Seraseq™
Inherited Cancer DNA Mix v1

Package Insert

PLEASE NOTE:
THESE REAGENTS MUST NOT BE SUBSTITUTED FOR THE MANDATORY POSITIVE AND NEGATIVE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

NAME AND INTENDED USE
The Seraseq™ Inherited Cancer DNA Mix v1 is intended for use with Next Generation Sequencing (NGS) assays that identify inherited (germline) variants in genes associated with inherited cancer such as *BRCA1* and *BRCA2*. The Seraseq Inherited Cancer DNA Mix v1 is intended as a quality reference material for translational and disease research testing and monitors library preparation, sequencing, and variant allele detection under a given set of bioinformatics pipeline parameters. *For Research Use Only. Not for use in diagnostic procedures.*

SUMMARY
A well-designed quality control program can provide added confidence in the reliability of results obtained for unknown specimens. The use of independent reference products may provide valuable information concerning assay accuracy and bioinformatics pipeline analysis.

PRINCIPLES OF THE PROCEDURE
Seraseq Inherited Cancer DNA Mix v1 is ready-to-use in Next Generation Sequencing (NGS) assays in steps that follow DNA isolation; no further purification or DNA isolation is needed. The reference materials should follow the same workflow as unknown samples. The product contains DNA at a concentration of 50 ng/µL. The Reference Material is formulated in 1 mM Tris / 0.1 mM EDTA pH 8.0, which is a buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

Seraseq Inherited Cancer DNA Mix v1 contains 23 mutations (not including those present in the GM24385 background) that are associated with inherited cancer (Table 1). The product is formulated to simulate the heterozygous state and the 50% variant allele frequency is confirmed by droplet digital PCR.

REAGENTS
Item No. 0730-0003. 1 vial, 200 µL per vial, 50 ng/µL concentration.

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
CAUTION: Handle Seraseq Inherited Cancer DNA Mix v1 and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq Inherited Cancer DNA Mix v1 is manufactured using genomic DNA extracted from the human cell line GM24385, which is a B-lymphocytic, male cell line from the Personal Genome Project offered by the NIGMS Human Genetic Cell Repository (https://catalog.coriell.org/1/NIGMS). Purified genomic DNA is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 aqueous buffer.

Safety Precautions
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. Dispose of all specimens and materials used in testing as though they contain infectious agents.

Handling Precautions
Avoid contamination of the product when opening and closing the vials.

STORAGE INSTRUCTIONS
Store Seraseq Inherited Cancer DNA Mix v1 frozen at -20 °C or colder. 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 v1 is a mixture of human genomic DNA and synthetic DNA constructs. It 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 Provided
Seraseq Inherited Cancer DNA Mix v1 is a mixture of human genomic DNA and synthetic DNA constructs in a 1 mM Tris / 0.1 mM EDTA pH 8.0 buffer. Two hundred (200) µL is provided per tube and the concentration is 50 ng/µL.

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 v1 should be integrated into library preparation after the DNA isolation step. If a DNA shearing step is part of the workflow, Seraseq Inherited Cancer DNA Mix v1 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.

Quality Control
Although Seraseq Inherited Cancer DNA Mix v1 is designed to simulate a heterozygous state and offered at a target mutation frequency of 50%, the product does not have assigned values for mutation frequencies. There are many reasons why assays may observe deviation from the representative data which may or may not be of significance. It is therefore recommended that each laboratory qualify the use of Seraseq Inherited Cancer DNA Mix v1 with each assay system prior to its routine use.

INTERPRETATION OF RESULTS
Detection of the variants within Seraseq Inherited Cancer DNA Mix v1 may vary with different types of tests and different test kit lots. Since the reference material does not have an assigned value, the laboratory must establish a range for each lot of Seraseq Inherited Cancer DNA Mix v1. 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 v1 MUST NOT BE SUBSTITUTED FOR THE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

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 v1 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.

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EXPECTED RESULTS

Specific detection of variants and variant allele frequencies will vary among different assays, different procedures, different lot numbers, and different laboratories. Each laboratory should establish its own acceptance criteria. For example, the acceptable range for each variant might include all values within two standard deviations of the mean of 20 data points obtained in 20 runs[2]. Table 1 lists the variants in the product and their target allele frequencies (verified by digital PCR).

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq Inherited Cancer DNA Mix v1 has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq Inherited Cancer DNA Mix v1 does not have assigned values. Procedures for implementing a quality assurance program and monitoring test performance on a routine basis must be established by each individual laboratory.

Table 1: List of mutations incorporated

Gene ID	Mutation Type	HGVS Nomenclature	Target allele frequency
BRCA2	Deletion	NM_000059.3:c.8975_9100del	50%
BRCA2	Deletion	NM_000059.3:c.1310_1313del	50%
BRCA2	Duplication	NM_000059.3:c.1813dupA	50%
BRCA2	Insertion	NM_000059.3:c.9342_9343insAluY	50%
BRCA1	Duplication	NM_007294.3:c.5266dupC	50%
BRCA1	Deletion	NM_007294.3:c.5177_5180del	50%
BRCA1	Deletion	NM_007294.3:c.3756_3759del	50%
BRCA1	Deletion	NM_007294.3:c.3481_3491del	50%
BRCA1	Substitution	NM_007294.3:c.3113A>G	50%
BRCA1	Deletion	NM_007294.3:c.3084_3094del	50%
BRCA1	Deletion-Insertion	NM_007294.3:c.2834_2836delinsC	50%
BRCA1	Deletion	NM_007294.3:c.68_69del	50%
MSH2	Substitution	NM_000251.2:c.942+3A>T	50%
MSH2	Deletion	NM_000251.2:c.1662-12_1677del	50%
MSH6	Deletion-Insertion	NM_000179.2:c.2056_2060delinsCTTCTACCTCAAAAA	50%
MSH6	Insertion	NM_000179.2:c.2308_2312delinsT	50%
MSH6	Deletion-Insertion	NM_000179.2:c.2641delinsAAAA	50%
MSH6	Duplication	NM_000179.2:c.3163dupG	50%
MLH1	Deletion	NM_000249.3:c.1852_1854del	50%
MLH1	Deletion-Insertion	NM_000249.3:c.232_243delinsATGTAAGG	50%
PMS2	Deletion	NM_000535.5:c.2243_2246del	50%
PMS2	Deletion	NM_000535.5:c.861_864del	50%
CDKN2A	Insertion	NM_000077.4:c.9_32dup24	50%

Note: Above list does not include variants present in the GM24385 background. Variant nomenclature provided complies with HGVS guidelines (http://varnomen.hgvs.org/). Certain assays may detect the presence of a PMS2 variant (NM_000535.5:c.2444C>G) which was used for internal development purposes only.

REFERENCES

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.
2. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition. NCCLS document C24-A2, 1999.