PRODUCT SHEET

Seraseq™ Tumor Mutation DNA Mix v2

REFERENCE MATERIAL FOR THE DEVELOPMENT OF SOMATIC MUTATION ASSAYS BY NGS

HIGHLIGHTS

SINGLE-VIAL FORMAT FOR EACH ALLELIC FREQUENCY IN BOTH HIGH AND LOW CONCENTRATIONS; IDEAL FOR SOMATIC MUTATION ASSAY DEVELOPMENT.

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

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

INTRODUCTION

Developing and optimizing somatic mutation assays is a difficult task, with variations in the amount of tumor cellularity and potential sources of variability across the NGS workflow. Successful assays require accuracy throughout the entire process, from sample DNA purification and quantitation, to library construction and template preparation, through bioinformatics parameters and variant annotation.

The Seraseq Tumor Mutation DNA Mix v2 is a multiplexed mixture of actionable biosynthetic DNA targets precisely blended with a single, well-characterized genomic background, offered in three allelic ratios (10%, 7% and 4%) in both high and low concentrations. Designed to assess the overall performance of your somatic mutation assay by next-generation sequencing (NGS), these unique products include a wide range of single nucleotide variants (SNVs), insertion-deletion mutations (indels), and structural variants (SVs).

PRODUCT FEATURES

- Single-vial format for each allele frequency (10%, 7% and 4%); offered in both high and low concentrations
- 40 unique multiplexed variants across 28 genes (Table 1); 20 SNVs, 5 SNVs that are part of homopolymers, 13 indels, and 2 SVs (Table 2)
- Mutation targets quantitated with digital PCR
- Well-characterized GM24385 human genomic DNA as background 'wild-type' material
- Manufactured under cGMP compliance in ISO 9001 and ISO 13485 certified facilities

GENES COVERED BY THE SERASEQ TUMOR MUTATION DNA MIX V2

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. See Table 2 for a detailed list of variants (40).

PRECISELY QUANTITATED MUTATION MIX

With the accuracy of digital PCR, Seraseq Tumor Mutation DNA Mix v2 provides a precisely quantitated mixture of mutations at a specific minor allele frequency, for somatic mutation NGS assay development and optimization (Figure 1). Run in parallel with clinical samples, it provides assurance in the ability to correctly call various types of mutations.

To help meet input requirements for your targeted enrichment assay, Seraseq Tumor Mutation DNA Mix v2 is offered in two concentrations: a high concentration (HC) at 25 ng/µL and a low concentration (LC) at 5 ng/µL.

AVERAGE ALLELE FREQUENCY BY DIGITAL PCR

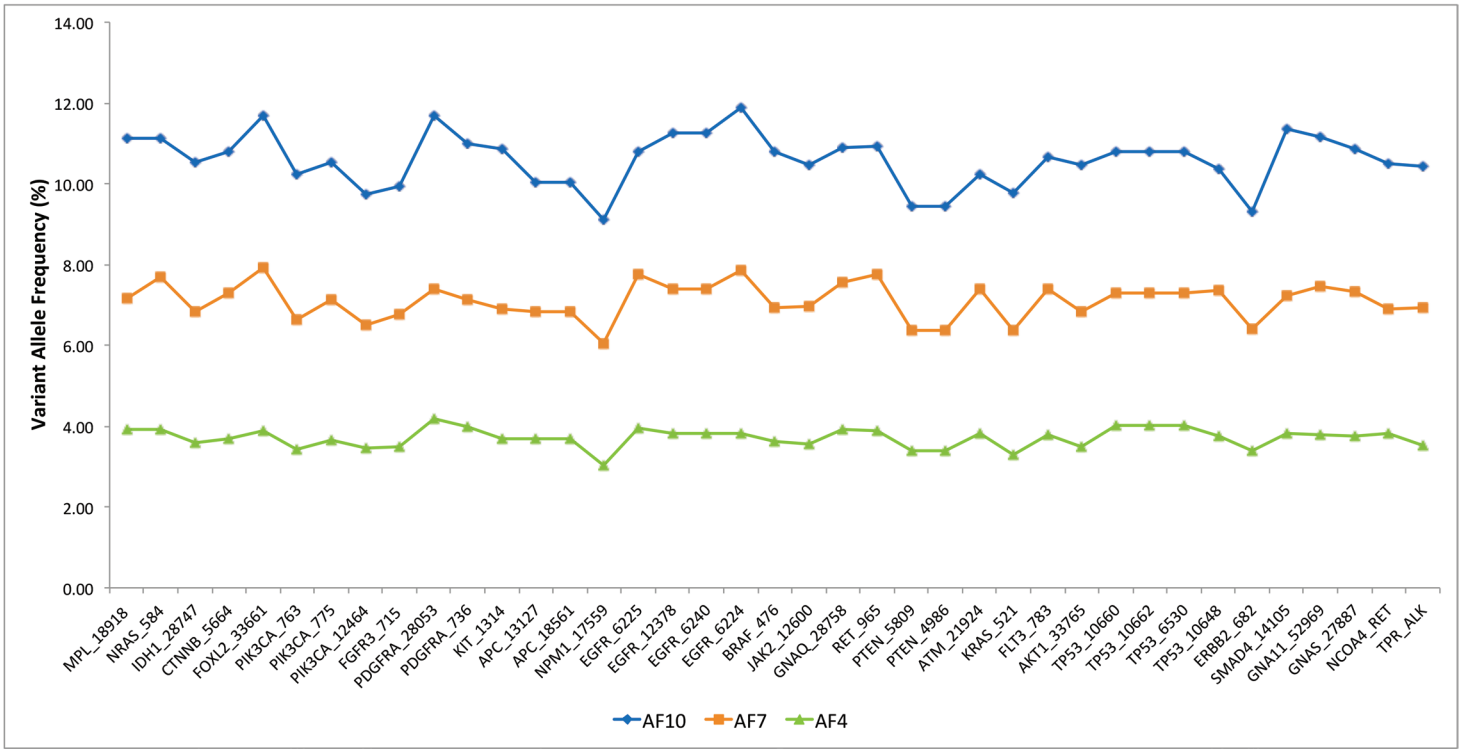

FIGURE 1: Digital PCR quantitation of individual mutations (39 out of the 40 total shown). Allele frequencies represent samples run on the Bio-Rad QX200™ Droplet Di

MUTATIONS INCLUDED IN THE SERASEQ TUMOR MUTATION DNA MIX V2

EXPANDED LIST OF ACTIONABLE MUTATIONS

In Seraseq Solid Tumor Mutation Mix-I (a predecessor product), 26 mutations were included based upon their relative evidentiary strength in the Sanger Wellcome Trust's "Catalog Of Somatic Mutations in Cancer" (COSMIC, http://cancer.sanger.ac.uk[1]) and inclusion in commonly used commercially available cancer 'hotspot' mutation NGS assays. As a result of clinical laboratory feedback, Seraseq Tumor Mutation DNA Mix v2 includes additional indel mutations (increasing the number from 4 indel mutations to 13), as well as several additional SNVs from the Actionable Genome Consortium. Two structural variants, NCOA4-RET and TPR-ALK, have also been included (Table 2).

These single nucleotide variants and insertion/deletion mutations have flanking sequences of at least 300 base-pairs (bp), and the structural variants have from 700 bp to 1100 bp on either side spanning the breakpoint. Each target is quantitated by digital PCR to have a 10%, 7% or 4% allele frequency in a GM24385 human genomic DNA background using either off-the-shelf or custom allele-specific digital PCR assays. The GM24385 genomic DNA has been extensively characterized by the Genome in a Bottle project[2] and originates from a participant in the Personal Genomes Project, public profile huAA53E0[3].

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

TABLE 2: List of mutations included in the Seraseq Tumor Mutation DNA Mix v2.
The presence of the 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.

ABOUT SERACARE

TRUSTED SUPPLIER TO THE DIAGNOSTIC TESTING INDUSTRY FOR OVER 30 YEARS.

HIGH-QUALITY CONTROL PRODUCTS, RAW BIOLOGICAL MATERIALS, AND IMMUNOASSAY REAGENTS.

INNOVATIVE TOOLS AND TECHNOLOGIES TO PROVIDE ASSURANCE IN DIAGNOSTIC ASSAY PERFORMANCE AND TEST RESULTS.

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: WWW.SERACARE.COM.

RELIABLE, CONSISTENT REFERENCE MATERIAL

As a manufactured reference material, developed under cGMP compliance in ISO 9001 and ISO 13485 certified facilities, Seraseq Tumor Mutation DNA Mix v2 provides a consistent source of reference material for your NGS assay. This product not only ensures a reliable supply of material which is consistent from 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.

ORDERING INFORMATION		
Material #	**Product**	**Fill Size**
0710-0075	Seraseq Solid Tumor Mutation DNA Mix v2 (AF10) HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
0710-0074	Seraseq Solid Tumor Mutation DNA Mix v2 (AF10) LC	1 vial, 25 µL at 5 ng/µL (125 ng total)
0710-0073	Seraseq Solid Tumor Mutation DNA Mix v2 (AF7) HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
0710-0072	Seraseq Solid Tumor Mutation DNA Mix v2 (AF7) LC	1 vial, 25 µL at 5 ng/µL (125 ng total)
0710-0071	Seraseq Solid Tumor Mutation DNA Mix v2 (AF4) HC	1 vial, 25 µL at 25 ng/µL (625 ng total)
0710-0070	Seraseq Solid Tumor Mutation DNA Mix v2 (AF4) LC	1 vial, 25 µL at 5 ng/µL (125 ng total)

LEARN MORE

To learn more about Seraseq Tumor Mutation DNA Mix v2 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. Forbes SA, Beare D, Gunasekaran P, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucl. Acids Res. 2015; 43:805-11. doi:10.1093/nar/gku1075

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

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