PRODUCT SHEET

Seraseq™ Tri-Level Tumor Mutation DNA Mix v2 HC

DAILY-RUN QC MATERIAL FOR NGS-BASED SOMATIC MUTATION ASSAYS

HIGHLIGHTS

SINGLE-SAMPLE MULTIPLEXED FORMAT; CONSERVES SPACE FOR PATIENT SAMPLES.

40 UNIQUE VARIANTS, QUANTITATED WITH DIGITAL PCR; ASSURES ACCURATE, PRECISE, AND CONSISTENT DETECTION OF SOMATIC MUTATIONS.

HIGH-QUALITY REFERENCE MATERIAL MANUFACTURED UNDER CGMP GUARANTEES CONSISTENT 'GROUND TRUTH.'

INTRODUCTION

Somatic tumor mutation profiling is a difficult task due to samples that are highly heterogeneous, as well as potential sources of variability across next-generation sequencing (NGS) workflows. Successful assays require accuracy throughout key steps of the entire process such as library construction, template preparation, bioinformatics analysis, and variant calling.

The Seraseq Tri-Level Tumor Mutation DNA Mix v2 High Concentration (HC) is a multiplexed mixture of 40 biosynthetic DNA targets precisely blended with a single, well-characterized genomic background. Produced under rigorous design control and manufacturing practices, this product can assess the performance of your NGS-based somatic mutation assay across a range of allele frequencies and mutation types.

BENEFITS

- Save time and cost with a convenient single-sample format that provides assurance of accuracy around the limit of detection (LOD) in a single run
- Ensure robust sensitivity using 40 therapeutically important and analytically challenging mutations (Table 2) across 28 genes (Table 1)
- Have confidence in lot-to-lot consistency through manufacture under cGMP compliance in ISO 9001- and ISO 13485-certified facilities
- Determine your assay's **specificity** through use of well-characterized GM24385 human genomic DNA as background 'wild-type' material

GENES COVERED BY THE SERASEQ TRI-LEVEL TUMOR MUTATION DNA MIX V2 HC

AKTI	FGFR3	JAK2	PDGFRA
APC	FLT3	KIT	PIK3CA
ATM	FOXL2	KRAS	PTEN
BRAF	GNA11	MPL	RET
CTNNB1	GNAQ	NCOA4-RET	SMAD4
EGFR	GNAS	NPM1	TP53
ERBB2	IDH1	NRAS/CSDE1	TPR-ALK

TABLE 1: List of 28 genes included in the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC. See Table 2 for a detailed list of variants (40).

A DIVERSE SET OF MUTATION TYPES CHALLENGES YOUR NGS ASSAY

In order to fulfill clinical labs' need for the most challenging variants, the 26-mutation panel from Seraseq Solid Tumor Mutation Mix-I (a predecessor product) was expanded to include additional insertion-deletion (INDEL) mutations (increasing the number from four INDEL mutations to 13), as well as two DNA structural variants; NCOA4-RET and TPR-ALK. Several additional SNVs from the Actionable Genome Consortium were included as well.

sequence both up- and down-stream to ensure compatibility with different target enrichment methods; the structural variants have from 700 bp to 1100 bp of native sequence on either side of the breakpoint. Targets are quantitated by digital PCR to have a 10%, 7%, or 4% allele frequency in a GM24385 human genomic DNA background. The GM24385 genomic DNA has been extensively characterized by the Genome in a Bottle project[1] and originates from a participant in the Personal Genomes Project (public profile huAA53E0[2]). Use of a single, well-characterized background eliminates mutation artifacts, allowing determination of assay specificity (false positive rate) in addition to sensitivity (false negative rate).

EFFECTIVELY MONITOR THE PERFORMANCE OF YOUR ASSAY

As a reference material manufactured under cGMP compliance in ISO 9001- and ISO 13485-certified facilities, the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC is very consistent from lot to lot. The use of qualified, highly sensitive digital PCR assays to establish minor allele frequencies ensures robust precision (Figure 1); therefore, unlike other sources of reference materials such as cell lines or residual patient samples, the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC may be considered an unchanging 'ground truth.' Having unlimited access to reliable, consistent reference materials not only saves you time and expense for developing, validating, and implementing an in-house source of QC materials, but also allows you to establish a baseline specific to your NGS assay so you can monitor for change over time. Because this 'ground truth' remains constant, any variation must be caused by a change in the NGS workflow that could possibly affect the fidelity of patient results.

Used as a daily reference material, the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC offers the most insights into the health of your clinical NGS test when paired with SeraCare's iQ NGS QC Management software. This allows comprehensive and intuitive access to all of your QC metrics, from variant calls and allele frequencies to instrument and reagent performance. The result is a proactive QC strategy that ensures you spend your time learning from QC data, rather than trying to generate and manage it.

This product is provided at a concentration of 25 ng/µL, and a Low Concentration (LC) version is also available at 5 ng/µL for NGS assays that require lower DNA input amounts. Additionally, the product is offered in a single-allele-frequency-per-vial format, where all 40 mutations in Table 2 are present at either 10%, 7%, or 4% minor allele frequency.

VARIANT ALLELE FREQUENCIES BY DIGITAL PCR ACROSS THREE DIFFERENT LOTS

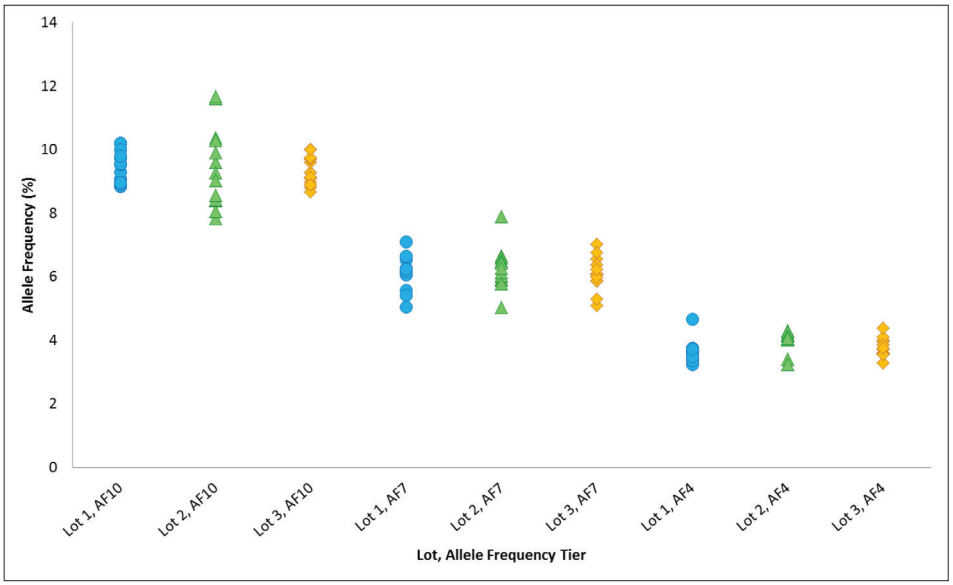

FIGURE 1. Digital PCR quantitation of individual mutations (39 out of the 40 total shown) across three different lots of the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC. For each lot, data are shown for the 14 mutations present at the 10% minor allele frequency level, the 13 mutations present at the 7% level, and 12 out of the 13 mutations that are present at the 4% level. The mutation TP53 c.263delC (COSMIC ID 18610) which is present at the 4% level is assessed using an NGS-based assay rather than digital PCR. Each point represents the average across three calls for samples run in triplicate on the Bio-Rad QX200™ Droplet Digital® PCR System.

MUTATIONS INCLUDED IN THE SERASEQ TRI-LEVEL TUMOR MUTATION DNA MIX V2 HC

Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid	Ladder Target AF
AKT1	COSM33765	Substitution	c.49G>A	p.E17K	10%
APC	COSM13127	Substitution	c.4348C>T	p.R1450*	10%
APC	COSM18561	Insertion in HP 7N	c.4666_4667insA	p.T1556fs*3	10%
ATM	COSM21924	Deletion	c.1058_1059delGT	p.C353fs*5	10%
ERBB2	COSM682/ 20959	Insertion	c.2324_2325ins12	p.A775_G776insYVMA	10%
GNA11	COSM52969	Substitution	c.626A>T	p.Q209L	10%
GNAQ	COSM28758	SNV in HP 3N	c.626A>C	p.Q209P	10%
KIT	COSM1314	Substitution	c.2447A>T	p.D816V	10%
MPL	COSM18918	Substitution	c.1544G>T	p.W515L	10%
NCOA4-RET	NA	Gene fusion	NCOA4{NC_00010.10}:r.1_1014+1312_RET	NA	10%
PDGFRA	COSM736	Substitution	c.2525A>T	p.D842V	10%
PIK3CA	COSM763	Substitution	c.1633G>A	p.E545K	10%
SMAD4	COSM14105	Insertion	c.1394_1395insT	p.A466fs*28	10%
TPR-ALK	NA	Gene fusion	TPR{NC_000001.10}:r.1_ 2185+ +246_ALK	NA	10%
CTNNB1	COSM5664	Substitution	c.121A>G	p.T41A	7%
EGFR	COSM6224	SNV in 3N	c.2573T>G	p.L858R	7%
GNAS	COSM27887	Substitution	c.601C>T	p.R201C	7%
JAK2	COSM12600	SNV in HP 3N	c.1849G>T	p.V617F	7%
KRAS	COSM521	Substitution	c.35G>A	p.G12D	7%
NPM1	COSM17559	Insertion	c.863_864insTCTG	p.W288fs*12	7%
NRAS/CSDE1	COSM584	Substitution	c.182A>G	p.Q61R	7%
PTEN	COSM4986	Insertion	c.741_742insA	p.P248fs*5	7%
PTEN	COSM5809	Deletion 6N > 5N	c.800delA	p.K267fs*9	7%
TP53	COSM10648	Substitution	c.524G>A	p.R175H	7%
TP53	COSM10660	Substitution	c.818G>A	p.R273H	7%
TP53	COSM10662	Substitution	c.743G>A	p.R248Q	7%
TP53	COSM6530	Deletion	c.723delC	p.C242fs*5	7%
BRAF	COSM476	Substitution	c.1799T>A	p.V600E	4%
EGFR	COSM12378	Insertion	c.2310_2311insGGT	p.D770_N771insG	4%
EGFR	COSM6225	Deletion	c.2236_2250del15	p.E746_A750delELREA	4%
EGFR	COSM6240	Substitution	c.2369C>T	p.T790M	4%
FGFR3	COSM715	Substitution	c.746C>G	p.S249C	4%
FLT3	COSM783	Substitution	c.2503G>T	p.D835Y	4%
FOXL2	COSM33661	Substitution	c.402C>G	p.C134W	4%
IDH1	COSM28747	Substitution	c.394C>T	p.R132C	4%
PDGFRA	COSM28053	Insertion	c.1694_1695insA	p.S566fs*6	4%
PIK3CA	COSM12464	Insertion	c.3204_3205insA	p.N1068fs*4	4%
PIK3CA	COSM775	Substitution	c.3140A>G	p.H1047R	4%
RET	COSM965	Substitution	c.2753T>C	p.M918T	4%
TP53	COSM18610	Deletion 5N >4N	c.263delC	p.S90fs*33	4%

TABLE 2: List of mutations included in the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC. The presence of a mutation in a particular assay depends upon the enrichment strategy and sequencing platform used. The mutation types are listed: HP = homopolymer, N = nucleotide, NA = not applicable. Because of ambiguity surrounding exact genomic coordinates for sequence deletions contained entirely within repetitive motifs such as homopolymers, analytic calls generated by certain analyses may differ relative to the mutation names presented in this table. In such cases, additional analysis would be required during concordance evaluation.

ABOUT SERACARE

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ORDERING INFORMATION		
Material #	**Product**	**Fill Size**
0710-0097	Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
RELATED PRODUCTS		
Material #	**Product**	**Fill Size**
4270-0010	iQ NGS QC Management software	NA
0710-0076	Seraseq Tri-Level Tumor Mutation DNA Mix v2 LC	1 vial, 25 µL at 5 ng/µL (125 ng total)
0710-0094	Seraseq Tumor Mutation DNA Mix v2 AF10 HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
0710-0095	Seraseq Tumor Mutation DNA Mix v2 AF7 HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
0710-0096	Seraseq Tumor Mutation DNA Mix v2 AF4 HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
0710-0074	Seraseq Tumor Mutation DNA Mix v2 AF10 LC	1 vial, 25 µL at 5 ng/µL (125 ng total)
0710-0072	Seraseq Tumor Mutation DNA Mix v2 AF7 LC	1 vial, 25 µL at 5 ng/µL (125 ng total)
0710-0070	Seraseq Tumor Mutation DNA Mix v2 AF4 LC	1 vial, 25 µL at 5 ng/µL (125 ng total)

LEARN MORE

To learn more about the Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC and SeraCare's products for precision oncology diagnostics, **visit www.seracare.com/oncology**.

Contact us at +1.508.244.6400 and 800.676.1881 or email info@seracare.com.

REFERENCES

1. Stanford University. GIAB Reference Materials and Data.

2. Available at: https://sites.stanford.edu/abms/content/giab-reference-materials-and-data. Accessed 13 April 2016.

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