Seraseq®
Inherited Cancer DNA Mix v2

Package Insert

NAME AND INTENDED USE

The Seraseq® Inherited Cancer DNA Mix v2 is a reference material intended for use in the development, validation, and evaluation of routine performance of Next Generation Sequencing (NGS) (and other amplified nucleic acid-based methods) that identify inherited (germline) variants in genes associated with hereditary cancer such as BRCA2 and MHS2. This reference material is suitable for use by clinical laboratories, research institutions, and diagnostic assay developers to ensure consistent and reliable results across different sequencing runs and laboratory conditions.

The Seraseq Inherited Cancer DNA Mix v2 is not intended for use in patient diagnosis, treatment, or in any therapeutic procedures. It is intended for use by trained laboratory personnel proficient in NGS technologies and familiar with proper laboratory practices and quality control procedures.
For Research Use Only (RUO). Not for use in diagnostic procedures.

REAGENT PROVIDED

Seraseq Inherited Cancer DNA Mix v2 is a mixture of synthetic DNA constructs and genomic DNA extracted from the human cell line GM24385. It contains 61 synthetic mutations in 55 cancer-predisposing genes (not including those present in the GM24385 background) (Table 2). The product is formulated to simulate a heterozygous state for each mutation at a 50% variant allele frequency (VAF) confirmed by droplet digital PCR and measured by NGS.

Table 1. Seraseq Inherited Cancer DNA Mix v2

Material No.	Product	Format
0730-0069	Seraseq Inherited Cancer DNA Mix v2	1x 20 µL

One (1) vial, 20 µL per vial, 500 ng total mass, at a nominal concentration of 25 ng/µL is provided. The product is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 aqueous buffer. Refer to the batch-specific Technical Product Report for exact concentration and VAF measured. Manufactured in the USA.

WARNINGS AND PRECAUTIONS

Safety and Handling Precautions

Handle Seraseq Inherited Cancer DNA Mix v2 and all materials derived from human blood products as though it is capable of transmitting infectious agents. Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens[1]. Do not pipette by mouth; do not smoke, eat, or drink in areas where specimens are being handled. Clean any spillage by immediately wiping up with 0.5% sodium hypochlorite solution. Avoid contamination of the product when opening and closing the vials. Dispose of all specimens and materials appropriately.

STORAGE INSTRUCTIONS

Store Seraseq Inherited Cancer DNA Mix v2 frozen between -30°C to -10°C. Once opened, a vial can be thawed and re-frozen up to five (5) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to five (5) or less.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq Inherited Cancer DNA Mix v2 should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and vials should be discarded.

PROCEDURE

Materials Required but not Provided

Refer to instructions supplied by manufacturers of the test kits to be used.

Instructions for Use

Allow the product vial to come to room temperature before use. Mix by vortexing to ensure a homogeneous solution and spin briefly. Seraseq Inherited Cancer DNA Mix v2 should be integrated into library preparation after the DNA isolation step; no further purification or DNA isolation is needed. If a DNA shearing step is part of the workflow, the reference material should be sheared and go through the target selection and library preparation in parallel with test specimens. Refer to standard assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS & INTERPRETATION OF RESULTS

Table 2 indicates each of the mutations represented in the Seraseq Inherited Cancer DNA Mix v2. While the presence and frequency of each variant in this product was confirmed during manufacture using digital PCR assays and NGS, there may be differences in observed allele frequencies due to assay characteristics. The Seraseq Inherited Cancer DNA Mix v2 does not have assigned values for allele frequencies of the variants present. Furthermore, specific detection of variants and variant allele frequencies within the product will vary among different assays, different procedures, different lot numbers, and different laboratories.

Each laboratory must establish an assay-specific expected value and acceptance range for each variant and lot of the Mutation Mix prior to its routine use. When results for the product are outside of the established acceptance range, it may indicate unsatisfactory test performance. Possible sources of error include: deterioration of test kit reagents, operator error, faulty performance of equipment, contamination of reagents or change in bioinformatics pipeline parameters.

LIMITATIONS OF THE PROCEDURE

Seraseq Inherited Cancer DNA Mix v2 MUST NOT BE SUBSTITUTED FOR THE MANDATORY POSITIVE AND NEGATIVE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

Seraseq Inherited Cancer DNA Mix v2 is not compatible with MLPA (Multiplex ligation-dependent probe amplification) assays and NGS analysis methods based only on coverage depth, since the large genomic rearrangements do not reflect copy losses or gains across the whole DNA sequence.

TEST PROCEDURES and *INTERPRETATION OF RESULTS* provided by manufacturers of test kits must be followed closely. Deviations from procedures recommended by test kit manufacturers may produce unreliable results. Seraseq Inherited Cancer DNA Mix v2 is not a calibrator and should not be used for assay calibration. Adverse shipping and storage conditions or use of outdated product may produce erroneous results.

REFERENCE

1. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings.

Human Genetic and Genomic Testing Using Traditional and High-Throughput Nucleic Acid Sequencing Methods. Third Edition. CLSI guideline MM09, 2023

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Table 2. List of Mutations

Gene	Mutation Type	Nucleotide change	Protein change	Transcript	GRCh37 Location	Variant Length
APC	SNV	c.4348C>T	p.R1450*	NM_000038.6	5:112175639	1
	Duplication	c.4666dup	p.T1556fs	NM_000038.6	5: 112175951-112175952	1
ATM	Deletion	c.640del	p.S214fs	NM_000051.4	11: 108114817	1
AXIN2	Duplication	c.1994dup	p.N666fs	NM_004655.4	17:63532585	1
BAP1	Duplication	c.1433_1449dup	p.P484fs	NM_004656.4	3:52437712_52437728	17
BARD1	Indel	c.1600_1634delinsGCG	p.T534fs	NM_000465.4	2:215617214_215617248	35
BMPR1A	Duplication	c.1131_1153dup	p.V385fs	NM_004329.3	10:88679191_88679213	23
BRCA1	Deletion	c.3481_3491del	p.E1161fs	NM_007294.4	17:41244057_41244067	11
BRCA2	Duplication	c.1813dup	p.I605fs	NM_000059.4	13:32907428	1
	Deletion	c.8954-8_9136del	Deletion	NM_000059.4	13:32953879_32954162	284
	Insertion	c.9342_9343insAluY	N/A	NM_000059.4	13:32968911_32968912	329
BRIP1	SNV	c.2392C>T	p.R798*	NM_032043.3	17:59793412	1
CDH1	Duplication	c.2037_2061dup	p.C688delinsHLRGQRV*	NM_004360.5	16: 68857396-68857397	25
CDK4	SNV	c.70C>T	p.R24C	NM_000075.4	12:58145431	1
CDKN1B	Duplication	c.59_77dup	p.S27fs	NM_004064.5	12: 12870830-12870831	19
CDKN2A	Insertion	c.9_32dup	p.A4_P11dup	NM_000077.5	9: 21974794-21974795	24
CHEK2	Deletion	c.1100del	p.T367fs	NM_007194.4	22: 29091856-29091857	1
DICER1	Duplication	c.682_724dup	p.V242fs	NM_177438.3	14: 95595818-95595819	43
EPCAM	Deletion	c.859-1462_*1999del	N/A	NM_002354.3	2:47610837_47615745	4,909
FH	Deletion	c.37_92del	p.P13fs	NM_000143.4	1:241682931_241682986	56
FLCN	Duplication	c.1285dup	p.H429fs	NM_144997.7	17: 17119708-17119709	1
HOXB13	Deletion	c.844_845del	p.A282fs	NM_006361.6	17:46804162_46804163	2

NOTE: Above list does not include variants present in the GM24385 background. Substitution refers to a single nucleotide variant; Indel is defined as a deletion/insertion less than 10 base pairs, and large genomic rearrangements (LGRs) (deletions or insertions) are defined as longer than 10 base pairs. Target variant allele frequency (VAF) at 50%.

Gene	Mutation Type	Nucleotide change	Protein change	Transcript	GRCh37 Location	Variant Length
KIT	SNV	c.1676T>C	p.V559A	NM_000222.2	4:55593610	1
MAX	Deletion	c.183_195del	p.Q62fs	NM_002382.5	14:65544731_65544743	13
MEN1	Duplication	c.1382_1404dup	p.E469fs	NM_001370259.2	11:64572250_64572272	23
MET	SNV	c.3281A>G	p.H1094R	NM_000245.4	7:116417464	1
MITF	Duplication	c.773_785dup	p.D263fs	NM_001354604.2	3: 69998210-69998211	13
MLH1	Deletion	c.1852_1854del	p.K618del	NM_000249.4	3:37089130_37089132	3
MSH2	SNV	c.942+3A>T	N/A	NM_000251.3	2:47641560	1
	Boland Inversion 3'	c.-125-9509096_1277-3165inv	N/A	NM_000251.3	2:38121110_47669522	9,548,413
MSH6	Duplication	c.3261dup	p.F1088fs	NM_000179.3	2: 48030639-48030640	1
MUTYH	Deletion	c.549_576del	p.L184fs	NM_001048174.2	1:45798276_45798303	28
NBN	Insertion	c.667_668insTTTATATTTTTATTATATAAAATATAATAAAGAAAACATTTATATTTTATAATATAAAAATATTTATATTATATATAAAATATAATAAAGAAAACATTATATA	p.K223delinsIYIFII*	NM_002485.5	8:90983435_90983436	102
NF1	Indel	c.2410-110_2850+65delinsAAAA		NM_001042492.3	17:29555933_29556548	616
	SNV	c.4600C>T	p.R1534*	NM_001042492.3	17:29588751	1
NTHL1	Duplication	c.417_436dup	p.L146fs	NM_002528.7	16: 2094719-2094720	20
PALB2	Deletion	c.3114-1_3201+2del	N/A	NM_024675.4	16:23625323_23625413	91
PDGFRA	SNV	c.2537A>T	p.D846V	NM_006206.6	4:55152105	1
PMS2	Deletion	c.2243_2246del	p.K748fs	NM_000535.7	7:6018256_6018259	4
	Deletion	c.861_864del	p.R287fs	NM_000535.7	7:6035204_6035207	4
POLD1	SNV	c.1433G>A	p.S478N	NM_002691.4	19:50909713	1

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Gene	Mutation Type	Nucleotide change	Protein change	Transcript	GRCh37 Location	Variant Length
POLE	SNV	c.1270C>G	p.L424V	NM_006231.4	12:133250250	1
PTCH1	Deletion	c.202-16_227del	N/A	NM_000264.5	9:98268856_98268897	42
PTEN	Deletion	c.750_751del	p.C250fs	NM_000314.8	10: 89717719-89717720	2
RAD50	Deletion	c.326_329del	p.T109fs	NM_005732.4	5:131911578_131911581	4
RAD51C	SNV	c.706-2A>G	N/A	NM_058216.3	7: 6037056	1
RAD51D	Indel	c.694_715delinsTGAGAGCTGAAGACCCTGGCCT	p.R232*	NM_002878.4	17:33430296_33430317	22
RET	SNV	c.2753T>C	p.M918T	NM_020975.6	10:43617416	1
SDHA	Indel	c.1785delinsCTTCTGGCGCGCATGCCAGG	p.E595fs	NM_004168.4	5:251574	20
SDHAF2	Deletion	c.267del	p.F89fs	NM_017841.4	11:61205478	1
SDHB	Insertion	c.42_43insCACTCTCCTTGAGGCGCCGGTTGCCG	p.A15delinsHSP*	NM_003000.3	1:17380472_17380473	26
SDHC	Deletion	c.250_251del	p.L84fs	NM_003001.5	1: 161326470-161326471	2
SDHD	Duplication	c.383_386dup	p.L129fs	NM_003002.4	11: 111965596-111965597	4
SMAD4	Duplication	c.1349_1376dup	p.A460fs	NM_005359.6	18: 48603038-48603039	28
SMARCA4	Deletion	c.917_941del	p.Q306fs	NM_003072.5	19: 11098390-11098414	25
STK11	Deletion	c.291-10_922del	N/A	NM_000455.5	19:1218406_1222985	4,580
TMEM127	Deletion	c.24_48del	p.L9fs	NM_017849.4	2:96931072_96931096	25
TP53	SNV	c.524G>A	p.R175H	NM_000546.6	17:7578406	1
TSC1	Indel	c.850_881delinsGCTTTCCTCATCGTT	p.R284fs	NM_000368.5	9:135787701_135787732	32
TSC2	SNV	c.2640-1G>A	N/A	NM_000548.5	16:2126068	1
VHL	SNV	c.481C>T	p.R161*	NM_000551.4	3:10191488	1

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