Seraseq®

Tumor Mutation DNA Mix v3 AF7%, AF10% and Tri-Level Package Insert

NAME AND INTENDED USE

The Seraseq® Tumor Mutation DNA Mix v3 AF10%, Seraseq Tumor Mutation DNA Mix v3 AF7% and Seraseq Tumor Mut DNA Mix v3 Tri-Level are intended for use with Next Generation Sequencing (NGS) assays that detect mutations in key oncogenes and tumor suppressor genes. These products are intended as a quality reference material for translational and disease research testing to monitor 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 Tumor Mutation DNA Mix v3 AF10%, AF7% and Tri-Level are 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 25 ng/µL. The reference material is formulated in 1 mM Tris / 0.1 mM EDTA pH 8.0, which is an aqueous buffer that is compatible with both PCR-based target amplification and hybridization-based target selection methods.

Seraseq Tumor Mutation DNA Mix products contain 112 mutations (not including those present in the GM24385 background) that are associated predominantly with druggable mutations relevant to solid tumors (see Table 2). Variant allele frequency (VAF) and copy gain, is confirmed by digital PCR. VAF is also measured by NGS as reported in the batch-specific TPR.

REAGENTS

Table 1. Seraseq Solid Tumor Mutation DNA Mixes v3

Material No.	Product
0710-3460	Seraseq Tumor Mutation DNA Mix v3 AF10%
0710-3461	Seraseq Tumor Mutation DNA Mix v3 AF7%
0710-3462	Seraseq Tumor Mutation DNA Mix v3 Tri-Level

Each Material No. is available as an individual product. Information in this Package Insert applies to all products.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

CAUTION: Handle Seraseq Solid Tumor Mutation DNA Mix v3 products and all materials derived from human blood products as though it is capable of transmitting infectious agents. Seraseq Solid Tumor Mutation DNA Mix v3 products are 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). Seraseq Solid Tumor Mutation DNA Mix v3 products are 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 it 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 Solid Tumor Mutation DNA Mix v3 products 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. When stored in this fashion Seraseq Solid Tumor Mutation DNA Mix v3 products will be stable through the expiration indicated on the vial label.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq Solid Tumor Mutation DNA Mix v3 products are 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 Solid Tumor Mutation DNA Mix v3 products are a mixture of human genomic DNA and synthetic DNA constructs containing variants mixed at defined allelic frequencies. Seraseq Solid Tumor Mutation Mixes v3 are formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0. 15 µL is provided per tube and the concentration is 25 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. Solid Tumor Mutation DNA Mix v3 products should be integrated into library preparation after the DNA isolation step. Refer to standard assay procedures in order to determine the amount of material to use.

Quality Control

Although Seraseq Solid Tumor Mutation DNA Mix v3 products are designed to assess DNA present at the indicated target VAF, the products do 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 Solid Tumor Mutation DNA Mix v3 products with each assay system prior to its routine use.

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INTERPRETATION OF RESULTS
Detection of the variants within Seraseq Solid Tumor Mutation DNA Mix v3 products 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 Solid Tumor Mutation DNA Mix v3 products. 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 Solid Tumor Mutation mix v3 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 Solid Tumor Mutation DNA Mix v3 products are not calibrators and should not be used for assay calibration. Adverse shipping and storage conditions or the use of outdated product may produce erroneous results.

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 2 lists the variants present in all three products and target allele frequencies for Seraseq Tumor Mutation DNA Mix v3 Tri-Level (verified by digital PCR).

Seraseq Tumor Mutation DNA Mix v3 AF10% is targeted at 10% VAF and +12 copies for CNVs (verified by digital PCR).

Seraseq Tumor Mutation DNA Mix v3 AF7% is targeted at 7% VAF and +6 copies for CNVs (verified by digital PCR).

SPECIFIC PERFORMANCE CHARACTERISTICS
Seraseq Solid Tumor Mutation DNA Mix v3 products are designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Solid Tumor Mutation DNA Mix v3 products do 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.

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– Fourth Edition. CLSI document C24, 2016.

Table 2: List of Mutations

Gene	Nucleotide Change	Transcript	Variant Type	Tri-Level Target AF & Amplification
ARID1A	c.5548del	NM_006015.6	Deletion	4%
BRAF	c.1799T>A	NM_004333.6	SNV	4%
BRCA2	c.7934del	NM_000059.4	Deletion	4%
CDK12	c.4382del	NM_016507.4	Deletion	4%
EGFR	c.2236_2250del	NM_005228.5	Deletion	4%
EGFR	c.2303G>T	NM_005228.5	SNV	4%
EGFR	c.2310_2311insGGT	NM_005228.5	Insertion	4%
EGFR	c.2369C>T	NM_005228.5	SNV	4%
EGFR	c.2389T>A	NM_005228.5	SNV	4%
ESR1	c.1613A>G	NM_000125.4	SNV	4%
EZH2	c.1937A>T	NM_004456.5	SNV	4%
FANCA	c.2778+1G>A	NM_000135.4	SNV	4%
FANCL	c.1096_1099dup	NM_018062.4	Duplication	4%

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Gene	Nucleotide Change	Transcript	Variant Type	Tri-Level Target AF & Amplification
FGFR3	c.746C>G	NM_000142.5	SNV	4%
FOXL2	c.402C>G	NM_023067.4	SNV	4%
IDH1	c.394C>T	NM_005896.4	SNV	4%
KRAS	c.34G>T	NM_004985.5	SNV	4%
MAP2K1	c.370C>T	NM_002755.4	SNV	4%
MLH1	c.232_243delinsATGTAAGG	NM_000249.4	INDEL	4%
MRE11	c.1100_1131del	NM_005591.4	Deletion	4%
MSH6	c.2056_2060delinsCTTCTACCTCAAAAA	NM_000179.3	INDEL	4%
NBN	c.1396del	NM_002485.5	Deletion	4%
NF1	c.3738_3747del	NM_001042492.3	Deletion	4%
NTRK1	c.1783G>A	NM_002529.4	SNV	4%
PALB2	c.839del	NM_024675.4	Deletion	4%
PIK3CA	c.3140A>G	NM_006218.4	SNV	4%
PIK3CA	c.3203dup	NM_006218.4	Insertion	4%
PIK3R1	c.1727_1729del	NM_181523.3	Deletion	4%
RAD51C	c.242C>A	NM_058216.3	SNV	4%
RAD51C	c.338dup	NM_058216.3	SNV	4%
RET	c.2753T>C	NM_020975.6	SNV	4%
TP53	c.267del	NM_000546.6	Deletion	4%
TSC1	c.1263+1G>T	NM_000368.5	SNV	4%
AR	c.2623C>T	NM_000044.6	SNV	7%
BRCA1	c.1961del	NM_007294.4	Deletion	7%
CDKN2A	c.9_32dup	NM_000077.5	Insertion	7%
CHEK1	c.676del	NM_001114122.3	Deletion	7%
CTNNB1	c.121A>G	NM_001904.4	SNV	7%
EGFR	c.2573T>G	NM_005228.5	SNV	7%
HRAS	c.182A>G	NM_005343.4	SNV	7%
HRAS	c.37G>C	NM_005343.4	SNV	7%
IDH2	c.419G>A	NM_002168.4	SNV	7%
KIT	c.2361+67_2361+72delTTTTTT	NM_000222.3	Deletion	7%
KRAS	c.35G>A	NM_004985.5	SNV	7%
MAP4K3	c.246-2475_246-2470delTTTTTT	NM_003618.4	Deletion	7%

Gene	Nucleotide Change	Transcript	Variant Type	Tri-Level Target AF & Amplification
MET	c.3082+1del	NM_001127500.3	Deletion	7%
MSH2	c.1662-12_1677del	NM_000251.3	Deletion	7%
MTOR	c.6644C>A	NM_004958.4	SNV	7%
NRAS	c.182A>G	NM_002524.5	SNV	7%
NTRK2	c.1915G>A	NM_006180.6	SNV	7%
PTCH1	c.2307_2308delinsTT	NM_000264.5	INDEL	7%
PTEN	c.741dup	NM_000314.8	Insertion	7%
PTEN	c.800del	NM_000314.8	Deletion	7%
PTPN11	c.226G>A	NM_002834.5	SNV	7%
RAD54L	c.636_637dup	NM_003579.4	Duplication	7%
RAF1	c.770C>T	NM_002880.4	SNV	7%
RB1	c.751C>T	NM_000321.3	SNV	7%
SLC7A8	c.-231_-224delTTTTTTTT	NM_012244.4	Deletion	7%
TERT	c.-124C>T	NM_198253.3	SNV	7%
TERT	c.-146C>T	NM_198253.3	SNV	7%
TP53	c.818G>A	NM_000546.6	SNV	7%
TP53	c.743G>A	NM_000546.6	SNV	7%
TP53	c.723del	NM_000546.6	Deletion	7%
TP53	c.524G>A	NM_000546.6	SNV	7%
TSC2	c.2640-1G>A	NM_000548.5	SNV	7%
AKT1	c.49G>A	NM_005163.2	SNV	10%
APC	c.4348C>T	NM_000038.6	SNV	10%
APC	c.4666dup	NM_000038.6	Insertion	10%
ATM	c.1058_1059del	NM_000051.4	Deletion	10%
BARD1	c.1600_1634delinsGCG	NM_000465.4	Indel	10%
BRIP1	c.157dup	NM_032043.3	SNV	10%
CHEK2	c.1116_1117delinsGT	NM_007194.4	INDEL	10%
EGFR	c.2235_2249del	NM_005228.5	Deletion	10%
ERBB2	c.2313_2324dup	NM_004448.4	Insertion	10%
IDH2	c.515G>A	NM_002168.4	SNV	10%
KIT	c.2447A>T	NM_000222.3	SNV	10%
KRAS	c.183A>C	NM_004985.5	SNV	10%

Gene	Nucleotide Change	Transcript	Variant Type	Tri-Level Target AF & Amplification
MAP4K3	c.998-35_998-30delAAAAAA	NM_003618.4	Deletion	10%
MSH2	c.942+20_942+29delAAAAAAAAA	NM_000251.3	Deletion	10%
NTRK3	c.1867G>A	NM_001012338.3	SNV	10%
PDGFRA	c.2525A>T	NM_006206.6	SNV	10%
PIK3CA	c.1633G>A	NM_006218.4	SNV	10%
PMS2	c.861_864del	NM_000535.7	Deletion	10%
RAD51D	c.392dup	NM_002878.4	SNV	10%
RAD51D	c.271A>T	NM_002878.4	SNV	10%
SMAD4	c.1394dup	NM_005359.6	Insertion	10%
SMARCB1	c.118C>T	NM_003073.5	SNV	10%
STK11	c.734+1G>T	NM_000455.5	SNV	10%
VHL	c.481C>T	NM_000551.4	SNV	10%
ZNF2	c.*1525_*1530delTTTTTT	NM_021088.4	Deletion	10%
CD74::NRG1	Intron 6::Intron 5	NM_001025159.3::NM_013964.5	Translocation	10%
CD74::ROS1	Intron 6::Intron 34	NM_001025159.3::NM_001378902.1	Translocation	10%
COL1A1::PDGFB	Intron 25::Intron 1	NM_000088.3::NM_002608.3	Translocation	10%
EML4::ALK	Intron 13::Intron 19	NM_019063.5::NM_004304.5	Translocation	10%
ETV6::NTRK3	Intron 5::Intron 14	NM_001987.5::NM_002530.4	Translocation	10%
FGFR2::BICC1	Intron 17::Intron 2	NM_000141.5::NM_001080512.3	Translocation	10%
FGFR3::TACC3	Exon 18::Intron 7	NM_000142.5::NM_006342.3	Translocation	10%
NCOA4::RET	Intron 7::Intron 11	NM_001145263.2::NM_020975.6	Translocation	10%
PML::NTRK2	Intron 2::Intron 12	NM_002675.4::NM_006180.6	Translocation	10%
TPM3::NTRK1	Intron 7::Intron 9	NM_153649.4::NM_002529.4	Translocation	10%
CCND1	Amplification	NM_053056.3	CNV	+12 copies
FGF19	Amplification	NM_005117.3	CNV	+12 copies
FGF3	Amplification	NM_005247.4:11	CNV	+12 copies
FGF4	Amplification	NM_002007.4	CNV	+12 copies
FGFR1	Amplification	NM_023110.3	CNV	+12 copies
MYCN	Amplification	NM_005378.6	CNV	+12 copies
ERBB2	Amplification	NM_004448.4	CNV	+3 copies
MET*	Amplification	NM_001127500.3	CNV	+3 copies

Gene	Nucleotide Change	Transcript	Variant Type	Tri-Level Target AF & Amplification
AKT2	Amplification	NM_001626.6	CNV	+6 copies
CCNE1	Amplification	NM_001238.4	CNV	+6 copies
CDK4	Amplification	NM_000075.4	CNV	+6 copies
MYC	Amplification	NM_002467.6	CNV	+6 copies

* MET gene is covered using overlapping DNA constructs.
The overlapping regions are expected to show higher copy number levels than the rest of the gene.

NOTE:

The above list does not include variants present in the GM24385 background. Indels are defined as insertion/deletions less than 10 base pairs.

Table 2 lists the variants and CNVs present in all three products and only includes target allele frequencies and copy gains for Seraseq Tumor Mutation DNA Mix v3 Tri-Level.

Seraseq Tumor Mutation DNA Mix v3 AF7% mix contains all variants at a target allele frequency of 7% and CNVs at +6 copies.

Seraseq Tumor Mutation DNA Mix v3 AF10% mix contains all variants at a target allele frequency of 10% and CNVs at +12 copies.