EasySeq® NGS Targeted Capture Kit

BRCA1 and BRCA2 Genes

> Single Molecule Tags for identifying unique capture molecules.

- Extremely easy workflow, one tube per sample
- Double tiling; both strands independently targeted
  - Detection of formalin induced sequencing artifacts
  - Detection of random sequencing errors
- Intrinsic sample ID and quality control
- Single Click Analysis with JSI SeqNext Software
- CNV detection on Germline DNA
- Includes detection of the *CHEK2* c.1100delC mutation

Technology

Using sequence capturing with single molecule Molecular Inversion Probes (smMIP)[1], followed by a barcoding amplification step, the system enables sequencing of the targeted regions in a very simple, fast and robust workflow, resulting in coverage with double probe tiling[2] and independent reading of both strands. The kits contain all reagents for a straightforward, one-step capturing and subsequently sample tagging for NGS sequencing. The resulting libraries are compatible with Illumina sequencing platforms.

Easy, robust and straightforward workflow (figure EasySeq® workflow)

All EasySeq® kits consist of a very limited number of hands-on steps:

1. Thaw the capture strips (purple) for the required number of samples, and just add DNA. Run the capturing program in a thermal cycler
2. Add the Exo MasterMix and run the exonuclease program in a thermal cycler.
3 Thaw an equal number (as in step 1) of barcoding strips (blue) and transfer a fixed aliquot from the capture strips to the same positions of the barcoding strips. Every well of the Barcoding PCR strips contain unique sample ID barcodes. Run the barcoding PCR program
4. Combine all wells in one tube, purify this single tube with AMPureXP: **Ready for Sequencing**.
5. Import the fastq files into JSI SeqNext software, load the predefined analysis- and ROI settings and simply press the analysis button

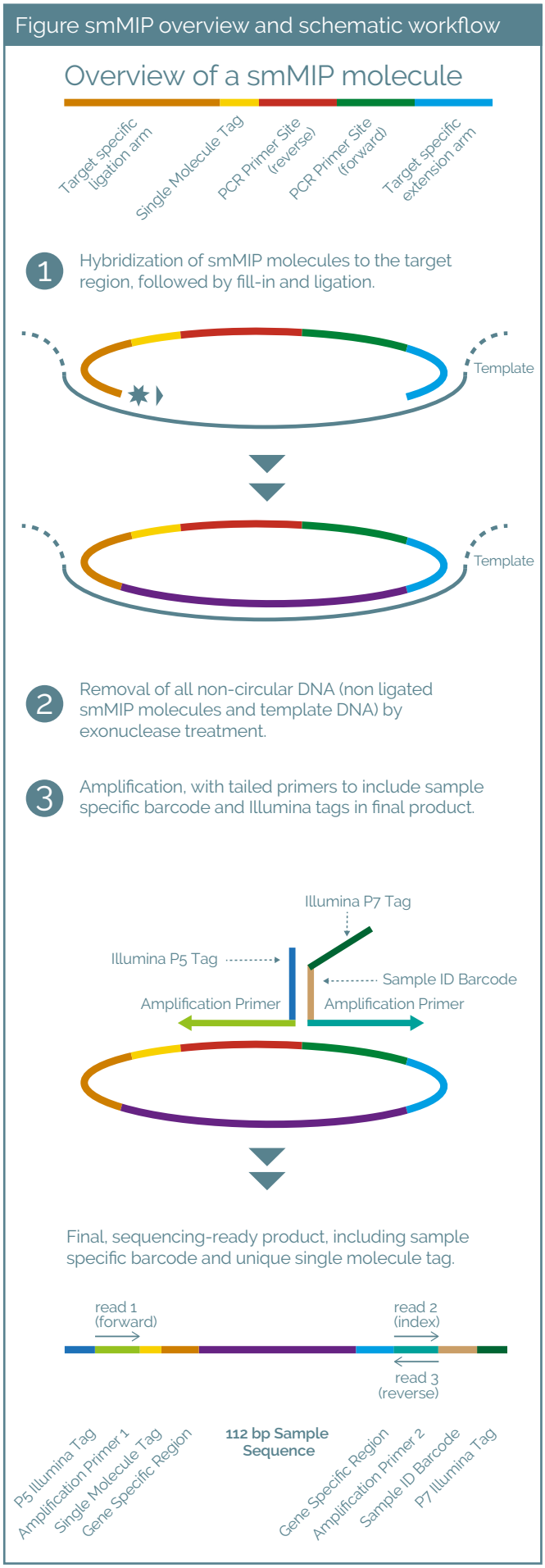
Data Analysis

For downstream data analysis, NimaGen recommends **SeqNext** from **JSI medical systems**. Analysis settings templates for the EasySeq® generated sequence data will be available thru JSI (www.JSI-medisys.de).

[1] Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure, J., Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res 2013;23:843–54.

[2] Kornelia Neveling et al., BRCA Testing by Single-Molecule Molecular Inversion Probes. Clinical Chemistry 2016; 63:2.

Figure smMIP overview and schematic workflow



EasySeq® workflow

1 Capture & Hybridize

Simply add DNA to Hybridization / ligation strip tubes

Incubate overnight in thermal cycler

2 Exo-treat

Add Exo MasterMix

Incubate 45' in thermal cycler

3 Barcoding

Transfer aliquot to Barcoding PCR strip (blue) by multichannel pipette

Barcoding PCR (45') in thermal cycler, followed by optional QC (agarose)

4 Pool and Sequence

Pool samples

Purify by single-tube AMPureXP

QC (tapestation), Quantify (Qubit) and dilute -> Illumina Run

5 Data analysis

Data analysis

Ready-to-go

Kit content

ESI-BRCA024 (24 Sample kit)	ESI-BRCA096 (96 Sample kit)
3x (Purple) Hybridization/ Ligation strips of 8 wells, containing 20 µL smMIP pool in Hybridization/Extension/ Ligation MasterMix each.	12x (Purple) Hybridization/ Ligation strips of 8 wells, containing 20 µL smMIP pool in Hybridization/Extension/ Ligation MasterMix each.
3x Tubes for Exo Treatment (17 µL Exonuclease I, orange, 17 µL Exonuclease III, yellow and 35 µL (2x) Exo Buffer, green).	3x Tubes for Exo Treatment (55 µL Exonuclease I, orange, 55 µL Exonuclease III, yellow and 105 µL (2x) Exo Buffer, green).
3x (Blue) Barcoding PCR strips of 8 wells, containing PCR MasterMix, Universal Forward PCR primer and in every well 1 of 24 unique barcoded primers.	12x (Blue) Barcoding PCR strips of 8 wells, containing PCR MasterMix, Universal Forward PCR primer and in every well 1 of 96 unique barcoded primers.
1x vial with 50 µL control DNA (20 ng/µL).	1x vial with 50 µL control DNA (20 ng/µL).
1x vial with 500 µL Elution Buffer (EB).	1x vial with 500 µL Elution Buffer (EB).
3x Tubes with spike-in Sequencing Primers (forward/r1, index, reverse/r2), 20 µL each at 100 µM.	3x Tubes with spike-in Sequencing Primers (forward/r1, index, reverse/r2), 20 µL each at 100 µM.

Features

The revolutionary smMIP based enrichment method, in combination with the straightforward kit design provides a number of unique features:

No need for DNA fragmentation

The targeted DNA sequences are captured by a pre-aliquoted MasterMix containing a balanced smMIP pool, in one single well per sample: Just add the DNA and start capturing.

Single Molecule analysis

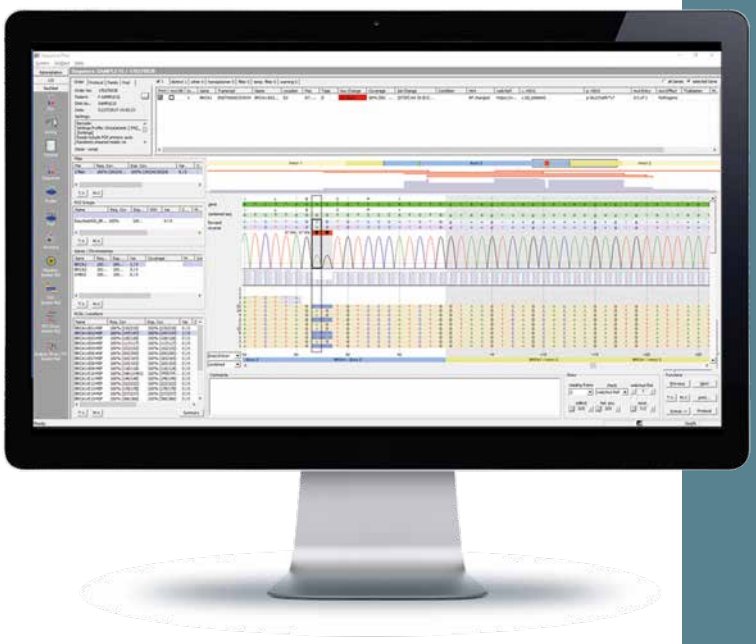
Due to the unique single molecule tags, the system generates consensuses of original molecular captures. This enables ultra-high sensitivity in detecting low mutation rates and ruling out sequencing errors and features intrinsic CNV detection for germline analysis on DNA derived from blood.

Sample Identification by SNP-smMIPs

Besides the probe pool for capturing the targeted hotspot regions, the assay contains additional identification smMIPs, targeting a set of high variable, genome-wide Single Nucleotide polymorphysms, for additional quality control (sample tracking), error detection (mixed DNA detection), and heritance checking.

Double Tiling

The targeted regions are covered by at least two independent smMIP reads on both strands, enabling identification of formalin induced sequence errors in FFPE samples.



SEQUENCE PILOT:
software for genetic analysis

With **SeqNext JSI medical systems** offers a powerful all-in-one solution for robust high-throughput variant detection and interpretation of your NGS data. Development and optimization in close cooperation with our customers guarantees user-friendliness, efficiency and optimal integration into your workflow:

- Ready to use system with an easy import of predefined and validated NimaGen analysis settings
- Benefit from the various advantages of molecular tags (smMIPs)
- Detection (high sensitivity) of base changes, deletions and insertions
- Parallel powerful CNV detection
- Customisable reduction (high specificity) of sequencing artefacts
- Powerful algorithms (mapping / alignment) to ensure high coverage
- Definition of pseudo gene / homology sequences for background reduction
- Compatible with all common sequencing platforms
- Export of FASTQ-, BAM- and VCF-files
- Detection of gene fusions and chromosomal rearrangements
- Analysis of FFPE samples
- Use **varSEAK** for a powerful filtering and interpretation of your variants
- Detailed and customisable patient reports

For more information please visit:
www.jsi-medisys.de
www.varSEAK.bio

JSI medical systems

Ordering information		
ESI-BRCA024	EasySeq® NGS Targeted Capture Kit for BRCA1/2	24 sample kit
ESI-BRCA096	EasySeq® NGS Targeted Capture Kit for BRCA1/2	96 sample kit

- For research use only -

Product and Company Information

Product name	EasySeq® NGS Targeted Capture Kit for BRCA1/2 Genes
Product use	For Research Use Only
Company	NimaGen BV Lagelandseweg 56 6545 CG Nijmegen The Netherlands
In collaboration with	Radboud university medical center, dpt. Human Genetics, Nijmegen, The Netherlands
Telephone	+31 (0)24 820 02 41
Email	info@nimagen.com
Website	www.nimagen.com