Implementation of Next Generation Sequencing to acess hereditary breast cancer risk PCR-based of multiple candidate genes

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INTRODUCTION

Tumor suppressor genes *BRCA1* and *BRCA2* are the two main breast and ovarian cancer susceptibility genes. Both of which encode large proteins, consequently it to sequence all coding regions using Sanger method is time-consuming and expensive[1]. Nevertheless, other genes have been shown to be associated with hereditary breast cancer phenotype[1]. The next generation sequencing (NGS) allows to analyze simultaneously different genes and samples, reducing the time of analysis and overall cost[2]. In this study, we propose to develop an approach using multiplex and Long-Range PCR-based assays for NGS screening of mutation in nine genes related with hereditary breast cancer in patients with clinical criteria.

MATERIAL AND METHODS

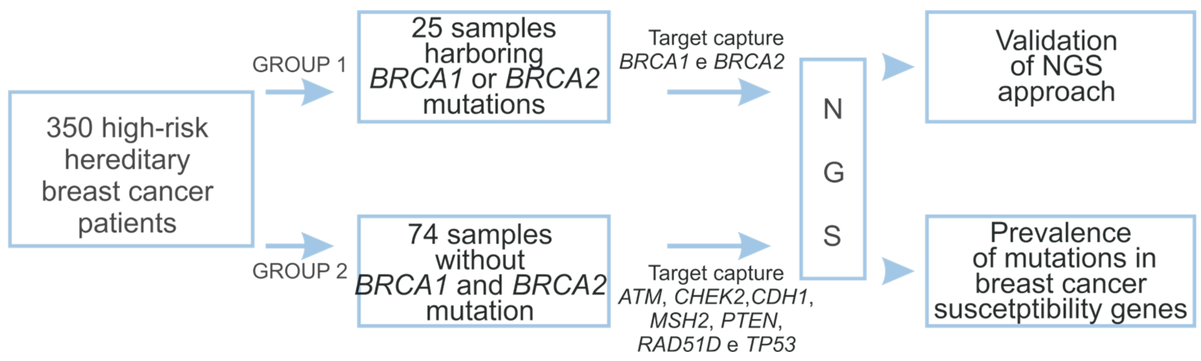

Figure 1. Summary of the methodology followed in the present study.

RESULTS AND DISCUSSION

The sequencing was initially divided in two experiments (Table 1). In the first experiment, a high variation of coverage was observed between all amplicons, 7.3% of the amplicons did not meet the coverage of at least 27x (Figure 2A). In the second one, after PCR optimization, just 1.53% of the amplicon did not reach 27x of coverage, and was observed an uniform coverage distribution (Figure 2B). To cover the complete coding regions with at least 27x of coverage, a minimum of 1.120.937 and 5.063.555 reads was sufficient for first and second experiment, respectively. Of the 214 sequence variants identified by Sanger sequencing, 201 were also identified by NGS (Table 2). Among 13 missed variants, eight were filtered out because coverage was <27x, three presented alternative allele ratio <20%, one of them was filtered out due the pipeline filters, and the last one was not detected by pipeline limitation. The overall sensitivity and the positive predictive value of NGS were estimated to be 93.9% and 82.7%, respectively. The concordance between NGS and Sanger sequencing was 78,82%. An estimate of the costs, taking into account the main inputs for the sequencing of the *BRCA1* and *BRCA2* genes was performed and showed a 5-fold cost reduction of the NGS in respect to the Sanger method. The PCR reactions for the other genes were standardized. A control sample was sequenced for the nine genes selected for this project, and in 21.8% of the amplicons the coverage <27x. We expect to perform the sequencing of the 25 samples of the group 2 in the next months, and be able to evaluate the read coverage for each target region and to optimize the methodology aiming a minimum coverage of 27x for each amplicon, with uniform coverage distribution among amplicons and samples.

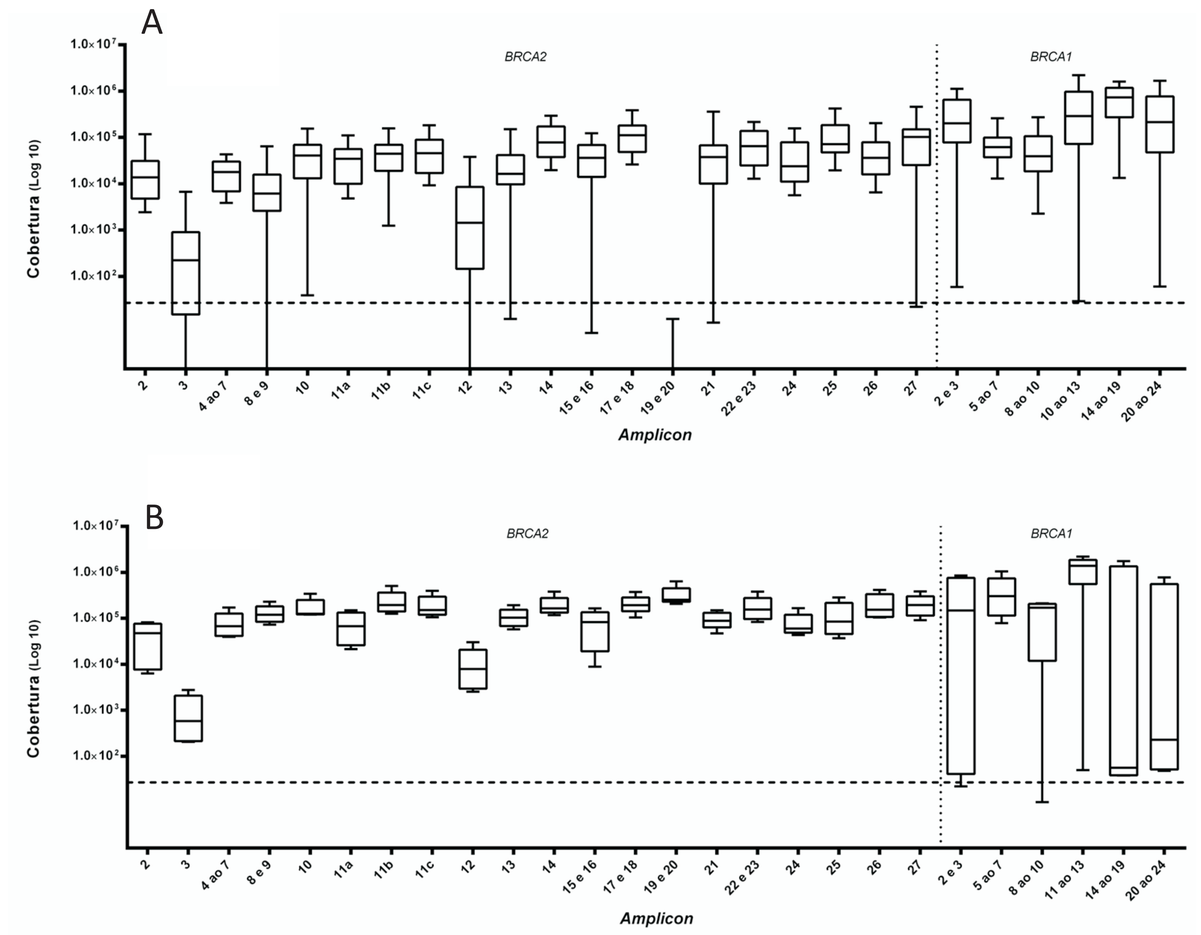

Figure 2. Distribution of coverage for *BRCA1* e *BRCA2* genes. A) First sequencing of 20 samples B) Second experiment of five samples. Horizontal dashed line represent 27x coverage. Vertical doted line divided *BRCA1* and *BRCA2* amplicons.

		SANGER SEQUENCING	
		Detected variants	*Undetected variants*
NGS	*Variants called*	201	41
	Variants not called	13	-

Table 2. Variants detected by NGS and Sager Sequencing.

	1ST RUN	2ND RUN
Samples	20	5
Mapped reads	88.208.276	37.210.732
Coverage (mean/median)	123.996/49.157	216.358/118.615
Q>30	91,4%	95,9%

Table 1. Overview of NGS experiments.

CONCLUSION

We showed that LR-PCR, multiplex-PCR followed by NGS of the *BRCA1* and *BRCA2* genes is an efficient protocol for germline mutation screening. Sensitivity, positive predictive value and concordance is high. NGS has advantage over Sanger sequencing when it comes to costs, throughput and turn-around time for the screening of genetic variations, and will be beneficial in clinical use.

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