PRODUCT SHEET

Seraseq® Tumor Mutation DNA Mix v2 HC

REFERENCE MATERIAL FOR THE DEVELOPMENT OF SOMATIC MUTATION ASSAYS BY NGS

HIGHLIGHTS

SINGLE-VIAL FORMAT FOR EACH ALLELE FREQUENCY; IDEAL FOR SOMATIC MUTATION ASSAY DEVELOPMENT.

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

HIGH-QUALITY REFERENCE MATERIAL MANUFACTURED UNDER cGMP; SAVES RESOURCE TIME PROCURING MATERIALS WITH SPECIFIC VARIANTS AND PRODUCING HOME-BREW REAGENTS.

INTRODUCTION

Developing and optimizing somatic mutation assays 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 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 may be used to establish and refine the performance of your NGS- based somatic mutation assay across a range of allele frequencies and mutation types.

BENEFITS

- Save time and cost by generating data across many different mutation types, all at the same minor allele frequency, 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 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 Tumor Mutation DNA Mix v2 HC. See Table 2 for a detailed list of variants (40).

PRECISELY QUANTITATED MUTATION MIX

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.

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% or 7% allele frequency (depending on the product) 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).

The Best Way to Establish and Optimize Test Accuracy and Precision

As a reference material manufactured under cGMP compliant and ISO 13485-certified facilities, the Seraseq 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 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 and refine the performance characteristics of your NGS assay to ensure you meet all of your design requirements. Because this 'ground truth' remains constant, the effects of improvements to your NGS workflow are very easy to determine.

This product is provided at a concentration of 25 ng/µL. Additionally, the product is also offered in a single-vial format for use as a routine run QC material (Seraseq® Tri-Level Tumor Mutation DNA Mix v2 HC), where 14 of the mutations in Table 2 are at 10% minor allele frequency, 13 of the mutations are at 7%, and the remaining 13 mutations are at 4%.

Average Allele Frequency by Digital PCR

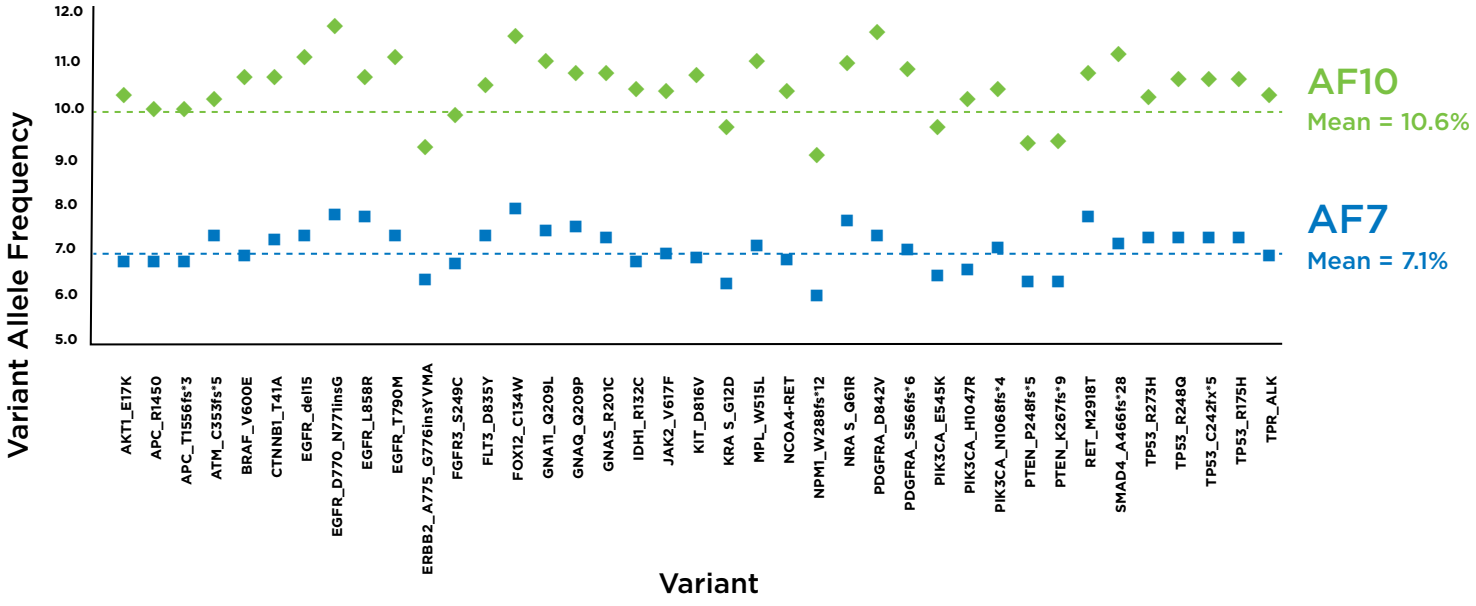

FIGURE 1: Digital PCR quantitation of individual mutations (39 out of the 40 total shown) for the 10% and 7% minor allele frequency levels offered for the Seraseq Tumor Mutation DNA Mix v2 HC. The mutation TP53 c.263delC (COSMIC ID 18610) was assessed using an NGS-based assay rather than digital PCR. 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 Tumor Mutation DNA Mix V2 HC

Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid
AKT1	COSM33765	Substitution	c.49G>A	p.E17K
APC	COSM13127	Substitution	c.4348C>T	p.R1450*
APC	COSM18561	Insertion in HP 7N	c.4666_4667insA	p.T1556fs*3
ATM	COSM21924	Deletion	c.1058_1059delGT	p.C353fs*5
BRAF	COSM476	Substitution	c.1799T>A	p.V600E
CTNNB1	COSM5664	Substitution	c.121A>G	p.T41A
EGFR	COSM6225	Deletion	c.2236_2250del15	p.E746_A750delELREA
EGFR	COSM12378	Insertion	c.2310_2311insGGT	p.D770_N771insG
EGFR	COSM6224	SNV in 3N	c.2573T>G	p.L858R
EGFR	COSM6240	Substitution	c.2369C>T	p.T790M
ERBB2	COSM682/20959	Insertion	c.2324_2325ins12	p.A775_G776insYVMA
FGFR3	COSM715	Substitution	c.746C>G	p.S249C
FLT3	COSM783	Substitution	c.2503G>T	p.D835Y
FOXL2	COSM33661	Substitution	c.402C>G	p.C134W
GNA11	COSM52969	Substitution	c.626A>T	p.Q209L
GNAQ	COSM28758	SNV in HP 3N	c.626A>C	p.Q209P
GNAS	COSM27887	Substitution	c.601C>T	p.R201C
IDH1	COSM28747	Substitution	c.394C>T	p.R132C
JAK2	COSM12600	SNV in HP 3N	c.1849G>T	p.V617F
KIT	COSM1314	Substitution	c.2447A>T	p.D816V
KRAS	COSM521	Substitution	c.35G>A	p.G12D
MPL	COSM18918	Substitution	c.1544G>T	p.W515L
NCOA4-RET	NA	Gene fusion	NCOA4{NC_000010.10}:r.1_1014+1312_RET {NC_000010.10}:r.2327-1437_5659	NA
NPM1	COSM17559	Insertion	c.863_864insTCTG	p.W288fs*12
NRAS/CSDE1	COSM584	Substitution	c.182A>G	p.Q61R
PDGFRA	COSM736	Substitution	c.2525A>T	p.D842V
PDGFRA	COSM28053	Insertion	c.1694_1695insA	p.S566fs*6
PIK3CA	COSM763	Substitution	c.1633G>A	p.E545K
PIK3CA	COSM775	Substitution	c.3140A>G	p.H1047R
PIK3CA	COSM12464	Insertion	c.3204_3205insA	p.N1068fs*4
PTEN	COSM4986	Insertion	c.741_742insA	p.P248fs*5
PTEN	COSM5809	Deletion 6N > 5N	c.800delA	p.K267fs*9
RET	COSM965	Substitution	c.2753T>C	p.M918T
SMAD4	COSM14105	Insertion	c.1394_1395insT	p.A466fs*28
TP53	COSM10660	Substitution	c.818G>A	p.R273H
TP53	COSM10662	Substitution	c.743G>A	p.R248Q
TP53	COSM6530	Deletion	c.723delC	p.C242fs*5
TP53	COSM10648	Substitution	c.524G>A	p.R175H
TP53	COSM18610	Deletion 5N >4N	c.263delC	p.S90fs*33
TPR-ALK	NA	Gene fusion	TPR{NC_000001.10}:r.1_2185+246_ ALK{NC_000002.11}:r.4125-550_6265	NA

TABLE 2: List of mutations included in the Seraseq 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.

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RELIABLE, CONSISTENT CONTROL MATERIAL

As a manufactured control material, developed under cGMP compliance in ISO 13485 certified facilities, Seraseq Solid Tumor Mutation Mix-II 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
0710-0094	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)

RELATED PRODUCT

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)

LEARN MORE

To learn more about Seraseq Solid Tumor Mutation Mix-II and 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. Availableat: https://sites.stanford.edu/abms/content/giab-reference-materials-and-data

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