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The molecular analysis of BRCA1 and BRCA2: Next-generation sequencing supersedes conventional approaches

Valeria D'Argenio [a,b], Maria Valeria Esposito [a], Antonella Telese [a], Vincenza Precone [a,b], Flavio Starnone [a], Marcella Nunziato [a,b], Piergiuseppe Cantiello [a], Mariangela Iorio [a], Eloisa Evangelista [a], Massimiliano D'Aiuto [c], Alessandra Calabrese [c], Giulia Frisso [a,b], Giuseppe D'Aiuto [c], Francesco Salvatore [a,b,d,*]

[a] CEINGE-Biotecnologie Avanzate, via G. Salvatore 486, 80145 Naples, Italy
[b] Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Via Pansini 5, 80131 Naples, Italy
[c] Department of Senology, Istituto Nazionale Tumori — IRCCS Fondazione Pascale, Naples, Italy
[d] IRCCS-Fondazione SDN, Naples, Italy

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ABSTRACT

Background: Accurate and sensitive detection of *BRCA*1/2 germ-line mutations is crucial for the clinical management of women affected by breast cancer, for prevention and, notably, also for the identification of at-risk healthy relatives. The most widely used methods for *BRCA1/2* molecular analysis are Sanger sequencing, and denaturing high performance liquid chromatography (dHPLC) followed by the Sanger method. However, recent findings suggest that next-generation sequencing (NGS)-based approaches may be an efficient tool for diagnostic purposes. In this context, we evaluated the effectiveness of NGS for *BRCA* gene analysis compared with dHPLC/Sanger sequencing.
Methods: Seventy women were screened for BRCA1/2 mutations by both dHPLC/Sanger sequencing and NGS, and the data were analyzed using a bioinformatic pipeline.
Results: Sequence data analysis showed that NGS is more sensitive in detecting *BRCA*1/2 variants than the conventional procedure, namely, dHPLC/Sanger.
Conclusion: Next-generation sequencing is more sensitive, faster, easier to use and less expensive than the conventional Sanger method. Consequently, it is a reliable procedure for the routine molecular screening of the *BRCA*1/2 genes.

1. Introduction

BRCA1 and *BRCA2* are the most important genes predisposing to inherited breast and ovarian cancers [1]. Germline mutations in these two highly penetrant genes can increase the lifetime risk of developing these tumors by as much as 80%, and are also associated with an earlier onset of disease. Moreover, other kinds of cancers (i.e. prostate, pancreatic and gastric cancers) are more common in *BRCA1* and *BRCA2* carriers than in the general population [2]. Therefore, early identification of carriers among women affected by breast or ovarian cancer is crucial to enable patient stratification and to guide clinicians in deciding the most appropriate therapeutic strategy and follow-up program. In addition, accurate genetic counseling can identify at-risk healthy members of affected families, who can then be enrolled in appropriate surveillance programs.

Direct Sanger sequencing continues to be the routine procedure for the molecular analysis of the *BRCA* genes [3,4]. However, given the large size of both *BRCA1* and *BRCA2* and the consequent cost of their direct sequencing, large-scale mutation scanning strategies such as denaturing high-performance liquid chromatography (dHPLC) and high-resolution melting, are also commonly used pre-sequencing methods [5,6]. By dramatically increasing the throughput of sequencing and reducing its costs, next-generation sequencing (NGS) has had a major impact on several fields of molecular research [7–9]. Furthermore, NGS is increasingly being applied in the field of diagnostics, including BRCA analysis [10–15].

Here, we report the outcome of an evaluation study conducted to assess the analytic performances of an NGS-based strategy for the molecular analysis of the *BRCA1* and *BRCA2* genes versus a well established, commonly used, dHPLC/Sanger sequencing strategy.

2. Materials and methods

2.1. Enrollment of patients and sample collection

Seventy women attending the Breast Unit, Istituto Nazionale dei Tumori, Fondazione G. Pascale in Naples, were consecutively enrolled

Abbreviations: BC, breast cancer; dHPLC, denaturing high-performance liquid chromatography; FN, false negative; FP, false positive; NGS, next-generation sequencing; TN, true negative; TP, true positive.

* Corresponding author at: CEINGE-Biotecnologie Avanzate, Dipartimento di Medicina Molecolare e Biotecnologie Mediche – Ed. 19, Via Sergio Pansini 5, 80131 Naples, Italy.
E-mail address: salvator@unina.it (F. Salvatore).

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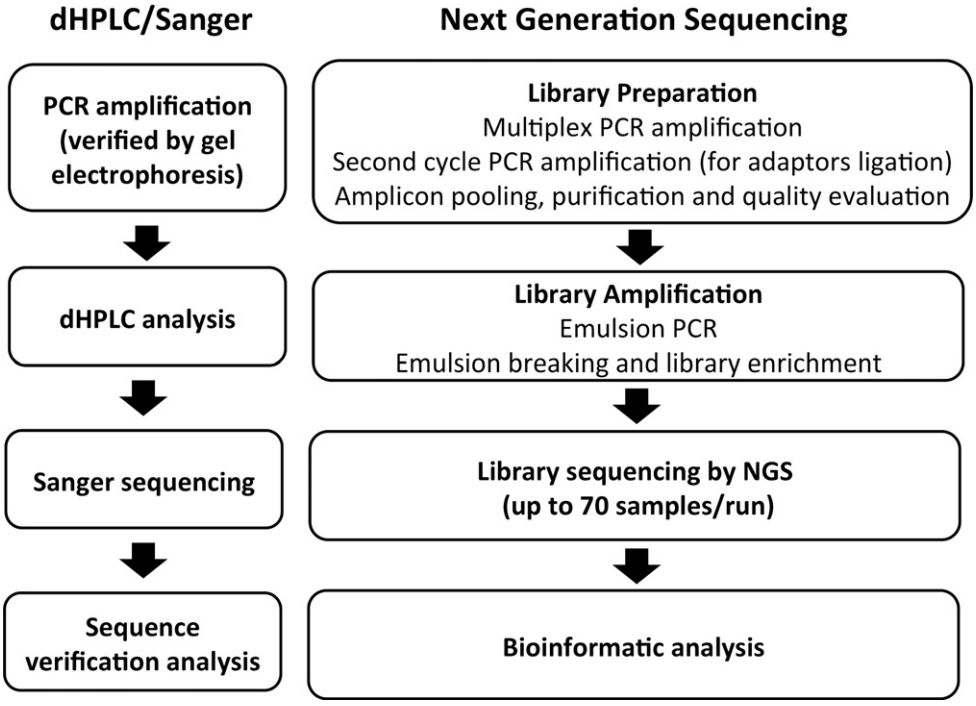

Fig. 1. Complete workflow of the two analytic strategies, dHPLC/Sanger and NGS, used to analyze the 70 at risk women.

in the study. All enrolled patients underwent pre-test counseling during which they were informed about the significance of molecular screening, provided information about their personal and familial history, and gave written informed consent to the study. The 70 enrolled women had at least one of the following conditions: early-onset breast cancer (BC) (diagnosed at 35 years or earlier); bilateral BC; multiple organ cancers, including BC; BC diagnosed at any age with at least one first- or two second-degree relatives with breast and/or ovarian cancer; BC diagnosed with advanced tumor staging; and patients with benign mammary alterations with a BC-positive family history. The latter group consisted of 18 patients and can be considered a small control cohort.

A blood EDTA sample was collected from each subject. Genomic DNA was isolated from peripheral blood using the Nucleon BACC3 Genomic DNA Extraction Kit (GE Healthcare, Life Sciences, Little Chalfont, UK), according to the manufacturer's instructions. The quality of DNA samples was assessed by agarose gel electrophoresis and their quantity was evaluated with the NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Table 1
BRCA1 variants identified in the analyzed population by dHPLC/Sanger and NGS.

Exon/intron	HGVS[1] cDNA	HGVS[1] protein	Mutation type	Clinical relevance (BIC)[2]	Reference ID according to NCBI	Number of times a variant was observed (dHPLC + Sanger/NGS)
IVS7	c.442-3_442 − 3delT	–	Deletion	Unknown	n.r.[3]	1/1
9	c.591C > T	p.Cys197Cys	Synonymous	Polymorphism	rs1799965	1/1
IVS10	c.671-12_671 + 12delG	–	Deletion	Unknown	n.r.[3]	1/1
11	c.1067A > G	p.Gln356Arg	Missense	Unknown	rs1799950	15/20
11	c.1911 T > C	p.Thr637Thr	Synonymous	Unknown	n.r.[3]	1/1
11	c.2077G > A	p.Asp693Asn	Missense	Polymorphism	rs4986850	14/17
11	c.2082C > T	p.Ser694Ser	Synonymous	Unknown	rs1799949	31/39
11	c.2311 T > C	p.Leu771Leu	Synonymous	Unknown	rs16940	30/38
11	c.2612C > A	p.Pro871Gln	Missense	Polymorphism	rs799917	23/42
11	c.3113A > G	p.Glu1038Gly	Missense	Polymorphism	rs16941	36/36
11	c.3119G > A	p.Ser1040Asn	Missense	Unknown	rs4986852	3/3
11	c.3418A > G	p.Ser1140Gly	Missense	Unknown	rs2227945	0/1
11	c.3419G > T	p.Ser1164Ile	Missense	Deleterious	n.r.[3]	1/1
11	c.3548A > G	p.Lys1183Arg	Missense	Polymorphism	rs16942	33/37
11	c.3711A > G	p.Ile1237Met	Missense	Unknown	rs80357388	0/1
13	c.4308 T > C	p.Ser1436Ser	Synonymous	Polymorphism	rs1060915	11/37
14	c.4484G > T	p.Arg1495Met	Missense	Deleterious	rs80357389	0/1
16	c.4837A > T	p.Ser1613Cys	Missense	Unknown	rs1799966	1/1
16	c.4837A > G	p.Ser1613Gly	Missense	Polymorphism	rs1799966	32/38
16	c.4843G > A	p.Ala1615Thr	Missense	Unknown	rs80356987	1/1
16	c.4956G > A	p.Met1652Ile	Missense	Unknown	rs1799967	0/1
16	c.4964_4982del	p.Ser1655_Glu1661fs	Frameshift	Deleterious	rs80359876	1/1
IVS18	c.5153-1G > C	–	Substitution	Deleterious	rs80358137	1/1

[1] All identified variants are indicated both by cDNA base sequence (second column) and by protein sequence (third column) according to the HGVS (Human Genome Variation Society) nomenclature guidelines.
[2] Breast Cancer Information Core (http://research.nhgri.nih.gov/bic/).
[3] n.r., not reported in NCBI (National Center for Biotechnology Information) database.

Table 2
BRCA2 variants identified in the analyzed population by dHPLC/Sanger and NGS.

Exon/intron	HGVS[1] cDNA	HGVS[1] Protein	Mutation type	Clinical relevance (BIC)[2]	Reference ID according to NCBI	Number of times a variant was observed (dHPLC + Sanger/NGS)
10	c.865A > C	p.Asn289His	Missense	Polymorphism	rs766173	6/6
10	c.865A > G	p.Asn289Asp	Missense	Unknown	rs766173	1/1
10	c.1114C > A	p.His372Asn	Missense	Polymorphism	rs144848	26/56
10	c.1124C > T	p.Pro375Leu	Missense	Unknown	rs80358409	0/1
10	c.1151C > T	p.Ser384Phe	Missense	Polymorphism	rs41293475	0/1
10	c.1365A > G	p.Ser455Ser	Synonymous	Polymorphism	rs1801439	8/8
IVS10	c.1909 + 12_1909 + 12delT	–	Deletion	Unknown	n.r.[3]	1/1
11	c.2229 T > C	p.His743His	Synonymous	Polymorphism	rs1801499	5/5
11	c.2971A > G	p.Asn991Asp	Missense	Polymorphism	rs1799944	4/6
11	c.3396A > G	p.Lys1132Lys	Synonymous	Polymorphism	rs1801406	28/29
11	c.3807 T > C	p.Val1269Val	Synonymous	Polymorphism	rs543304	19/31
11	c.3824 T > C	p.Ile1275Thr	Missense	Unknown	rs80358625	0/1
11	c.4131_4132insTGAGA	p.Asn1377_Thr1378	In Frame Insertion	Deleterious	rs80359429	1/2
11	c.4563G > A	p.Lys1521Lys	Synonymous	Polymorphism	rs206075	27/69
11	c.4585G > A	p.Gly1529Arg	Missense	Polymorphism	rs28897728	1/1
11	c.5199C > T	p.Ser1733Ser	Synonymous	Polymorphism	rs28897734	1/2
11	c.5312G > A	p.Gly1771Asp	Missense	Polymorphism	rs80358755	1/3
11	c.3515C > G	p.Ser1172Trp	Missense	Unknown	rs80358600	0/2
11	c.5744C > T	p.Thr1915Met	Missense	Polymorphism	rs4987117	3/1
11	c. 6037A > T	p.Lys2013Ter	Nonsense	Deleterious	rs80358840	1/1
22	c.6486_6489delACAA	p.Lys2162_Gln2163fs	Frameshift	Deleterious	rs80359598	1/1
11	c.6513C > G	p.Val2171Val	Synonymous	Polymorphism	rs206076	45/69
14	c.7242A > G	p.Ser2414Ser	Synonymous	Polymorphism	rs1799955	16/23
14	c.7354A > G	p.Asn2452Asp	Missense	Unknown	rs398122580	1/1
IVS16	c.7806-14 T > C	–	Deletion	Unknown	rs9534262	1/1
27	c.9976A > T	p.Lys3326Ter	Nonsense	Polymorphism	rs11571833	1/1

[1] All identified variants are indicated both by cDNA base sequence (second column) and by protein sequence (third column) according to the HGVS (Human Genome Variation Society) nomenclature guidelines.
[2] Breast Cancer Information Core (http://research.nhgri.nih.gov/bic/).
[3] n.r., not reported in NCBI (National Center for Biotechnology Information) database.

2.2. dHPLC/Surveyor nuclease and Sanger sequencing

The combined dHPLC/SURVEYOR® Nuclease approach (Transgenomic, Omaha, NE, USA) was used for molecular *BRCA1* and *BRCA2* screening, as previously described [5]. Briefly, PCR amplifications were performed using specific primer pairs to amplify all the *BRCA1* and *BRCA2* coding exons and their flanking regions using a multiamplicon approach (Supplemental Table 1). After amplification, each PCR reaction/sample was digested by SURVEYOR® Nuclease using 15 μL of a 1:1 mixture of DNA amplicons of each sample and wild-type DNA, 1.5 μL of 0.15 mM MgCl2, 1.5 μL of Enhancer Cofactor and 1.0 μL of SURVEYOR Nuclease (Transgenomic). The reactions were incubated at 42 °C for 60 min and stopped by adding 1.5 μL of stop buffer. For each amplicon pool, a gradient was chosen using Navigator software (Transgenomic) based on the size of the PCR fragments. Fragments were analyzed by HPLC on the Transgenomic WAVE Nucleic Acid High Sensitivity Fragment Analysis System (WAVE HS system; Transgenomic). Amplicon reactions that showed a dHPLC profile suggestive of mutations were selected for Sanger sequencing confirmation. Direct sequencing was performed with an ABI 3100 capillary sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sanger electropherogram evaluation was carried out using the SeqMan tool (DNASTAR, Inc., Madison, Wisconsin, USA).

2.3. NGS sequencing

Multiple amplicon DNA libraries, covering all the *BRCA1* and *BRCA2* coding exons and their flanking regions, were obtained using the BRCA MASTR v2.1 Assay kit (Multiplicom, Niel, Belgium), following the manufacturer's instructions. In brief, for each patient, 250 ng of genomic DNA was used to perform 5 multiplex PCR reactions able to amplify the entire target region. Then, a 1:1000 dilution of each multiplex PCR was re-amplified using hybrid primers to univocally tag all the multiplexes from the same patient with a barcode sequence (MID). These primers contained a universal adaptor sequence that is required for downstream sequencing reactions. The multiplexed tagged reactions were purified using Agencourt AMPure XP Beads (Beckman Coulter, Brea, CA, USA), quantified using the NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and quality-assessed using the Experion DNA 1 kb Analysis kit (Bio-Rad, Hercules, CA, USA). Equimolar amounts of the 5 multiplexed tagged reactions from the

Table 3
Analytic performances of DHPLC/Sanger and NGS methods in detecting BRCA 1/2 sequence variants.

	DHPLC/Sanger		NGS	
	BRCA1	BRCA2	BRCA1	BRCA2
N total variants	237	198	320	323
N unique variants	19 (7 SNPs, 9 UCV, 3 causative mutations)	22 (15 SNPs, 3 UCV, 4 causative mutations)	23 (7 SNPs, 12 UCV, 4 causative mutations)	26 (16 SNPs, 6 UCV, 4 causative mutations)
Analytic sensitivity % (95% CI)	67.6 (63.9–71.1)		100 (99.4–100)	
Analytic specificity % (95% CI)	100 (100)		100 (100)	
PPV %	100		100	
NPV %	99.98		100	

N, number; SNP, single nucleotide polymorphism; UCV, unknown significance variant; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

same sample were pooled to create a library/sample. Up to 35 different libraries were pooled and sequenced using the Genome Sequencer FLX System (454-Life Science and Roche, Branford, CO, USA), according to the manufacturer's instructions. Causative mutations or doubtful variants were confirmed by Sanger sequencing.

2.4. NGS data analysis

NGS sequence data were analyzed using the SeqPilot software (version 3.5.2) from JSI Medical Systems (www.jsi-medisys.de). The *BRCA1* (ENSG00000012048 for gene reference and ENST00000357654 for transcript) and *BRCA2* (ENSG00000139618 and ENST00000380152 respectively) sequences from the NCBI-database (http://www.ncbi.nlm.nih.gov) were used as reference sequences. Raw sequencing data were directly uploaded in the software and, for each MID, blasted against a target reference sequence. Thus, we obtained a report/patient containing the sequence-coverage/exon and the list of high confidence variants. According to the manufacturer's protocol, a minimum absolute coverage/exon of 40× is allowed and only variants present in both directions and with a minimum coverage of 10% default, excluding homopolymers, were contemplated in the genetic analysis. All sequence variants are named according to the nomenclature used by the Human Genome Variation Society, HGVS (http://www.hgvs.org). The significance of variants was attributed according to the Breast Cancer Mutation database, BIC (http://research.nhgri.nih.gov/projects/bic/), and the Ensemble Database (http://www.ensembl.org).

2.5. Assessment of methodology performance

The sensitivity and specificity of the described procedures were assessed by evaluating their ability to correctly identify all the base changes identified in the same population by direct Sanger sequencing of all *BRCA1* and *BRCA2* exons. As specified above, missing Sanger data were obtained after the NGS analysis to determine if a specific variant was present or not, and were used as gold standard to identify each substitution as true positive (TP), true negative (TN), false positive (FP) or false negative (FN), and to assess the analytic performances of the two approaches used. Sensitivity and specificity were calculated using the following formulas: sensitivity = TP / (TP + FN); specificity = TN / (TN + FP). Confidence intervals for sensitivity and specificity were estimated with the Pearson–Klopper method, and the R statistical software environment.

3. Results

The *BRCA1* and *BRCA2* genes were analyzed in the 70 enrolled subjects using the two analytic strategies described under Section 2 (Fig. 1). The combined dHPLC/Surveyor® nuclease strategy revealed 237 and 198 variants in *BRCA1* and *BRCA2*, respectively, and these were confirmed by Sanger sequencing. These variants correspond to 19 *BRCA1* variants, i.e., 7 polymorphisms, 9 variants of unknown significance (UCVs) and 3 causative mutations (Table 1), and to 22 *BRCA2* variants, i.e., 15 polymorphisms, 3 UCVs, and 4 causative mutations (Table 2). The NGS-based strategy revealed 320 and 323 variants in *BRCA1* and *BRCA2*, respectively. These variants correspond to 23 *BRCA1* variants, namely, 7 polymorphisms, 12 UCVs, and 4 causative mutations (Table 1), and 26 *BRCA2* variants, namely, 16 polymorphisms, 6 UCVs, and 4 causative mutations (Table 2).

Comparative analysis of the results obtained with the two analytic strategies showed that all the variants detected by the routinely used dHPLC/Sanger method were identified also by NGS. Interestingly, the NGS technique identified variants missed by conventional screening (Tables 1 and 2). Additional Sanger sequencing was carried out to assess all these discrepancies and confirmed the NGS data.

We assessed the reliability of the NGS-based approach by comparing the NGS results to the Sanger sequencing results (Table 3). While the specificity was 100% for both methods, the NGS-based procedure had a higher sensitivity (100% versus 67.6%), which is in line with a previous report [16]. In addition, in our study the NGS-based method had also a higher diagnostic sensitivity since it was able to detect a causative BRCA1 mutation missed by traditional screening (Table 3).

In terms of analytical time, it took about three months to analyze all the 70 enrolled subjects using the dHPLC/Sanger approach, and only 10 days using NGS. In addition, the cost of NGS reagents was half that of the reagents required for the dHPLC/Sanger procedure.

4. Discussion

Thanks to the recent development of NGS technologies, and the consequent decreased cost of DNA sequencing, a wide range of technical possibilities are now available for studies aimed at elucidating the molecular basis of human diseases [17]. In addition, these technologies are now beginning to be exploited for diagnostic purposes. Therefore, it is conceivable that in the near future NGS may become an invaluable tool for both clinical biochemists and clinicians. In fact, increasing evidence indicates that information about the *BRCA1* and *BRCA2* mutation status, especially if available in a timely fashion, will enable a patient and her/his health-care provider to make informed decisions about cancer prevention, screening and treatment [18].

In this optics, we evaluated the efficacy of an NGS-based method for the molecular analysis of *BRCA1* and *BRCA2* in 70 patients using both dHPLC/Sanger and NGS-based strategies. Here, we demonstrate that NGS is more sensitive than the conventional approaches in detecting sequence variants. In addition, since we analyzed up to 70 samples in the same sequencing run, also the time and cost of the analysis were greatly reduced. Given these results, this NGS-based approach to the detection of *BRCA1*/*BRCA2* mutations is suitable for use in a routine diagnostic workflow.

Although several NGS-based procedures have been proposed for the molecular diagnosis of the predisposition for breast cancer, our study demonstrates that NGS-based tests are more sensitive, faster, easier to use, and less expensive than the conventional Sanger method, which is the most widely test currently used and is considered the gold standard test.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2015.03.045.

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References

[1] Narod SA. Breast cancer in young women. Nat Rev Clin Oncol 2012;9:460–70.
[2] Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer 2011;12:68–78.
[3] Gerhardus A, Schleberger H, Schlegelberger B, Gadzicki D. Diagnostic accuracy of methods for the detection of BRCA1 and BRCA2 mutations: a systematic review. Eur J Hum Genet 2007;15:619–27.
[4] Vuttariello E, Borra M, Calise C, Mauriello E, Greggi S, Vecchione A, et al. A new rapid methodological strategy to assess BRCA mutational status. Mol Biotechnol 2013;54:954–60.
[5] Pilato B, De Summa S, Danza K, Papadimitriou S, Zaccagna P, Paradiso A, et al. DHPLC/SURVEYOR nuclease: a sensitive, rapid and affordable method to analyze BRCA1 and BRCA2 mutations in breast cancer families. Mol Biotechnol 2012;52:8–15.
[6] Jiménez Ide J, Esteban Cardeñosa E, PalancaSuela S, González EB, BoluferGilabert P. Group of Cancer Genetic Counselling Program of Valencia Community. Advantages

of the high resolution melting in the detection of BRCA1 or BRCA2 mutation carriers. Clin Biochem 2009;42:1572–6.
[7] Ten Bosch JR, Grody WW. Keeping up with the next generation: massively parallel sequencing in clinical diagnostics. J Mol Diagn 2008;10:484–92.
[8] D'Argenio V, Notomista E, Petrillo M, Cantiello P, Cafaro V, Izzo V, et al. Complete sequencing of Novosphingobium sp. PP1Y reveals a biotechnologically meaningful metabolic pattern. BMC Genomics 2014;15:384.
[9] D'Argenio V, Casaburi G, Precone V, Salvatore F. Comparative metagenomic analysis of human gut microbiome composition using two different bioinformatic pipelines. Biomed Res Int 2014;2014:325340.
[10] De Leeneer K, De Schrijver J, Clement L, Baetens M, Lefever S, De Keulenaer S, et al. Practical tools to implement massive parallel pyrosequencing of PCR products in next generation molecular diagnostics. PLoS One 2011;6:e25531.
[11] Walsh T, Lee MK, Casadei S, Thornton AM, Stray SM, Pennil C, et al. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. Proc Natl Acad Sci U S A 2010;107:12629–33.
[12] Michils G, Hollants S, Dehaspe L, Van Houdt J, Bidet Y, Uhrhammer N, et al. Molecular analysis of the breast cancer genes BRCA1 and BRCA2 using amplicon-based massive parallel pyrosequencing. J Mol Diagn 2012;14:623–30.
[13] Feliubadaló L, Lopez-Doriga A, Castellsagué E, del Valle J, Menéndez M, Tornero E, et al. Next-generation sequencing meets genetic diagnostics: development of a comprehensive workflow for the analysis of BRCA1 and BRCA2 genes. Eur J Hum Genet 2013;21:864–70.
[14] Hernan I, Borràs E, de Sousa Dias M, Gamundi MJ, Mañé B, Llort G. Detection of genomic variations in BRCA1 and BRCA2 genes by long-range PCR and next-generation sequencing. J Mol Diagn 2012;14:286–93.
[15] Vaca-Paniagua F, Alvarez-Gomez RM, Fragoso-Ontiveros V, Vidal-Millan S, Herrera LA, Cantú D, et al. Full-exon pyrosequencing screening of BRCA germline mutations in Mexican women with inherited breast and ovarian cancer. PLoS One 2012;7:e37432.
[16] D'Argenio V, Frisso G, Precone V, Boccia A, Fienga A, Pacileo G, et al. DNA sequence capture and next-generation sequencing for the molecular diagnosis of genetic cardiomyopathies. J Mol Diagn 2014;16:32–44.
[17] Chrystoja CC, Diamandis EP. Whole genome sequencing as a diagnostic test: challenges and opportunities. Clin Chem 2014;60:724–33.
[18] Metcalfe KA, Kim-Sing C, Ghadirian P, Sun P, Narod SA. Health care provider recommendations for reducing cancer risks among women with a BRCA1 or BRCA2 mutation. Clin Genet 2014;85:21–30.