PRODUCT SHEET

Seraseq® BRCA1/2 Large Genomic Rearrangement Products

The first comprehensive reference materials for NGS-based BRCA assay development, validation, and routine QC use

Genetic testing of the tumor suppressor genes BRCA1 and BRCA2 allows for the identification of DNA variants which are associated with a significantly elevated lifetime risk of breast, ovarian, pancreatic and prostate cancer.

Large genomic rearrangements (LGRs) include deletions, duplications and/or insertions often involving whole exons. Usually pathogenic, they have been reported to account for up to 27% of the overall BRCA1[1] and 5% of BRCA2 disease-causing mutations[2] with a strong founder effect accounting for about 1/3rd of all disease diagnosis.

Accurate detection of a BRCA1 or BRCA2 pathogenic variant has immense impact on clinical management of disease including patient's eligibility for new PARP inhibitors. Thus, it is crucial to determine germline as well as somatic BRCA1/2 mutations in those patients[3] and screening for large genomic rearrangements in both BRCA1 and BRCA2 is strongly recommended[4].

However, these LGRs are frequently missed by PCR-based methods and targeted NGS assays that do not detect partial or complete exon losses or gains. Given the difficulty in detecting LGRs, there is a need for a comprehensive BRCA1/2 testing algorithm including reference materials that incorporate pathogenic LGRs to support NGS assays that analyze for these mutations at both germline and somatic levels.

To help clinical labs performing NGS-based BRCA testing to better develop, characterize, validate, and routinely assess amplicon and hybrid capture-based targeted NGS assays, LGC Clinical Diagnostics has designed novel reference materials containing 20 pathogenic variants including 11 exon-level large rearrangements. Available as either full-process FFPE (for somatic testing) or gDNA (for germline or somatic testing) formats, they constitute the first set of comprehensive NGS assay reference materials for the profiling of BRCA-linked cancers.

This unique product made thanks to innovative technology and expertise, combines BRCA variants in a well-characterized genomic background (GM24385) at clinically relevant allele frequencies, precisely quantitated by digital PCR assays and analyzed by NGS. These include 10 variants in BRCA1 and 10 variants in BRCA2 ranging in size from SNVs to insertions and/or deletions over 500 bp, resulting in a variety of phenotypic alterations at the amino acid level, including missense, nonsense, frameshift, stop-gain/loss, splice-site, and insertion/deletion of partial or up to two exons (Table 1).

FEATURES AND BENEFITS

- Develop, monitor, validate and challenge your BRCA NGS assays with confidence using a highly multiplexed reference material containing biomarkers important in BRCA-driven cancers.
- 20 pathogenic BRCA1/2 variants (11 LGRs, 9 deletions, 5 SNVs, 4 indels, and 2 insertions).
- Available as full process (FFPE curl) or purified genomic DNA at various allele frequencies suited for either somatic or germline testing.
- Mutation targets quantitated by highly sensitive dPCR assays, and orthogonally analyzed by NGS.
- All mutations are blended against the well-characterized GM24385 human genomic DNA as 'wild-type' background material.

HIGHLIGHTS

First-to-market comprehensive reference standard for BRCA variants detection including 11 pathogenic exon-level rearrangements

Support both somatic and germline testing

Highly multiplexed containing 20 clinically-significant BRCA1/2 variants

High-quality reference material manufactured under cGMP compliance in ISO 13485 certified facilities

ORDERING INFORMATION

Product	Material	Conc.	Fill Volume	Total Mass	VAF
Seraseq FFPE BRCA1/2 LGR Reference Material	0730-0564	1 FFPE curl/vial	10 µm	>100 ng*	~15%**
Seraseq gDNA BRCA1/2 LGR Somatic Mutation Mix	0730-0567	15 ng/µl	25 µl	375 ng	~10%
Seraseq gDNA BRCA1/2 LGR Inherited Mutation Mix	0730-0568	15 ng/µl	25 µl	375 ng	~50%

*Based on Qiagen QIAamp DNA FFPE Tissue Kit and the Qubit dsDNA HS Assay. **Average variant allele frequencies (VAFs) as determined by digital PCR (dPCR; Bio-Rad QX200) with 3 replicates. The biosynthetic constructs bearing the BRCA1/2 variants were targeted between 5-20% VAFs. See the Technical Product Report for more details. Quality was assessed using the Agilent Genomic DNA ScreenTape assay for the Agilent TapeStation. Extracted DNA was analyzed using the Paragon Genomics CleanPlex® BRCA1 & BRCA2 Kit v3 amplicon based NGS assay.

To place an order, please contact us at +1.508.244.6400 and +1 800.676.1881 or email CDx-CustomerService@lgcgroup.com.

TABLE 1

Gene ID	Variant Type	Nucleotide change	Protein change	GRCh37	GRCh38	Transcript	Variant Length (bp)	Exon Involved*
BRCA1	Deletion	c.4487_4675+2del	Splice variant	17:41226346_41226536	17:43074329_43074519	NM_007294.4	191	Whole exon 12
	Insertion	c.4186_4357dup	p.R1397Yfs*2	17:41234421_41234592	17:43082404_43082575	NM_007294.4	172	Whole exon 14
	Deletion	c.2071_2171del	p.R691*	17:41245377_41245477	17:43093360_43093460	NM_007294.4	101	Part of exon 10
	Deletion	c.4987_5074del	p.V1665Sfs*8	17:41219625_41219712	17:43067608_43067695	NM_007294.4	88	Whole exon 16
	Deletion	c.5279_5332del	p.I1760_D1778delinsN	17: 41203080_41203133	17: 43051063_43051116	NM_007294.4	54	Part of exon 19
	INDEL	c.5209_5248delinsTC	p.R1737Sfs*80	17:41209098_41209137	17:43057081_43057120	NM_007294.4	40	
	INDEL	c.2820_2830delinsAAGATAAGCCAGTTTGATAA	p.D940_C944delinsER*	17:41244718_41244728	17:43092701_43092711	NM_007294.4	11	
	SNV	c.1961del	p.K654Sfs*47	17:41245587	17:43093570	NM_007294.4	1	
	SNV	c.4327C>T	p.R1443*	17:41234451	17:43082434	NM_007294.4	1	
	Deletion	c.441+2T>G	Splice variant	17:41256137	17:43104120	NM_007294.4	1	
BRCA2	Deletion	c.8755-2_9023del	Splice variant	13:32953452_32953956	13:32379315_32379819	NM_000059.4	505	Whole exon 22 and part of exon 23
	Deletion	c.2886_3144del	p.H962Qfs*6	13:32911378_32911636	13:32337241_32337499	NM_000059.4	259	Part of exon 11
	Deletion	c.68_316del	p.D23_L105del	13:32893214_32893462	13:32319077_32319322	NM_000059.4	249	Whole exon 3
	INDEL	c.5150_5226delinsTACTTAATACTTATTAAGTATTA	p.E1717_N1742delinsVLNTY*	13:32913642_32913718	13:32339505_32339581	NM_000059.4	77	
	INDEL	c.891_899delinsGATACTTCAG	p.T298Ifs*7	13:32906506_32906514	13:32332369_32332377	NM_000059.4	9	
	Deletion	c.5436del	p.E1812Dfs*3	13:32913927	13:32339790	NM_000059.4	1	
	SNV	c.8167G>C	p.D2723H	13:32937506	13:32363369	NM_000059.4	1	
	SNV	c.8331+2T>A	Splice variant	13:32937672	13:32363535	NM_000059.4	1	
	SNV	c.910G>T	p.E304*	13:32906525	13:32332388	NM_000059.4	1	
	Insertion	c.2407dup	p.Y803Lfs*2	13:32910898	13:32336761	NM_000059.4	1	

NOTE: Above list does not include variants present in the GM24385 background. The annotations of nucleotide and protein changes refer to transcripts NM_007294.4 (BRCA1) and NM_000059.4 (BRCA2).
*Exon information provided only for LGRs involving at least 50bp.

1. Wallace AJ. New challenges for BRCA testing: a view from the diagnostic laboratory. Eur J Hum Genet. 2016 Sep;24 Suppl 1(Suppl 1):S10-8. doi: 10.1038/ejhg.2016.94. PMID: 27514839; PMCID: PMC5141576.
2. James PA, Sawyer S, Boyle S, Young MA, Kovalenko S, Doherty R, McKinley J, Alsop K, Beshay V, Harris M, Fox S, Lindeman GJ, Mitchell G. Large genomic rearrangements in the familial breast and ovarian cancer gene BRCA1 are associated with an increased frequency of high risk features. Fam Cancer. 2015 Jun;14(2):287-95. doi: 10.1007/s10689-015-9785-0. PMID: 25678442.
3. N. Valtcheva, B.D. Nguyen, U. Wagner, S. Freiberger, Z. Varga, C. Britschgi, K.J. Dedes, M. Rechsteiner. Reliable detection of BRCA1 and BRCA2 large genomic rearrangements in FFPE tissue: A new diagnostic benchmark for somatic BRCA testing. Annals of Oncology. 2021 Suppl5 (vol32):S928. doi:10.1016/j.annonc.2021.08.786.
4. Woodward AM, Davis TA, Silva AGS, et alLarge genomic rearrangements of both BRCA2 and BRCA1 are a feature of the inherited breast/ovarian cancer phenotype in selected familiesJournal of Medical Genetics 2005;42:e31.

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