risks of hereditary breast and ovarian cancer. Over the years capillary Sanger sequencing and MLPA were used to detect causal variants in breast cancer families. Next generation sequencing technologies allowed implementing new strategies thereby reducing sequencing costs without losing sensitivity. Today sequencing costs of targeted NGS-based strategies are largely determined by enrichment procedures. Molecular inversion probes (MIPs) have shown to be a cost-effective enrichment used in multiplex, particularly if used in large cohorts[1]. MIPs use probes with two locus-specific regions at the ends that form a partial circle when hybridized to the target region. The gap is filled by extension and ligation. Exonucleases remove any non-circularized DNA (Fig.1).

Here we present a workflow of re-sequencing *BRCA1* and *BRCA2* using single molecule molecular inversion probes (smMIPs).

Methods

- The strategy involves all coding exons and their flanking intronic sequences (*BRCA1*:NM_007294.3, *BRCA2*: NM_000059.3). The design consists of 422 overlapping smMIPs on both strands avoiding known SNPs in binding sites.
- Every base was targeted by at least two independent smMIPs.
- Paired end sequencing (2x150bp) was done on a NextSeq500 using a mid-output flow cell (130M reads, Illumina™).
- Sequencing results were analyzed with the SeqNext module of JSI SeqPilot™ (version 4.2.0 build 503).
- In total 152 BRCA mutation carriers were tested (86 *BRCA1* and 66 *BRCA2* comprising positive *BRCA1/2* test requests from January 2010 to July 2014).

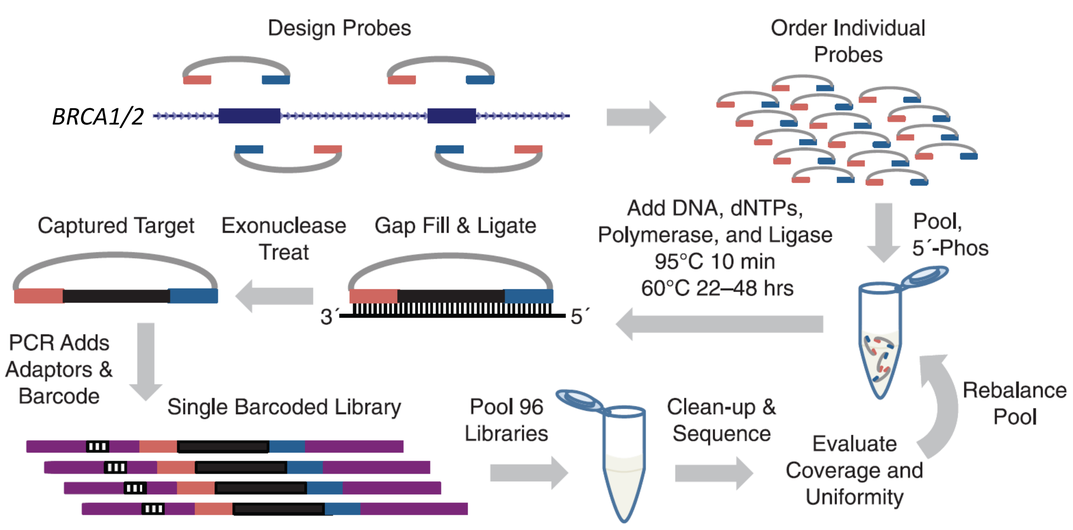


Fig.1 Schematic showing a general workflow of a MIP method (adapted from O'Roak et al).

Results

Coverage statistics BRCA1/2	Run 1	Run 2	Run 3
Average coverage smMIP *BRCA1* (stdev)	416 (129)	360 (195)	302 (153)
Average coverage smMIP *BRCA2* (stdev)	297 (99)	296 (170)	276 (141)
% *BRCA1* covered >40x (>2 smMIPs)	100%	100%	98%
% *BRCA2* covered > 40x (>2 smMIPs)	97.6%	97.6%	97.6%
% *BRCA1* covered > 100x (>2 smMIPs)	96 %	100 %	95.3 %
% *BRCA2* covered > 100x (>2 smMIPs)	95.2%	94.8%	92.3%
Horizontal coverage *BRCA1* [-20..20] >40x	100%	100%	100%
Horizontal coverage *BRCA2* [-20..20] >40x	100%	100%	100%

Variant Statistics			
No. of samples	54	57	58
No. of pathogenic variants	54	57	58
% Subsititutions	31.5%	42.1%	39,7%
% Dup/ins	18.5%	21.1%	12.1%
% Del	50%	36.8%	48.2%
% True positive	100%	100%	100%
% False positive	0 %	0 %	0%
% False negative	0 %	0 %	0%

Sensitivity and specificity

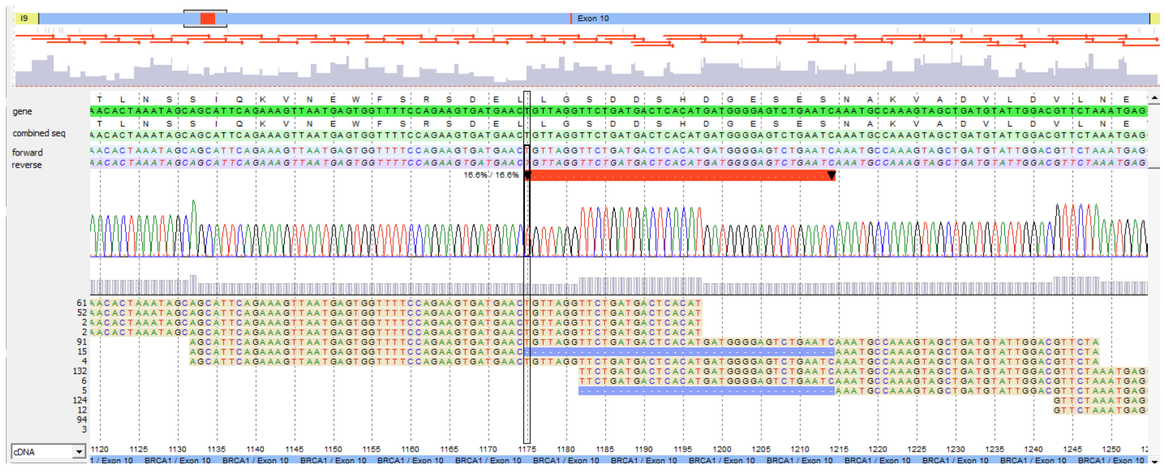
Number of samples*	152	
Total of sequenced bases	2,933,424	
True positive (TP)	1,821	(path. mutations+SNPs)
False positive (FP)	0	(false positive calls)
True negatives (TN)	2,931,603	(bases identical to reference)
False negative (FN)	0	(missed variants)
True positive rate	100 %	= TP / (TP+FN)
False positive rate	0%	= FP / (FP+TN)
Accuracy	100%	= (TP+TN)/(TP+TN+FP+FN)
Precision	100%	= (TP/(TP+FP)
True negative rate	100%	= TN/(FP+TN)

*17 samples have not been included in this calculation because in the original sequencing analysis only the pathogenic mutation containing amplicon was tested.

Conclusion

- We have demonstrated the development and validation of a smMIP workflow for routine diagnostic testing of *BRCA1* and *BRCA2*.
- The analytical sensitivity and specificity are matching those of the gold standard Sanger and NGS-based BRCA sequencing workflows.
- With the use of smMIPs sequencing costs are markedly reduced because of the library-free target enrichment.
- FFPE derived DNA also provides good results (data not shown).
- Future developments will include optimizing CNV detection.

Fig.2 Screenshot of a *BRCA1* exon 10 c.1175_1214del pathogenic mutation



[1] O'Roak et al, Science (2012) 338(6114):1619-22)

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