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Massive parallel sequencing for diagnostic genetic testing of BRCA genes - A single center experience

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Abstract

The aim of this study was to implement massive parallel sequencing (MPS) technology in clinical genetics testing. We developed and tested an amplicon-based method for resequencing the BRCA1 and BRCA2 genes on an Illumina MiSeq to identify disease-causing mutations in patients with hereditary breast or ovarian cancer (HBOC). The coding regions of BRCA1 and BRCA2 were resequenced in 96 HBOC patient DNA samples obtained from different sample types: peripheral blood leukocytes, whole blood drops dried on paper, and buccal wash epithelia. A total of 16 random DNA samples were characterized using standard Sanger sequencing and applied to optimize the variant calling process and evaluate the accuracy of the MPS-method. The best bioinformatics workflow included the filtration of variants using GATK with the following cut-offs: variant frequency > 14%, coverage (> 25×) and presence in both the forward and reverse reads. The MPS method had 100% sensitivity and 94.4% specificity. Similar accuracy levels were achieved for DNA obtained from the different sample types. The workflow presented herein requires low amounts of DNA samples (170 ng) and is cost-effective due to the elimination of DNA and PCR product normalization steps.

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Keywords

Amplicon sequencing, BRCA, Diagnostics, Massive parallel sequencing, NGS