

Seraseq[®] Inherited Cancer Reference Material

SUPPORTING THE DEVELOPMENT OF GERMLINE MUTATION ASSAYS BY NEXT-GENERATION SEQUENCING

INTRODUCTION

Targeted Next-Generation Sequencing (NGS) panels are increasingly being used to discover causative variants for inherited cancer, such as Hereditary Breast and Ovarian Cancer. As such panels continue to expand, there is a growing demand for multiplexed reference materials that cover a broad range of pathogenic variants to expedite test development and validation. The traditional practice of using reference materials from public biobanks or remnant patient samples with single pathogenic variants can be extremely tedious, inefficient, and expensive.

HIGHLIGHTS

SINGLE-VIAL FORMAT FOR 24 HETEROZYGOUS VARIANTS ACROSS 7 CANCER GENES. IDEAL FOR DEVELOPMENT AND VALIDATION OF GERMLINE CANCER ASSAY

VARIANTS QUANTITATED WITH DIGITAL PCR. ASSURES PRECISE DETECTION OF GERMLINE MUTATIONS

HIGH-QUALITY MANUFACTURED REFERENCE MATERIAL SAVES TIME AND COST PROCURING SAMPLES OR PRODUCING HOMEBREW REAGENTS WITH SPECIFIC VARIANTS Seraseq Inherited Cancer DNA Mix v1 addresses the lack of multiplexed reference materials for targeted NGS assays with an expert-designed product¹ and published methodology² focused on seven genes associated with inherited cancer including BRCA1 and BRCA2. This unique product combines over 20 variants³ in a well-characterized genomic background that can be used for assay development, analytical validation or routine QC.

BENEFITS

- Single vial format for 24 unique variants across 7 cancer genes
- Save \$1000s in sequencing and validation costs with this highly multiplexed configuration
- Assess common, rare as well as technically challenging variants (TABLE 1)

FEATURES

- Mutation targets quantitated with digital PCR (dPCR)
- Well-characterized GM24385 human genomic DNA as background 'wild-type' material
- Manufactured under cGMP compliance and ISO 13485 certified facilities

PRODUCT DESIGN:

Biosynthetic constructs were designed to provide a mix of common, hard to find and hard to sequence inherited cancer specific variants in a single reference sample. Each individual mutation is engineered in the center of approximately 1,000 bp of wild-type gene sequence (Figure 1).

Variant Type	Number of variants	
Very large INDEL (>100bp)	2	
Large INDEL (11-30bp)	6	
Small INDEL (1-10bp)	6	
Homopolymer associated	2	
Tri-nucleotide or quad-nucleotide repeat associated	4	
In PMS2 exons 12-15	2	
Single nucleotide variants	2	
Total	24	

TABLE 1: List of mutation types (not including those in GM24385) present in the inherited cancer DNA mix v.1.



Figure 1: Design methodology for Seraseq Inherited Cancer DNA Mix v1. Schematic drawing for the design and formulation of the reference material. Six representative variants are shown here with the full list of variants listed in Table 3.

The constructs are precisely quantitated by a digital PCR assay and mixed into a single genomic DNA background (GM24385) to ensure a target allele frequency of 50%. The GM24365 genomic DNA has been extensively characterized by the Genome in a Bottle project³ and is originally derived from a participant in the Personal Genome Project, public profile huAA53EO⁴. The final product, therefore, contains both the engineered (biosynthetic) variants (TABLE 3) as well as endogenous variants contained in the GM24385 cell line (TABLE 2). This technology offers significant advantages over single-variant genomes or mixtures of various unrelated genomes while performing identically to authentic patient genomic DNA in NGS-based inherited disease assays².

Gene ID HGVS Nomenclature		dbSNP	
BRCA2	NM_000059.3:c.3396A>C, NM_000059.3:c.4563A>G NM_000059.3:c.5744C>T, NM_000059.3:c.6513G>C NM_000059.3:c.7242A>G, NM_000059.3:c.7397C= NM_000059.3:c.7806-14T>C	rs1801406, rs206075 rs4987117, rs206076 rs1799955, rs169547 rs9534262	
MLH1 NM_000249.3:c.655A>G		rs1799977	
MSH2 NM_000251.2:c.211+9C>G, NM_000251.2:c. 2006-6T>A		rs2303426, rs2303428	
MSH6	NM_000179.2:c.116G>A	rs1042821	
PMS2	NM_000535.5:c.705+17A>G, NM_000535.5:c.780C>G NM_000535.5:c.1408C>T, NM_000535.5:c.1621G>A NM_000535.5:c.2253T>C	rs62456182, rs1805319 rs1805321, rs2228006 rs1805325	

TABLE2: List of mutations present in the GM24385 background cell line

PRECISELY QUANTITATED MUTATION MIX

With the accuracy of digital PCR, Seraseq Inherited Cancer DNA Mix v1 provides a precisely quantitated mixture of heterozygous mutations. This highly multiplexed reference material is suitable for validation and monitoring of the complex NGS assays detecting germline cancer mutations. During the development and validation stage, Seraseq Inherited Cancer DNA Mix v1 can challenge the bioinformatic pipeline to ensure the detection of some challenging sequence variants. When run in parallel with clinical samples, it assures the ability to correctly call various types of mutations.

Gene ID	HGVS Nomenclature	dbSNP	Variant Length	Target Allele Frequency	Allele Frequency by NGS⁵
BRCA1	NM_007294.3:c.68_69del	rs386833395	2	50%	47.4%
BRCA1	NM_007294.3:c.2834_2836delinsC	rs386134270	3	50%	46.1%
BRCA1	NM_007294.3:c.3084_3094del	rs80357647	11	50%	45.5%
BRCA1	NM_007294.3:c.3113A>G	rs16941	1	50%	48.3%
BRCA1	NM_007294.3:c.3481_3491del	rs80357877	11	50%	43.8%
BRCA1	NM_007294.3:c.3756_3759del	rs80357868	4	50%	50.5%
BRCA1	NM_007294.3:c.5177_5180del	rs80357867	4	50%	51.5%
BRCA1	NM_007294.3:c.5266dupC	rs397507246	1	50%	49.2%
BRCA2	NM_000059.3:c.1310_1313del	rs80359277	4	50%	52.9%
BRCA2	NM_000059.3:c.1813dupA	rs397507277	1	50%	49.1%
BRCA2	NM_000059.3:c.8975_9100del	rs80359736	126	50%	Detected*
BRCA2	NM_000059.3:c.9342_9343insAluY	-	343	50%	Detected*
MSH2	NM_000251.2:c.942+3A>T	rs193922376	1	50%	35.8%
MSH2	NM_000251.2:c.1662-12_1677del	-	28	50%	36.2%
MSH6	NM_000179.2:c.2056_2060delinsCTTCTACCTCAAAAA	-	15	50%	41.6%
MSH6	NM_000179.2:c.2308_2312delinsT	rs864622585	6	50%	44.7%
MSH6	NM_000179.2:c.2641delinsAAAA	rs63751408	5	50%	48.1%
MSH6	NM_000179.2:c.3163dupG	-	1	50%	49.7%
MLH1	NM_000249.3:c.232_243delinsATGTAAGG	-	12	50%	42.6%
MLH1	NM_000249.3:c.1852_1854del	rs121912962	3	50%	51.9%
PMS2	NM_000535.5:c.861_864del	rs267608154	4	50%	44.8%
PMS2	NM_000535.5:c.2243_2246del	rs267608173	4	50%	34%
PMS2*	NM_00535.5:c.2444C>G	-	1	N/A	N/A
CDKN2A	NM_000077.4:c.9_32dup24	rs587780668	24	50%	42.1%

TABLE 3: The two very large indels (*) were detected by the bioinformatics pipeline, but the variant caller did not asses allele frequency. Note: The above list does not include variants present in the GM24385 background.

*Certain assays may detect the presence of a PMS2 variant (NM_000535.5:c.2444C>G) which was used for internal development purposes only.

RELIABLE, CONSISTENT CONTROL MATERIAL

As a manufactured reference material, developed under cGMP compliance and ISO 13485 certified facilities, Seraseg Inherited Cancer DNA Mix v1 provides a consistent source of reference material for your NGS assay. This product not only ensures a reliable supply which is consistent lot-to-lot; it also eliminates the need to obtain, characterize, blend, and document your own mixes of cell lineages, saving you time and resources in your assay development and validation efforts.

ORDERING INFORMATION

Material #	Product	Fill Size	
0730-0003	Seraseq® Inherited Cancer DNA Mix v1	1 vial x 200 μL per vial at 50 ng/ μL concentration (10 μg total)	
	The above product can be customized with additional mutations.	Custom fill size	

LEARN MORE

To learn more about Seraseg Inherited Cancer Reference Material and SeraCare's product offering for other inherited diseases, visit https://www.seracare.com/Controls---Reference-Materials-NGS-Inherited-Disease/. Contact us at 508.244.6400 and 800.676.1881 or email info@seracare.com.

INNOVATIVE TOOLS AND TECHNOLOGIES TO PROVIDE ASSURANCE IN DIAGNOSTIC ASSAY PERFORMANCE AND TEST RESULTS

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