Seraseq™ Solid Tumor Mutation Mix-I (AF20)

Assuring detection quality for somatic mutations by next-generation sequencing.

Seraseq Solid Tumor Mutation Mix-I (AF20) is a highly multiplexed synthetic plasmid DNA mixed with well-characterized genomic background for assessing the overall performance of a somatic mutation assay by next-generation sequencing.

- 26 unique variants including SNVs, SNVs as part of homopolymer tracts, deletions and insertions
- Synthetic variants quantitated with droplet digital PCR
- 20% allele-frequency ratio with GM24385 human genomic DNA as background material
- 6 base-pair synthetic Internal Quality Marker inserted within 25 base-pairs of variant guards against potential amplicon contamination, simplifies analysis
- Eliminate the need to obtain, characterize, blend and document mixes of cell lineages
- Manufactured in ISO 9001 and ISO 13485 certified facilities with full cGMP compliance and over 20 years of experience

Somatic tumor mutation profiling 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 process, from sample DNA purification and quantitation, to library construction and template preparation, down to bioinformatics parameters and variant annotation.

By providing well-characterized DNA reference materials, SeraCare offers a precisely quantitated and well-defined mixture of mutations at a defined minor allele frequency for routine use. While assay-specific variation does occur (Figure 1), use of a sample control gives assurance in the ability to correctly call different types of mutations.

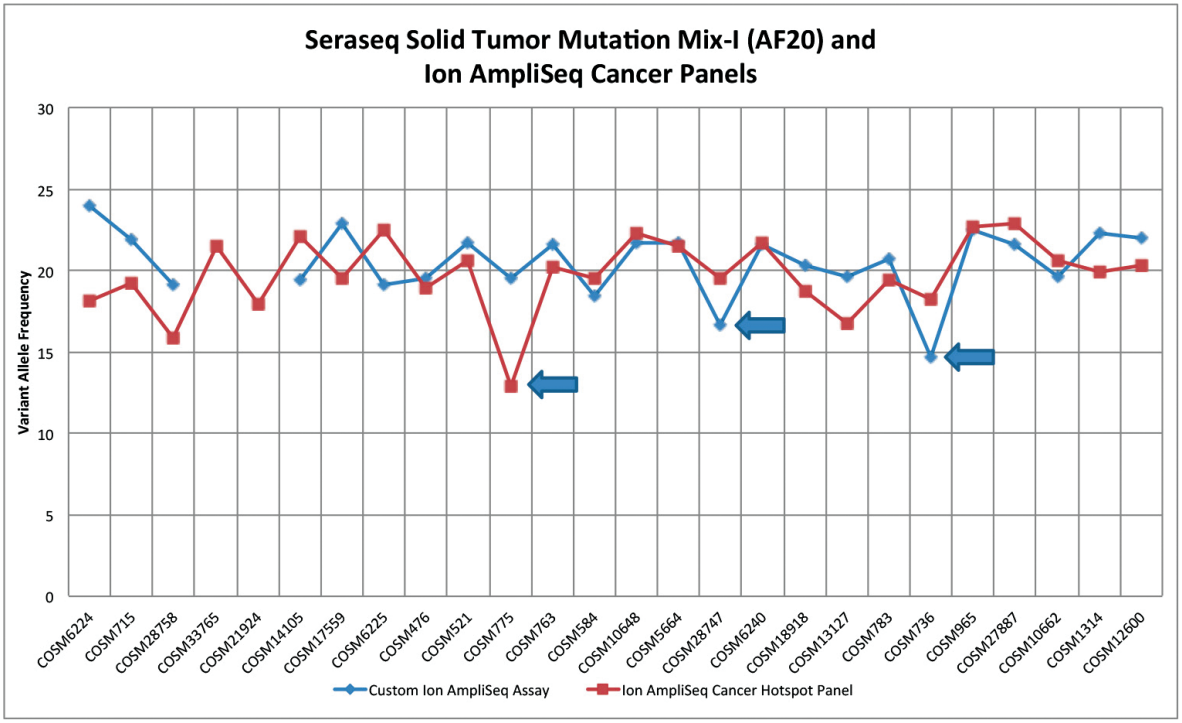

Figure 1: *Typical results using the Seraseq Solid Tumor Mutation Mix-1 (AF20) on an Ion Torrent PGM™ Sequencing System with two different cancer hotspot mutation panels: a laboratory developed custom panel and the Ion AmpliSeq™ Cancer Hotspot Panel v2. Note the apparent lower frequency mutations that are assay-dependent (arrows).*

All of the 26 mutations were chosen 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] in addition to the inclusion of the mutations in commonly used, commercially available cancer 'hotspot' mutation NGS assays. Several of these mutations are in homopolymer tracts, others are insertion or deletions (indels), while others are Single Nucleotide Variants (SNVs) (Table 1).

These mutations are individually engineered on individual 1kb stretches of DNA and quantitated by digital PCR to have a 20% allele frequency in a GM24385 human genomic DNA background. This genomic DNA has been extensively characterized by the Genome in a Bottle project[2] and originally comes from a participant in the Personal Genomes Project, public profile huAA53E0.[3]

	Gene	COSMIC ID of Mutation	Position (hg19)	CDS	Mutation Type	Amino Acid Change	Target VAF	Ion AmpliSeq™ Cancer Hotpot v2	Illumina TruSeq™ Cancer Panel
1	EGFR	COSM6224	55259515	c.2573T>G	SNV (Homopolymer)	p.L858R	20%	●	○
2	FGFR3	COSM715	1803568	c.746C>G	SNV (Homopolymer)	p.S249C	20%	●	⊙
3	GNAQ	COSM28758	80409488	c.626A>C	SNV (Homopolymer)	p.Q209P	20%	●	⊙
4	AKT1	COSM33765	105246551	c.49G>A	SNV (Homopolymer)	p.E17K	20%	●	●
5	ATM	COSM21924	108117846	c.1058_1059delGT	Small Deletion	p.C353fs*5	20%	●	○
6	SMAD4	COSM14105	48603093	c.1394_1395insT	Small Insertion	p.A466fs*28	20%	●	●
7	NPM1	COSM17559	170837547	c.863_864insTCTG	Large Insertion	p.W288fs*12	20%	●	●
8	EGFR	COSM6225	55242465	c.2236_2250del15	Large Deletion	p.E746_A750delELREA	20%	●	●
9	BRAF	COSM476	140453136	c.1799T>A	SNV	p.V600E	20%	●	●
10	KRAS	COSM521	25398284	c.35G>A	SNV	p.G12D	20%	●	●
11	PIK3CA	COSM775	178952085	c.3140A>G	SNV	p.H1047R	20%	●	●
12	PIK3CA	COSM763	178936091	c.1633G>A	SNV	p.E545K	20%	●	●
13	NRAS	COSM584	115256529	c.182A>G	SNV	p.Q61R	20%	●	●
14	TP53	COSM10648	7578406	c.524G>A	SNV	p.R175H	20%	●	●
15	CTNNB1	COSM5664	41266124	c.121A>G	SNV	p.T41A	20%	●	●
16	IDH1	COSM28747	209113113	c.394C>T	SNV	p.R132C	20%	●	●
17	EGFR	COSM6240	55249071	c.2369C>T	SNV	p.T790M	20%	●	●
18	MPL	COSM18918	43815009	c.1544G>T	SNV	p.W515L	20%	●†	●
19	APC	COSM13127	112175639	c.4348C>T	SNV	p.R1450*	20%	●	●
20	FLT3	COSM783	28592642	c.2503G>T	SNV	p.D835Y	20%	●	●
21	PDGFRA	COSM736	55152093	c.2525A>T	SNV	p.D842V	20%	●	●
22	RET	COSM965	43617416	c.2753T>C	SNV	p.M918T	20%	●	⊙
23	GNAS	COSM27887	57484420	c.601C>T	SNV	p.R201C	20%	●	○
24	TP53	COSM10662	7577538	c.743G>A	SNV	p.R248Q	20%	●	⊙
25	KIT	COSM1314	55599321	c.2447A>T	SNV	p.D816V	20%	●	○
26	JAK2	COSM12600	5073770	c.1849G>T	SNV	p.V617F	20%	●	⊙

Legend:

● Mutation observed to appear at ~20% allelic frequency

⊙ Mutation observed at ~5%-15% allelic frequency due to assay primer consideration

○ Mutation not observed due to assay primer consideration

† Some minor variability observed with strand bias and inadequate coverage

Table 1: *List of mutations included in the Seraseq Solid Tumor Mutation Mix-I (AF20). 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 ~20% allelic frequency using the Ion AmpliSeq™ Cancer Hotspot Panel v2 on the Ion Torrent PGM™ Sequencing system, while 17 of these mutations have been observed to appear at ~20% allelic frequency using the Illumina TruSeq® Amplicon - Cancer Panel on the MiSeq® System.*

In addition, within 25 base-pairs of the mutation a six-base artificial Internal Quality Marker has been inserted to provide a simple method to determine the presence of a minor allele control sequence, as well as alert the user should an amplicon contamination event occur.

With over 20 years of manufacturing experience and full cGMP compliance in 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 somatic mutation calling with the Seraseq Solid Tumor Mutation Mix-I (AF20).

Seraseq™ Solid Tumor Mutation Mix-I (AF20)
Item Number: 1600-0098
1 vial, 25 µL per vial at 25 ng/µL concentration

For Research Use Only. Not for use in Diagnostic Procedures.

To learn more about Seraseq™ precision oncology products, visit http://seracare.com/oncology

References

1. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, Ding M, Bamford S, Cole C, Ward S, Kok CY, Jia M, De T, Teague JW, Stratton MR, McDermott U, Campbell PJ. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res. 2015 Jan; 43(Database issue):D805-11.doi:10.1093/nar/gku1075

2. https://sites.stanford.edu/abms/content/giab-reference-materials-and-data

3. https://my.pgp-hms.org/profile/huAA53E0

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