Seraseq™ Solid Tumor Mutation Mix-I (AF1-10)

Test the lower limit of somatic tumor mutation detection and confirm sensitivity for nucleotide variants.

Using the major benchtop next-generation sequencing platforms, there have been several publications that detect single nucleotide variants down to 5% or 10% allele frequency. These experiments were performed with extensive dilution series with characterized cell lines, typically with only a few mutations to examine against a large gene panel.[1,2]

Identical in design to the Seraseq Solid Tumor Mutation Mix-I (AF20), the AF1-10 product features a set of variants across a range of allele frequencies.

- 26 unique variants including SNVs, SNVs as part of homopolymer tracts, deletions and insertions
- Synthetic variants quantitated with digital PCR
- Five discrete allele-frequency ratios: 1%, 3%, 5%, 8% and 10% in one kit with GM24385 human genomic DNA as background material
- Manufactured in ISO 9001 and ISO 13485 certified facilities with full cGMP compliance and over 20 years of experience

Seraseq Solid Tumor Mutation Mix-I (AF1-10) eliminates the need to obtain, characterize, blend, quantitate and document mixes of cell lineages. It confirms the lower limit of detection across a range of mutations and mutation types.

By providing a range of mutations spanning single-digit frequencies, you can evaluate for yourself the practical lower limit of detection of your routine somatic mutation assays. Table 1 provides a list of observed allele frequencies against each of the 26 mutations from the commonly used Ion AmpliSeq™ Cancer Hotspot Panel v2.

Gene ID	Allele Name	AF10	AF8	AF5	AF3	AF1
MPL	COSM18918	11.9	9.7	6.8	3.3	ND
NRAS	COSM584	14.7	9	7.1	3.1	ND
IDH1	COSM28747	10.5	11.2	6.2	3.1	ND
CTNNB1	COSM5664	12	10.3	6.6	2.9	2.1
PIK3CA	COSM763	10.4	8.7	5.7	2.3	ND
PIK3CA	COSM775	6.8	6.1	5.7	1.9	ND
FGFR3	COSM715	11.4	9.2	5.2	3	ND
PDGFRA	COSM736	9.8	6.8	4.6	2.3	ND
KIT	COSM1314	13	10.7	7.4	3.7	ND
APC	COSM13127	11.3	9.4	5.6	2.8	ND
NPM1	COSM17559	14.2	9.1	8	3.5	ND
EGFR	COSM6225	14.9	11.6	8	3.5	ND
EGFR	COSM6240	14.4	11.4	7	3.5	ND
EGFR	COSM6224	14.7	10.8	6.4	3.6	ND
BRAF	COSM476	11.6	10.9	6	2.8	ND
JAK2	COSM12600	13.5	10.7	6.3	3.1	ND
GNAQ	COSM28758	9.7	7.7	6.1	3.6	ND
RET	COSM965	14.4	10.4	6.6	4.4	ND
ATM	COSM21924	11.2	8.6	5.7	2.9	ND
KRAS	COSM521	11.8	9.8	5.5	2.6	ND
FLT3	COSM783	12.1	9	6.6	2.8	ND
AKT1	COSM33765	13.2	12	6.8	2.9	ND
TP53	COSM10662	14	10.7	8	3.7	ND
TP53	COSM10648	13.9	10.7	7.5	4.3	ND
SMAD4	COSM14105	11	10.3	7	3.2	ND
GNAS	COSM27887	15.3	9.8	7.1	2.9	1.8
Average AF:	12.4	9.8	6.5	3.1	NA	

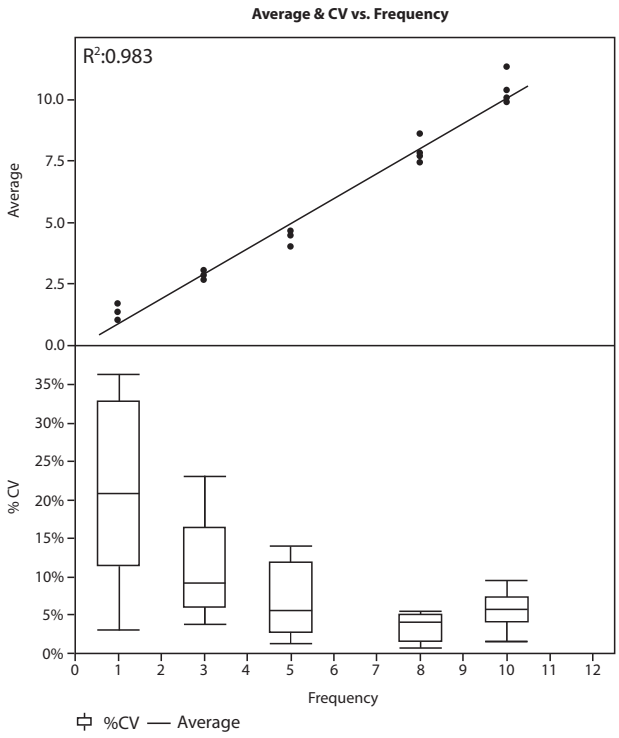
Table 1: Results using the Seraseq Solid Tumor Mutation Mix-I (AF1-10) using the Ion AmpliSeq™ Cancer Hotspot Panel v2 in conjunction with the Ion Torrent™ PGM Sequencing System.

All of the 26 mutations included in Seraseq Solid Tumor Mutation Mix-I (AF1-10) were chosen based upon their relative evidentiary strength in the Sanger Wellcome Trust's 'Catalogue of Somatic Mutations in Cancer (COSMIC)' in addition to the inclusion of the mutations in commonly used commercially-available cancer 'hotspot' mutation NGS assays.[3] Several of these mutations are in homopolymer tracts, others are insertion or deletions (indels), while others are Single Nucleotide Variants (SNVs). (Table 2).

	Gene	COSMIC ID of Mutation	Position (hg19)	CDS	Mutation Type	Amino Acid Change
1	EGFR	COSM6224	55259515	c.2573T>G	SNV (Homopolymer)	p.L858R
2	FGFR3	COSM715	1803568	c.746C>G	SNV (Homopolymer)	p.S249C
3	GNAQ	COSM28758	80409488	c.626A>C	SNV (Homopolymer)	p.Q209P
4	AKT1	COSM33765	105246551	c.49G>A	SNV (Homopolymer)	p.E17K
5	ATM	COSM21924	108117846	c.1058_1059delGT	Small Deletion	p.C353fs*5
6	SMAD4	COSM14105	48603093	c.1394_1395insT	Small Insertion	p.A466fs*28
7	NPM1	COSM17559	170837547	c.863_864insTCTG	Large Insertion	p.W288fs*12
8	EGFR	COSM6225	55242465	c.2236_2250del15	Large Deletion	p.E746_ A750delELREA
9	BRAF	COSM476	140453136	c.1799T>A	SNV	p.V600E
10	KRAS	COSM521	25398284	c.35G>A	SNV	p.G12D
11	PIK3CA	COSM775	178952085	c.3140A>G	SNV	p.H1047R
12	PIK3CA	COSM763	178936091	c.1633G>A	SNV	p.E545K
13	NRAS	COSM584	115256529	c.182A>G	SNV	p.Q61R
14	TP53	COSM10648	7578406	c.524G>A	SNV	p.R175H
15	CTNNB1	COSM5664	41266124	c.121A>G	SNV	p.T41A
16	IDH1	COSM28747	209113113	c.394C>T	SNV	p.R132C
17	EGFR	COSM6240	55249071	c.2369C>T	SNV	p.T790M
18	MPL	COSM18918	43815009	c.1544G>T	SNV	p.W515L
19	APC	COSM13127	112175639	c.4348C>T	SNV	p.R1450*
20	FLT3	COSM783	28592642	c.2503G>T	SNV	p.D835Y
21	PDGFRA	COSM736	55152093	c.2525A>T	SNV	p.D842V
22	RET	COSM965	43617416	c.2753T>C	SNV	p.M918T
23	GNAS	COSM27887	57484420	c.601C>T	SNV	p.R201C
24	TP53	COSM10662	7577538	c.743G>A	SNV	p.R248Q
25	KIT	COSM1314	55599321	c.2447A>T	SNV	p.D816V
26	JAK2	COSM12600	5073770	c.1849G>T	SNV	p.V617F

Table 2: List of mutations included in the Seraseq Solid Tumor Mutation Mix-I (AF1-10). The presence of the mutation in a particular assay depends upon the enrichment strategy and sequencing platform used. The 26 mutations listed above have been observed to appear at the appropriate allelic frequency using the Ion AmpliSeq™ Cancer Hotspot Panel v2 on the Ion Torrent™ PGM™ Sequencing system, while 17 of these (unshaded mutations in table) will be expected to appear at the appropriate allelic frequency using the Illumina TruSeq® Amplicon - Cancer Panel on the MiSeq® System.

These mutations are individually engineered on individual 1kb stretches of DNA and quantitated by digital PCR to have a 1%, 3%, 5%, 8% or 10% allele frequency in a GM24385 human genomic DNA background (refer to Figure 1 for digital PCR quantitation data). This genomic DNA has been extensively characterized by the Genome in a Bottle project[4] and originates from a participant in the Personal Genomes Project, public profile huAA53E0.[5]



Mutations: BRAF (COSM476), MPL (COSM18918), NPM1 (COSM17559) Large Insertion, PIK3CA (COSM775), EGFR (COSM6225) Large Deletion, KRAS (COSM521)

Figure 1: Seraseq Solid Tumor Mutation Mix-I (AF1-10) Digital PCR Quantitation Data

With over 20 years of manufacturing experience in full cGMP compliant, ISO 9001 and ISO 13485 certified facilities, SeraCare has provided routine testing laboratories worldwide consistent and reliable infectious disease reference standards with our ACCURUN® line for assay monitoring. We now offer that same quality for examining low-frequency somatic mutation calling with the Seraseq Solid Tumor Mutation Mix-I (AF1-10).

Seraseq™ Solid Tumor Mutation Mix-I (AF1-10)
Item Number: 0710-0001
5 vials, 25 µL each at 25 ng/µL concentration

References

1. Tsongalis GJ, Peterson JD, de Abreu FB, Tunkey CD, Gallagher TL, Strausbaugh LD, Wells WA, Amos CI. Routine use of the Ion Torrent AmpliSeq™ Cancer Hotspot Panel for identification of clinically actionable somatic mutations. Clin Chem Lab Med. 2014 52(5):707-14. doi: 10.1515/cclm-2013-0883. PubMed PMID:24334431.
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