

Next generation sequencing, a new gold standard for clinical gene panel testing

The adaption of the Next Generation Sequencing (NGS) technology to clinical laboratories has revolutionized the molecular diagnostics by reducing the costs and increasing the throughput. Clinical use of NGS enables simultaneous testing of multiple genes (gene panels), the entire exome or genome using a limited quantity of DNA, in addition to allowing for multiple patient samples to be tested in a single experiment.

Highly penetrant mutations in the cancer susceptibility genes, *BRCA1* and *BRCA2*, increase the risk of a person to develop Hereditary Breast and Ovarian Cancer (HBOC) to as high as 70-80%. The identification of *BRCA* mutations is important for early cancer diagnostic, determination of therapeutic strategies, and individual and familial genetic counseling.

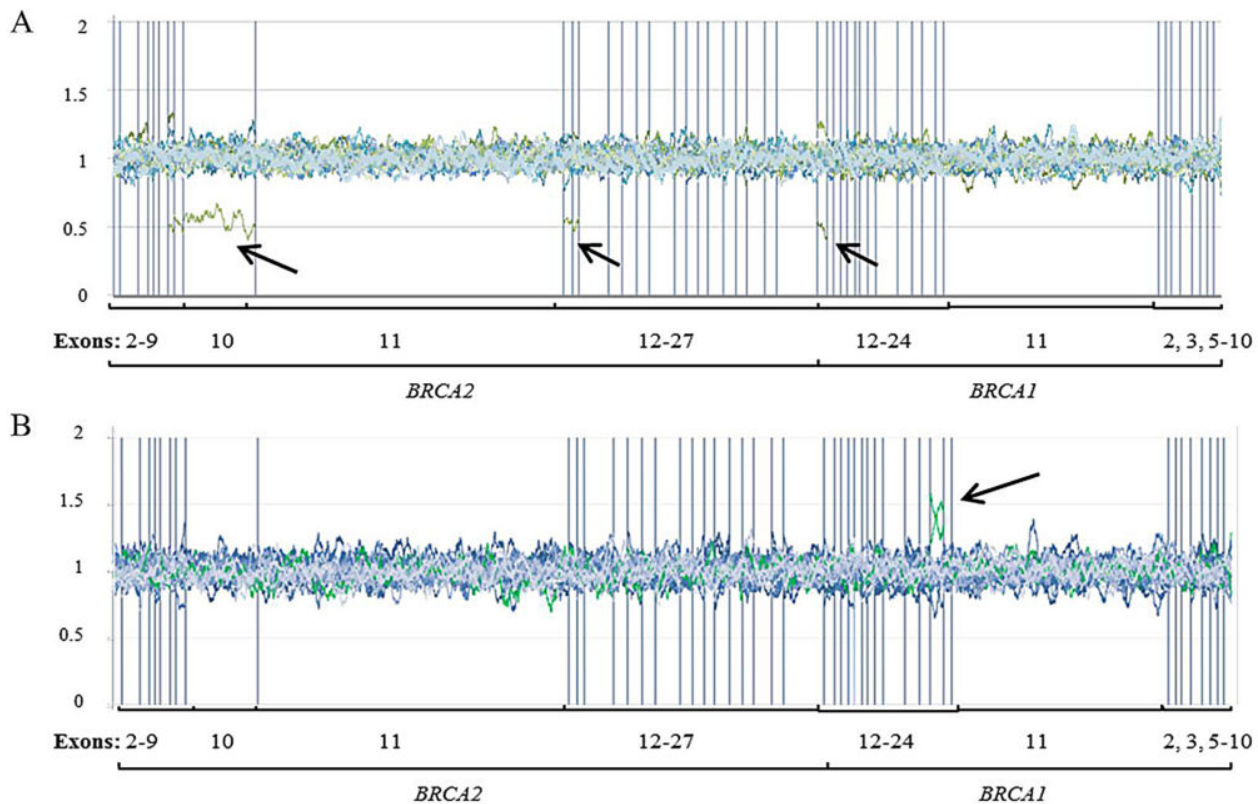


Fig. 1. RD plot demonstrating deletion detection (A) and gene duplication (B). Each horizontal line is one patient (total=24); Gene and exon locations are indicated (X-axis); Vertical blue lines indicate exon boundaries; Y- axis represents normalized RD data [for autosomal genes 1= 2 chromosome copies, 0.5= 1 chromosome copy (deletion), 1.5= 3 chromosome copies (duplication)].

Currently, the most common approaches for clinical diagnostics of HBOC involve Sanger sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA) which detect sequence variants (point mutations) and copy-number variants (large deletions and duplications), respectively. This procedure, although very sensitive, is relatively expensive and requires a high amount of experimental workload and hands-on time. This abstract describes the clinical validation of an NGS-based gene panel for *BRCA1/BRCA2* testing that can detect both point mutations and copy-number alterations in a single assay.

The panel was designed to process 24 patient samples in a single assay, covers all 46 exons *BRCA1* and *BRCA2* genes along with 20 nucleotides of intronic boundaries of each exon. The number of reads that align to every genomic region in NGS (Read Depth-RD) is proportional to the number of copies of that region present in the sample, therefore, this assay allows for parallel assessment of copy-number variants along with sequence changes in these genes, at a sensitivity of classical “gold-standard” approaches. Figure 1 shows the sequence alignment and RD for each nucleotide after bioinformatics normalization of the data in *BRCA1* and *BRCA2* genes for 24 patients. In this example, we demonstrate a detection of a *BRCA1* exon 24 deletion, and another complex *BRCA2* deletion involving exons 8-10 and 12-13 (Fig. 1A); as well, two cases of *BRCA1* duplication involving exon 13 (Fig. 1B) in patients with hereditary breast cancer.

A total of 402 patients who had previously been tested with Sanger sequencing and MLPA, as well as 240 clinical patients without previous testing were included for the validation of the assay. 183 variants (point mutations and copy number variants) were detected. The NGS panel yielded 100% sensitivity (true positive rate) and 100% specificity (true negative rate) as compared to Sanger sequencing and MLPA in detecting *BRCA1/BRCA2* mutations. A high RD rate along with low intra and inter-sample coverage variability utilized in this approach allowed accurate estimation of copy number change.

We have expanded this approach which is currently in routine use in a clinical laboratory setting at the London Health Sciences Molecular Genetic Laboratory on many clinical gene panels, including Charcot Marie Tooth syndrome, hereditary cancer syndrome, epilepsy, dyslipidemia, mitochondrial genome sequencing and others, all of which meet or outperform the quality criteria of “gold standard” approaches, with significant cost, capacity, and throughput improvements.

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Publication

[Clinical Next-Generation Sequencing Pipeline Outperforms a Combined Approach Using Sanger](#)

[Sequencing and Multiplex Ligation-Dependent Probe Amplification in Targeted Gene Panel Analysis.](#)

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