Seraseq® Tumor Mutation DNA Mix v3

Comprehensive reference material for development, validation and daily run controls for solid tumor assays

INTRODUCTION

As the utility of precision oncology continues to expand, next-generation sequencing (NGS) is rapidly emerging as the standard for detecting somatic mutations that guide cancer diagnosis, prognosis, and targeted therapy selection. NGS-based comprehensive genomic profiling (CGP) assays enable the simultaneous detection of a broad range of genomic alterations across hundreds of cancer-related genes in a single run. The complexity of these assay workflows, variability in patient sample quality, and the need for accurate detection of low-frequency mutations present significant challenges for assay development and validation. Comprehensive high-quality reference materials play a critical role in supporting such efforts for evaluating assay performance and ensuring regulatory compliance.

LGC's SeraCare brand continues to lead in providing innovative, fit-to-purpose reference standards to meet the evolving needs of NGS assays. The new Seraseq® Tumor Mutation DNA Mix v3 reference materials represent our most complex product to date, containing the highest number of biosynthetic variants in a single mix. These products are specifically designed to support NGS assay development, validation and routine QC monitoring.

Seraseq® Tumor Mutation DNA Mix v3 reference materials, engineered to mimic patient samples, contain 112 clinically relevant variants (SNVs, indels, CNVs, translocations) and support mutation detection across varied allele frequencies. All mutations are blended against a well-characterized GM24385 background and precisely quantified by highly sensitive digital PCR to offer high confidence in assay validation studies including determination of assay sensitivity, specificity and Limit of Detection (LoD). They are easy to source and readily available, reducing time and cost particularly associated with sourcing finite patient samples, which often contain limited mutations. Manufactured under rigorous design control procedures, these products ensure lot-to-lot consistency and accuracy, ultimately supporting high-quality genomic testing outcomes.

HIGHLIGHTS

Highly multiplexed containing 112 variants across 92 solid tumor genes - all in a single vial

Broad variant coverage including translocations, CNVs, MMR, HRR and MSI markers

Manufactured under cGMP compliant ISO certified laboratories

ORDERING INFORMATION

Material #	Product	Format	Target Concentration*	Fill Volume	Total Mass
0710-3460	Seraseq® Tumor Mutation DNA Mix v3 AF10%	gDNA	25 ng/µL	15 µL	375 ng
0710-3461	Seraseq® Tumor Mutation DNA Mix v3 AF7%	gDNA	25 ng/µL	15 µL	375 ng
0710-3462	Seraseq® Tumor Mut DNA Mix v3 Tri-Level	gDNA	25 ng/µL	15 µL	375 ng

*target concentrations are based on Qubit dsDNA BR assay

FEATURES AND BENEFITS

- 112 unique multiplexed biosynthetic DNA variants across 92 genes covering 51 SNVs, 5 INDELs, 25 deletions, 9 insertions, 10 translocations, and 12 CNVs, in a single reference material
- 94 variants recognized as FDA drug targets support comprehensive detection of clinically actionable mutations
- Three unique products offered at varied allele frequencies: 7%, 10% and Tri-Level (4%, 7% and 10% VAF tiers)
- Mutation targets precisely quantified with digital PCR and orthogonally validated by NGS
- Blended with well-characterized GM24385 human genomic DNA as background wild-type material
- Manufactured under cGMP-compliance and ISO 13485-certified facilities

MUTATIONS PRESENT IN THE SERASEQ® TUMOR MUTATION MIX V3 PRODUCTS (AF7%, AF10%, TRI-LEVEL)

GENE ID	NUCLEOTIDE CHANGE	TRANSCRIPT	VARIANT TYPE	TRI-LEVEL TARGET AF & AMPLIFICATION (#refer footnote for AF7 & AF10 target AF and copies)
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%
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%

MUTATIONS PRESENT IN THE SERASEQ® TUMOR MUTATION MIX V3 PRODUCTS (AF7%, AF10%, TRI-LEVEL) - CONTINUED

GENE ID	NUCLEOTIDE CHANGE	TRANSCRIPT	VARIANT TYPE	TRI-LEVEL TARGET AF & AMPLIFICATION (#refer footnote for AF7 & AF10 target AF and copies)
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%

MUTATIONS PRESENT IN THE SERASEQ® TUMOR MUTATION MIX V3 PRODUCTS (AF7%, AF10%, TRI-LEVEL) - CONTINUED

GENE ID	NUCLEOTIDE CHANGE	TRANSCRIPT	VARIANT TYPE	TRI-LEVEL TARGET AF & AMPLIFICATION (#refer footnote for AF7 & AF10 target AF and copies)
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%
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%

MUTATIONS PRESENT IN THE SERASEQ® TUMOR MUTATION MIX V3 PRODUCTS (AF7%, AF10%, TRI-LEVEL) - CONTINUED

GENE ID	NUCLEOTIDE CHANGE	TRANSCRIPT	VARIANT TYPE	TRI-LEVEL TARGET AF & AMPLIFICATION (#refer footnote for AF7 & AF10 target AF and copies)
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%
MAP4K3	c.998-35_998-30delAAAAAA	NM_003618.4	Deletion	10%
MSH2	c.942+20_942+29delAAAAAAAAAA	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%

MUTATIONS PRESENT IN THE SERASEQ® TUMOR MUTATION MIX V3 PRODUCTS (AF7%, AF10%, TRI-LEVEL) - CONTINUED

GENE ID	NUCLEOTIDE CHANGE	TRANSCRIPT	VARIANT TYPE	TRI-LEVEL TARGET AF & AMPLIFICATION (#refer footnote for AF7 & AF10 target AF and copies)
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		10%
COL1A1::P-DGFB	Intron 25::Intron 1	NM_000088.3::NM_002608.3		10%
EML4::ALK	Intron 13::Intron 19	NM_019063.5::NM_004304.5		10%
ETV6::NTRK3	Intron 5::Intron 14	NM_001987.5::NM_002530.4		10%
FGFR2::BICC1	Intron 17::Intron 2	NM_000141.5:: NM_001080512.3		10%
FGFR3::TACC3	Exon 18::Intron 7	NM_000142.5::NM_006342.3		10%
NCOA4::RET	Intron 7::Intron 11	NM_001145263.2:: NM_020975.6		10%
PML::NTRK2	Intron 2::Intron 12	NM_002675.4::NM_006180.6		10%
TPM3::NTRK1	Intron 7::Intron 9	NM_153649.4::NM_002529.4		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

MUTATIONS PRESENT IN THE SERASEQ® TUMOR MUTATION MIX V3 PRODUCTS (AF7%, AF10%, TRI-LEVEL) - CONTINUED

GENE ID	NUCLEOTIDE CHANGE	TRANSCRIPT	VARIANT TYPE	TRI-LEVEL TARGET AF & AMPLIFICATION (#refer footnote for AF7 & AF10 target AF and copies)
MET*	Amplification	NM_001127500.3	CNV	+3 copies
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 **but** includes **only** 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.

AVERAGE VARIANT ALLELE FREQUENCY (VAF) MEASURED BY DIGITAL PCR

Figure 1 shows the average allele frequency of each variant assayed by digital PCR for three products: AF7%, AF10%, and Tri-Level. Tri-Level targets variants at three VAF tiers: 4%, 7%, and 10%. The measured VAFs fall within ±30% of their respective target allele frequencies, underscoring the stringent product release testing measures and precise quantification of variants at low allele frequencies.

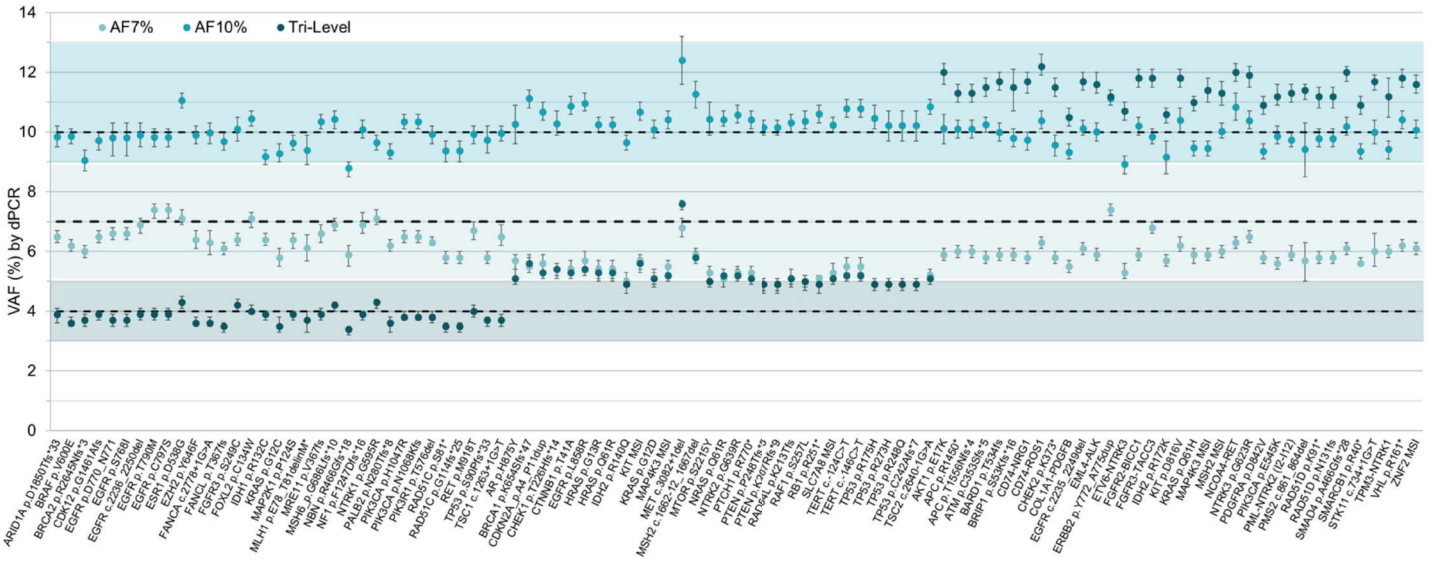

FIGURE 1: Average VAF for each variant in the Seraseq Mutation DNA mix v3 materials as measured by dPCR (n=3). Error bars represent 95% confidence intervals. Dashed lines are target VAFs and shaded regions represent acceptable range for each target.

CORRELATION OF VAFs ACROSS NGS ASSAYS AND DIGITAL PCR PLATFORMS

Figure 2a demonstrates the correlation between VAFs measured by TSO 500 and digital PCR assays across all three products—AF10%, AF7% and Tri-Level. Figure 2b and 2c shows correlation between VAFs measured across different NGS chemistries—TSO 500 vs AmpliSeq and TSO 500 vs VariantPlex respectively. The strong correlation highlights the reliability and accuracy of these reference materials across different platforms and chemistries. Slightly reduced VAFs for some variants reported by TSO 500 compared to digital PCR are consistent with expectations for NGS based assays that apply stringent filtering.

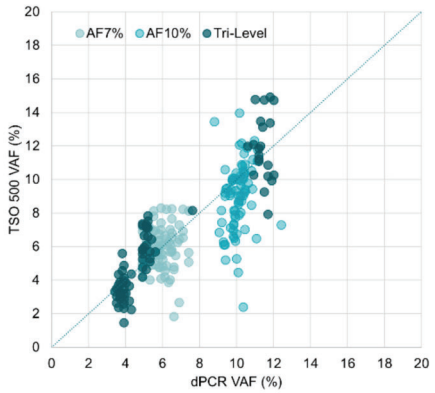

FIGURE 2A: Correlation between VAFs measured by digital PCR and TSO 500

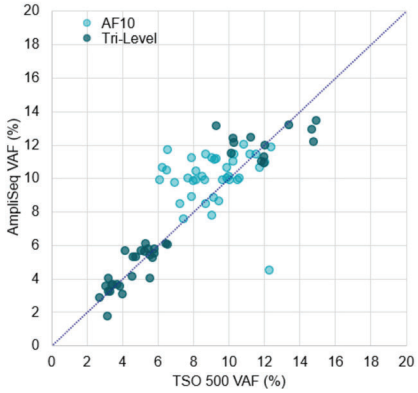

FIGURE 2B: Correlation between VAFs measured by AmpliSeq and TSO 500

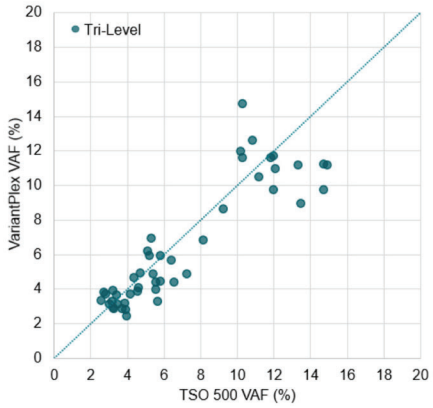

FIGURE 2C: Correlation between VAFs measured by VariantPlex and TSO 500

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

ABOUT US

SeraCare offers a comprehensive portfolio of reference materials for oncology and reproductive health, designed and manufactured to meet the precision demanded by NGS assays. The portfolio includes high quality ground-truth RNA, ctDNA and genomic DNA-based reference materials that are NGS platform agnostic for tumor profiling, immuno-oncology, liquid biopsy, NIPT and germline cancer assay workflows. For more information visit seracare.com

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