PRODUCT SHEET

Seraseq® Tri-Level Tumor Mutation DNA Mix v2 HC

ROUTINE QC MATERIAL FOR NEXT-GENERATION SEQUENCING-BASED SOMATIC MUTATION ASSAYS

HIGHLIGHTS

SINGLE-SAMPLE MULTI-PLEXED FORMAT; CONSERVES SPACE FOR PATIENT SAMPLES.

40 UNIQUE VARIANTS, QUANTITATED WITH DIGITAL PCR. ASSURES PRECISE 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 and template preparation, and 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 compliant 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 TUMOR MUTATION DNA MIX V2 HC			
AKT1	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 4 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.

Each single nucleotide variant and insertion/deletion mutation is present with at least 300 base-pairs (bp) of native 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 project1 and originates from a participant in the Personal Genomes Project, public profile huAA53E02. 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).

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 {NC_000010.10}:r.2327-1437_5659	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 {NC_000002.11}:r.4125-550_6265	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	COSM783	Substitution	c.2503G>T	p.D835Y	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.

EFFECTIVELY MONITOR THE PERFORMANCE OF YOUR ASSAY

As a reference material manufactured under cGMP compliant and ISO 13485 certified facilities, 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, 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.

This product is provided at a concentration of 25 ng/µL. Additionally, the product is offered in a different format of single-allele-frequency-per-vial format (Seraseq® Tumor Mutation DNA Mix v2 HC AF10/7), where all 40 mutations (Table 2) are present at either 10% or 7% minor allele frequency, respectively.

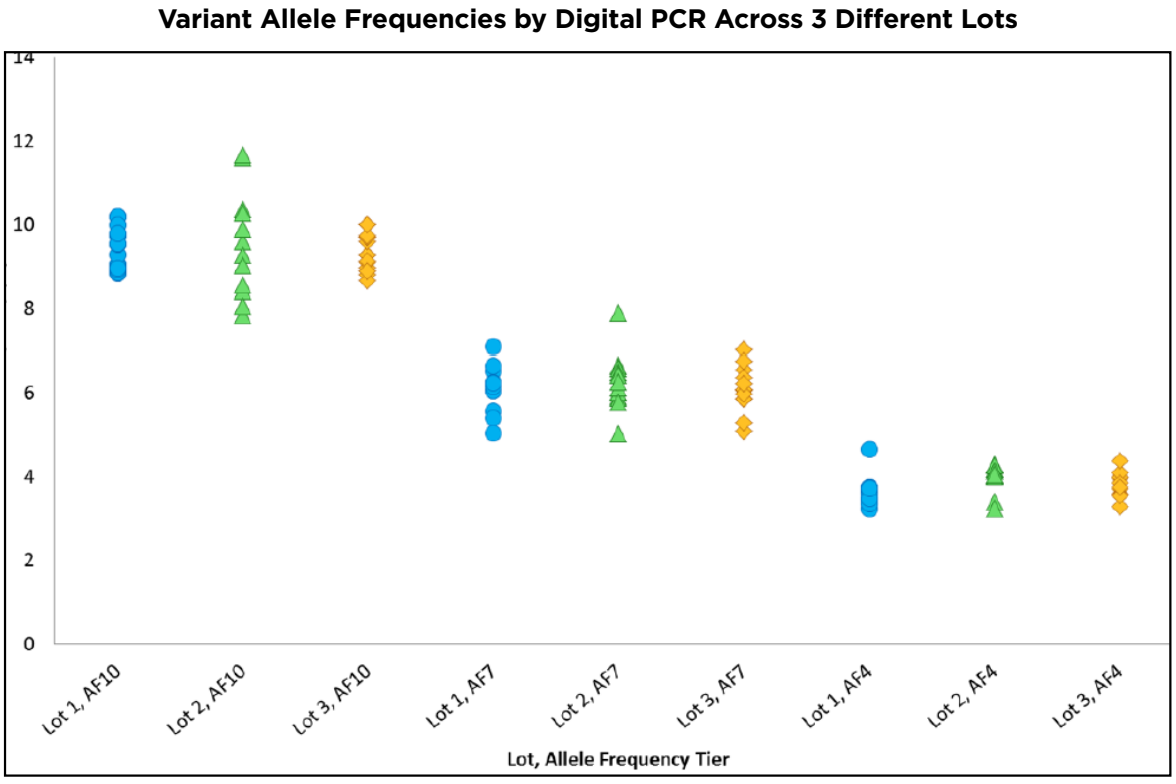

FIGURE 1: Digital PCR quantitation of individual mutations (39 out of the 40 total shown) across three different lots of Seraseq Tri-Level Tumor Mutation DNA Mix v2 HC. For each lot, data are shown for the 14 mutations present at the 10% minor allele frequency level, the 13 mutations present at the 7% 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.

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RELIABLE, CONSISTENT CONTROL MATERIAL

As a manufactured control material, developed under cGMP compliance in ISO 13485 certified facilities, Seraseq Tri-Level Tumor Mutation DNA Mix v2 provides a consistent source of reference material for your NGS assay. This product not only ensures a reliable supply which is consistent lot-to-lot; it also eliminates the need to obtain, characterize, blend, and document your own mixes of cell lineages, saving you time and resources in your assay development and validation efforts.

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
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)

LEARN MORE

To learn more about SeraCare's product offering for precision oncology diagnostics, visit www.seracare.com/oncology. Contact us at 508.244.6400 and 800.676.1881 or email info@seracare.com.

REFERENCES

1. Stanford University. GIAB Reference Materials and Data. Available at: https://sites.stanford.edu/abms/content/giab-reference-materials-and-data Accessed 13 April 2016.

2. Personal Genome Project. Public Profile–huAA53E0. Available at: https://my.pgp-hms.org/profile/huAA53E0 Accessed 13 April 2016.

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