NIMAGEN

B-Pure™

LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

EasySeq™ PCR Plates

For BRCA1 and BRCA2 sequencing

- √ Based on the Gold Standard Sanger Sequencing
- √ Sample in -> Result out: No need for batching up samples
- √ Thorough coverage: 100% Gene resequencing
- √ Fast, Straightforward, Robust
- √ Minimal hands-on time
- √ Compatible with:
  - Standard DyeTerminator Sequencing workflows
  - BrilliantDye® Terminator Cycle Sequencing Kit
- √ Primers tailed with M13 sequences: Universal Cycle Sequencing

Introduction

BRCA1 and *BRCA2* genes are tumor suppressor genes. The gene products are directly involved in the human DNA repair system and prevent uncontrolled cell growth.

Both *BRCA1* and *BRCA2* are comprehensive genes, comprising 23 and 27 exons, respectively. Mutations, found in both *BRCA* genes are not located in hotspots, but are distributed throughout the gene coding region. Functional mutations have been correlated with an increased cancer risk [1,2,3]. Ongoing *BRCA1* and *BRCA2* research has highlighted the need for a straightforward mutation detection workflow.

Principal

The EasySeq™ PCR plates were developed to facilitate an easy, robust, fast and cost effective sequencing workflow for detection of mutations in the BRCA genes. Other methods, like Next Generation Sequencing (NGS) or heteroduplex based pre-screening methods (for example dHPLC, HiRes Melting curve analysis, CSCE), introduce additional, non-robust and time consuming steps in the workflow. They also implicate the need for batching up samples. This can introduce unwanted delays in sample-to-result timelines.

Offering an off-the-shelf solution, this EasySeq™ approach provides an ultra-straightforward protocol. The workflow is based on on the industry-standard Sanger sequencing chemistry and Genetic Analyzers.

Kit content

The product is available as semi-skirted PCR plates, pre-spotted with dried-down primer pairs, to generate 80 PCR products covering both genes. Primers have been optimized for use with commercial available PCR master-mixes.

Primer Design

Making use of the Human Genome build NCBI36 (BRCA1: Acc. nr: cDNA: NM_007294.3, BRCA2: Acc. nr: NM_000059.3), M13 tailed PCR primers were designed and optimized for 100% coverage of the coding sequence of the *BRCA1* and *BRCA2* genes, including sections of approximately 50 bp up- and downstream of each exon. All amplicons can be amplified and sequenced using one universal set of PCR conditions. Cycle Sequencing is done with universal (-21M13 / M13REV) sequencing primers.

For more information about primer sequences and amplicon design, please contact Life Technologies India Pvt. Ltd.

Non-Template control wells

To provide maximum flexibility and easy conversion of your workflow, the EasySeq™ plates contain 15 multiplex Non-Template control wells to check the specificity of the results (see table 1). This set of multiplexes contain all primer and can be verified by agarose gel electrophoresis or spectrophotometric DNA concentration measurement to prove that there is no amplification.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BRCA1 exon 1	BRCA1 exon 10	BRCA1 exon 11/8	BRCA1 exon 15	BRCA1 exon 23	BRCA2 exon 8	BRCA2 exon 11/3	BRCA2 exon 11/11	BRCA2 exon 13	BRCA2 exon 20	BRCA1 MP-1	BRCA1 MP-3
B	BRCA1 exon 2	BRCA1 exon 11/1	BRCA1 exon 11/9	BRCA1 exon 16	BRCA1 exon 24	BRCA2 exon 9	BRCA2 exon 11/4	BRCA2 exon 11/12	BRCA2 exon 14/1	BRCA2 exon 21	BRCA1 MP-2	BRCA2 MP-4
C	BRCA1 exon 3	BRCA1 exon 11/2	BRCA1 exon 11/10	BRCA1 exon 17	BRCA2 exon 1	BRCA2 exon 10/1	BRCA2 exon 11/5	BRCA2 exon 11/13	BRCA2 exon 14/2	BRCA2 exon 22	BRCA1 MP-3	BRCA2 MP-5
D	BRCA1 exon 5	BRCA1 exon 11/3	BRCA1 exon 11/11	BRCA1 exon 18	BRCA2 exon 2	BRCA2 exon 10/2	BRCA2 exon 11/6	BRCA2 exon 11/14	BRCA2 exon 15	BRCA2 exon 23&24	BRCA1 MP-4	BRCA2 MP-6
E	BRCA1 exon 6	BRCA1 exon 11/4	BRCA1 exon 11/12	BRCA1 exon 19	BRCA2 exon 3	BRCA2 exon 10/3	BRCA2 exon 11/7	BRCA2 exon 11/15	BRCA2 exon 16	BRCA2 exon 25	BRCA1 MP-5	BRCA2 MP-7
F	BRCA1 exon 7	BRCA1 exon 11/5	BRCA1 exon 12	BRCA1 exon 20	BRCA2 exon 4	BRCA2 exon 10/4	BRCA2 exon 11/8	BRCA2 exon 11/16	BRCA2 exon 17	BRCA2 exon 26	BRCA1 MP-6	BRCA2 MP-8
G	BRCA1 exon 8	BRCA1 exon 11/6	BRCA1 exon 13	BRCA1 exon 21	BRCA2 exon 5&6	BRCA2 exon 11/1	BRCA2 exon 11/9	BRCA2 exon 11/17	BRCA2 exon 18	BRCA2 exon 27/1	BRCA2 MP-1	BRCA2 MP-9
H	BRCA1 exon 9	BRCA1 exon 11/7	BRCA1 exon 14	BRCA1 exon 22	BRCA2 exon 7	BRCA2 exon 11/2	BRCA2 exon 11/10	BRCA2 exon 12	BRCA2 exon 19	BRCA2 exon 27/2	BRCA2 MP-2	EM PTY

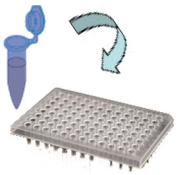
Table 1: Positions of the Amplicon-Specific primers and No-Template Controls in the EasySeq™ plates

Quality

All primers are produced under highly controlled conditions and are of B-Pure™, PAGE purified quality. After LC/MS and functional quality tests, the primer pairs are spotted and dried down under controlled conditions in semi-skirted 96-well plates with barcode. We have shown average sequence quality of >QV40 in the Regions of Interest. This enables unidirectional sequencing for *BRCA1/2*[5] as an option.

Steps:

1. Mix DNA with PCR Mastermix[1] and simply dispense In EasySeq Plate



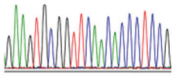
2. PCR amplification

3. Cleanup[2] PCR Product and Cycle Sequence with -21M13 and M13REV primers

4. Purify Sequencing Reactions

5. Run and Analyse On 31xx, 35xx or 37xx series Genetic Analyzer

6. Data Analysis



[1] In the last two columns (multiplex NTC wells), add only PCR Mastermix with water
[2] When using BD Direct or C-Pure, the PCR cleanup can be eliminated
*Genetic Analyzer is a product of Thermo Fisher Scientific Inc.

References

1. Irminger-Finger, Siegel BD, Leung WC (1999) The functions of breast cancer susceptibility gene 1 (BRCA1) product and its associated proteins. BiolChem 380(2)117-128.

2. Wooster R, Bignell G, Lancaster J et al. [1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789-792.

3. Casey G (1997) The BRAC1 and BRAC2 breast cancer genes. CurrOpinOncol 9:88-93.

4. J. Theelen, M. Nelen, N. Arts, H. Ouchene, A. Felton, C. Davidson, W. Hettenna, R. Petraroli, M. Ligtenberg (2010). A robust and straightforward approach for screening of BRCA1 and BRCA2 genes by direct resequencing.ESHG poster P06.017

5. S. Ellard, B. Shields, C. Tysoe, R. Treacy, S. Yau, C. Mattocks, A. Wallace (2009]. Semi-automated Unidirectional Sequence Analysis for Mutation Detection in a Clinical Diagnostic Setting. GEN. TESTING AND MOL. BIOMARKERS Vo113 (3):381-386

Name	Description	P/N
B-pure™ EasySeq™ PCR Plates 96-well	5 pcs / 5 analyses	350596
B-pure™ EasySeq™ PCR Plates 96-well	25 pcs / 25 analyses	352596

Download Protocol

Contact Us:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com