EasySeq™ PCR Plates for BRCA1/2 Sequencing

User manual

EasySeq™ PCR Plates

For BRCA1 and BRCA2 sequencing

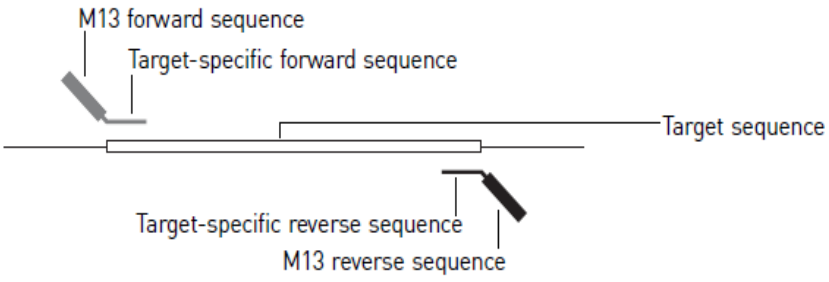
- √ Based on the Gold Standard Sanger Sequencing
- √ Sample in -> Result out: No need for batching up samples
- √ Thorough coverage: 100% Gene resequencing
- √ Fast, Straightforward, Robust
- √ Minimal hands-on time
- √ Compatible with:
  - Standard DyeTerminator Sequencing workflows
  - BrilliantDye®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

Product Information

Store the kits at -18 to -22 °C. Use the *B-Pure*™ EasySeq™ PCR plates for *BRCA1* and *BRCA2* for setting up PCR reactions, followed by cycle sequencing and capillary electrophoresis, to screen for mutations in the complete coding region including ±50 bp up- and downstream of each coding exon of the *BRCA1* and *BRCA2* genes. The product consists of semi skirted PCR plates with barcode, containing dried down primer pairs in optimized concentrations, covering the both BRCA genes. The columns 11 and 12 contain all amplicons, in a total of 15 PCR multiplexes, to be used as a No-Template Control (NTC), to prove all results are specific. All primer sets have been optimized in concentration and design to be compatible with key chemistries.

Al primers have been tailed with universal tails: Forward primers with -21M13 and reverse primers with M13Rev to enable cycle sequencing with universal primers:

M13 Forward primer (-21M13):	5' TGTAAAACGACGGCCAGT 3'
M13 Reverse primer (M13Rev):	5' CAGGAAACAGCTATGACC 3'



Guidelines for Genomic DNA quality

The performance of the *B-Pure*™ EasySeq™ PCR plates for *BRCA1* and *BRCA2* sequencing is **highly dependent** on the quality and quantity of the used genomic DNA. NimaGen strongly recommends to check quality and quantity of your DNA sample by A260/A280 ratio analysis. Follow the recommended quantity of DNA in the original PCR setup (25-50 ng/µl). Too much input DNA will result in possible inhibition and/or overloaded signals.

Setting up the PCR reactions

1. For each sample (EasySeq plate), prepare two PCR Mixes in 2 mL vials:

A. PCR Mix

PCR Mastermix (2x)	880 µl
Human Genomic DNA (25 – 50 ng/µl)	90 µl
Water (molecular biology grade)	790 µl
TOTAL	***1760 µl***

B. No-Template-Control (NTC) Mix

PCR Mastermix (2x)	165 µl
Water (molecular biology grade)	165 µl
TOTAL	***330 µl***

2. Dispense 20 µl of the PCR (A) mix in all wells of columns 1 – 10 of the EasySeq PCR plate (yellow wells in picture 1)

3. Dispense 20 µl of the NTC (B) mix in all wells of columns 11 and 12 except well H12, of the EasySeq PCR plate (pink wells in picture 1)

	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	EMPTY

Picture 1

4. Seal the plate with adhesive (PCR) film, then spin the plate briefly.
   Note: make sure to tightly seal all wells, to prevent evaporation during PCR
5. Run the reactions in the thermal cycler, followed by agarose gel electrophoresis for checking NTC's (well A11-G12)

HOLD	95°C	10 min
Cycle (32 cycles)	95°C	15 sec
	60°C	30 sec
	72°C	60 sec
HOLD	72°C	7 min
HOLD	4°C	∞

PCR conditions

Recommendations for post-PCR processing

1. **Cleanup of PCR:** For best results, use the AmpliClean PCR cleanup kit from Nimagen (p/n AP-005, AP-050, AP-500) according to this protocol:
   a. Gently shake the AmpliClean to resuspend the magnetic particles to obtain a homogeneous suspension.
   b. Add 36 µl of AmpliClean to the 20 µL PCR reactions
   c. Mix thoroughly by pipetting up and down and incubate for 3 - 5 minutes at Room Temperature
   d. Place the reaction plate onto a Alpaqua Magnetic Ring Plate for 2 minutes
   e. While sitting on the magnet, remove the cleared solution from the reaction plate and discard by pipetting from the center of the bottom of the wells. Make sure the removed solution is fully cleared and not to remove any magnetic particles
   f. While leaving the plate on the magnet, immediately dispense 150 µL of 70% ethanol to each well of the reaction plate and incubate for 30 seconds at room temperature.
   g. Remove the ethanol and discard. Repeat step f followed by a complete removal of Ethanol with the last aspiration. Check if all wells are free of liquid after this step
   h. Dry for 5 minutes at RT. Take plate from the Magnet.
   i. While plate is **off the magnet**, add 40 µL water or elution buffer to each well of the reaction plate and homogenize the beads in the elution buffer.
   j. Place the reaction plate onto the Magnet Plate for 2 minutes to separate beads from the solution and transfer the max. 30 µL of the eluant, containing the purified PCR products to a new plate.

2. **Cycle Sequencing**

Note: Optionally check the purified PCR products on agarose gel (apply 5 µL)
Note: For forward and reverse sequencing, perform steps a to c in duplo

a. Prepare Cycle Sequencing Mastermix:
   - 88 µL BrilliantDye™ Sequencing Mix
   - 132 µL 5x Sequencing Buffer
   - 88 µL -21M13 (Forward) **or** M13Rev (Reverse) primer (3-5 pMol/ µL)
   - 484 µL Molecular Biology grade water

   TOTAL 792 µL

b. Dispense 9 µL of this Mastermix in the 80 wells of column 1-10 of a new 96-well plate
c. Transfer 1 µL of the cleaned PCR products to the corresponding wells of the prepared sequencing plate
d. Seal the plate tightly and perform cycle sequencing:

initial denaturation	96°C	60 sec
28 cycles	96°C	10 sec
	50°C	5 sec
	60°C	1 min. 15 sec.
hold	4°C	∞

3. Sequence Cleanup: Nimagen recommends to use the D-Pure Cycle Sequencing cleanup kit (DP-005, DP-050, DP-500) according to the general protocol, or use a commercial available sephadex-based cleanup method.
4. Capillary Electrophoresis: Analyze the purified sequence samples on an Applied Biosystems / Hitachi Genetic Analyzer of the 3130, 3500 or 3730 series, preferably using a 50 cm array, NanoPOP-7 and with a *standard* or *fast* sequencing run module.

Note: It might be needed to lower the injection voltage and/or time to prevent over exposure.

* Applied Biosystems, Genetic Anlayzer and NanoPOP-7 are products of Thermo Fisher Scientific Inc.

India Contact:

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