

Seq-Ready™ TE MultiSample BRCA 1/2 Panel—Excellent Sequencing Coverage for Easy Implementation in Clinical Research Labs

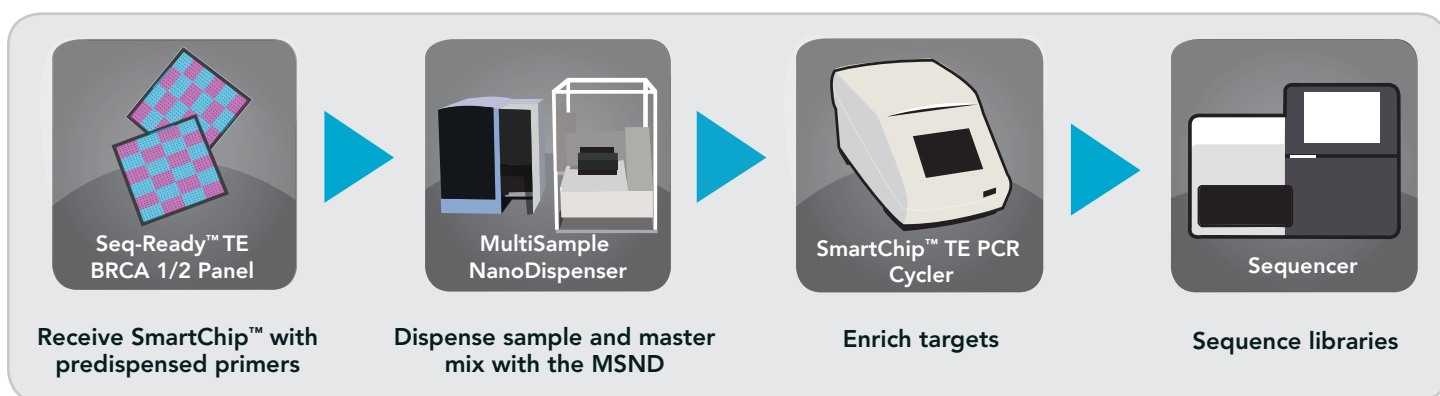
The Seq-Ready™ TE MultiSample BRCA 1/2 Panel contains 139 unique PCR primer pairs that target 100% of the coding regions of BRCA1 and BRCA2 tumor suppressor genes. Mutations in BRCA1 and BRCA2 have been linked to the development of breast and ovarian cancers. This panel was designed and verified in collaboration with leading clinical research laboratories.

Powered by the SmartChip™ massively-parallel singleplex PCR technology, Seq-Ready™ TE is an affordable and simple one-step target enrichment and library preparation solution that delivers excellent sequencing coverage for easy implementation in clinical research labs.

"The Seq-Ready™ TE MultiSample BRCA 1/2 Panel enabled us to develop a fast and easy high-throughput workflow requiring minimal pipetting and hands-on time. Of the 96 samples we tested, we detected all 82 expected unique mutations confirmed by Sanger sequencing."

— Mohammad R. Akbari, M.D., Ph.D., Molecular Human Geneticist, Dalla Lana School of Public Health University of Toronto, Early Access Customer

Streamlined Workflow for Clinical Research Labs



SmartChip™ Technology for Massively-Parallel Singleplex PCR

Seq-Ready™ TE MultiSample panels, powered by the SmartChip™ technology, take advantage of massively-parallel, singleplex PCR to simultaneously enrich a large number of target regions and incorporate sequencing adapters and indices to produce sequencing-ready libraries in one simple step. Unlike singleplex PCR, multiplex PCR reactions are subject to primer-primer interaction and inhibition, which can result in decreased target coverage due to amplicon dropouts. Without the concerns of primer competition, singleplex PCR yields higher coverage, which can result in more confident variant calling. This eliminates the need for orthogonal testing ("gap filling") using Sanger sequencing, saving additional reagents, time and labor.

Seq-Ready™ TE BRCA 1/2 Panel

Targets	Coding regions of BRCA1 and BRCA2 genes
Total target ROI	19 Kb
DNA input	As low as 60 ng (using NA12878)
Time-to-results	3 hr 45 min (DNA to sequencing-ready libraries)
Species	Human
Recommended pooling	48 samples (2 SmartChip™) on a MiSeq™ (2 x 250 bp) (96 indexes are available)

Seq-Ready™ TE Workflow: One-Step Target Enrichment and Library Prep

Target enrichment workflows based on hybrid capture or multiplexed PCR are long, difficult and labor intensive, making them a challenge to integrate into clinical research labs. The Seq-Ready™ TE ligation-free workflow simultaneously enriches target regions and incorporates sequencing adapters and indices to produce sequencing-ready libraries in one simple step (Figure 1). An operator can complete the streamlined workflow in less than 4 hours, with less than 45 minutes of hands-on time. A single Seq-Ready™ TE MultiSample BRCA 1/2 SmartChip™ is used to simultaneously prepare 24 libraries, enabling a throughput of up to several hundred samples per day.

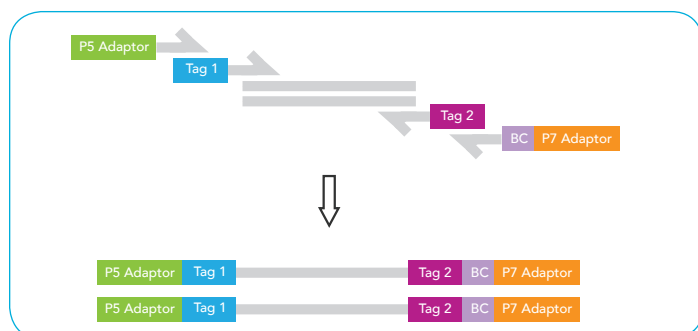


Figure 1. Nested primer sets simultaneously enrich a large number of target regions and incorporate sequencing adapters and indices to produce sequencing-ready libraries.

Seq-Ready™ TE Workflow Advantages

- One step target enrichment and library preparation
- Ligation-free workflow saves valuable labor, reagents and time
- No post-enrichment amplification required, saving reagents
- No sample-to-sample normalization required
- Minimal hands-on time
- Up to 24 samples per sequencing run

Complete Coverage

The Seq-Ready™ TE MultiSample BRCA 1/2 Panel targets 19 Kb of the human genome and yields excellent depth of coverage and uniformity (Figure 2). Over a hundred samples were sequenced on an Illumina MiSeq™ (2 x 250 bp), yielding 100% concordance with Sanger sequencing. The sequencing results show a uniformity of coverage of 99.3% at >5% of the mean coverage, with as little as 60 ng of input genomic DNA. Variant calling and zygosity determination were 100% accurate for BRCA 1/2. The SmartChip™ technology is ideal for clinical research laboratories who demand complete coverage of the target regions and the highest uniformity of coverage.

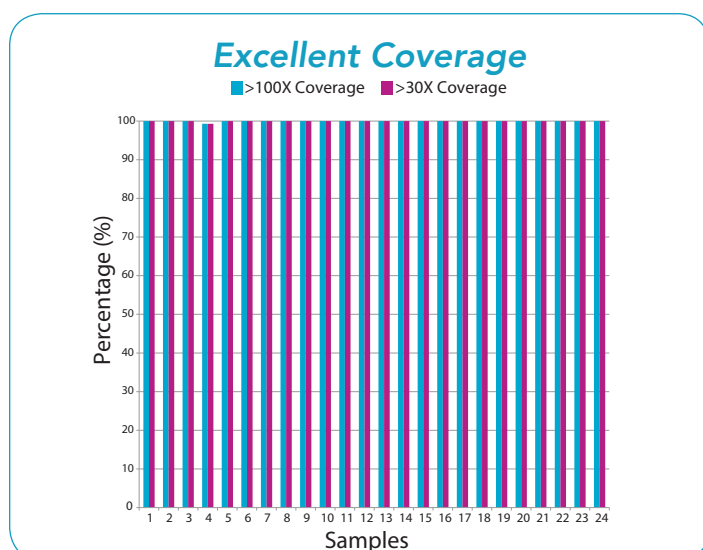


Figure 2. 24 samples were prepared on a single Seq-Ready™ TE MultiSample BRCA 1/2 SmartChip™. An average coverage of >100X for all regions of interest across the entire panel was obtained.

Optimal Coverage of Critical Regions

The Seq-Ready™ TE MultiSample BRCA 1/2 panel is designed with primers that have been optimized for 100% coverage of all targeted coding exons and exon-intron boundaries of BRCA1 and BRCA2 genes. Amplicons span at least 45 bp beyond the targeted coding exon and exon-intron boundaries to ensure that the regions of interest are sequenced. Amplicons overlap to reduce the chance of false negatives due to rare variants and polymorphisms. To reduce the possibility of dropouts, no SNPs with frequency >0.5% are included in the last 10 nucleotides of the 3' end of primers. Primer redundancy allows for maximum coverage in SNP dense regions.

Coverage Analysis Summary

Bases in target regions	19,092
Percent aligned base reads on-target	>97%
Mean coverage depth (N=24)	~5,000x
Uniformity of base coverage (> 0.05 of mean)	99.3%
Uniformity of amplicon coverage (> 0.05 of mean)	99.8%
Average target base coverage at 1x	100%
Average target base coverage at 30x	100%
Average target base coverage at 100x	100%

Ordering Information

Part Number	Product Name	Description
Fixed-Content DNA Panel Requirements		
430-000136	Seq-Ready™ TE MultiSample BRCA 1/2 Panel	Includes: 4 boxes (24 samples per box, total of 96 samples per kit) Each box includes: <ul style="list-style-type: none">• Seq-Ready™ TE SmartChip™ predispensed with Seq-Ready™ TE BRCA 1/2 Primers• Seq-Ready™ TE Loading Kit• TE Collection Kit
430-000138	Seq-Ready™ TE MultiSample Reagent Kit 1-24 (ILMN)	Includes: <ul style="list-style-type: none">• Seq-Ready™ TE Master Mix• Seq-Ready™ TE Universal Primer• Illumina-compatible indices• Illumina-compatible sequencing primers Enough reagents are supplied for 4 Seq-Ready™ TE BRCA 1/2 SmartChips™ (PN: 430-000136)
430-000139	Seq-Ready™ TE MultiSample Reagent Kit 25-48 (ILMN)	
430-000140	Seq-Ready™ TE MultiSample Reagent Kit 49-72 (ILMN)	
430-000141	Seq-Ready™ TE MultiSample Reagent Kit 73-96 (ILMN)	
System Requirements		
420-000029	Seq-Ready™ TE MultiSample System	Includes: <ul style="list-style-type: none">• MutliSample NanoDispenser• Centrifuge adapter (pair)• 2 SmartChip TE PCR Cyclers
Optional Systems		
420-000006	SmartChip™ Real-Time PCR Cycler	System hardware to enable quality control of target enriched libraries prior to sequencing.

To learn more, visit www.wafergen.com/seq-ready/brca

To place an order please contact us:

7400 Paseo Padre Parkway orders@wafergen.com www.wafergen.com
Fremont, CA 94555 (877) 923-3746 (877-WAFERGN)



Enabling clinical sequencing through complete coverage

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