Seraseq® ctDNA MRD Panel Mix

0710-2146

This product datasheet provides more details on the supplementary materials such as sequencing data files generated to support the new Seraseq ctDNA MRD Panel Mix.

NGS Variant Data Files:

The Seraseq ctDNA MRD Panel Mix is based on a well-characterized cancer cell line, its SNP-matched normal cell line and targetable DNA biosynthetic constructs present in the Seraseq ctDNA Complete mutation mix, making it a unique product for its composition of both synthetic and tumor-derived variants. The variant information we provide is from both the pre-fragmentation (HWM DNA) and post-fragmentation (ctDNA-like) production steps of the ctDNA MRD panel mix. The following files are available:

- *HMW_MRD_WES*
- *Archer_ctDNA_0_MRD_[batch#]*
- *Archer_ctDNA_P5_MRD_[batch#]*
- *P5_MRD_[batch#]*
- *P05_MRD_[batch#]*
- *P005_MRD_[batch#]*
- *0_MRD_[batch#]*

Pre- & Post-Fragmentation Data (Available upon Request)

1. **Whole Exome Sequencing (WES) Analysis**
   Whole Exome Sequencing (WES) of the cancer cell line was performed and analyzed by an in-house informatics pipeline to characterize non-synonymous somatic mutations. The first VCF file *HMW_MRD_WES* contains WES data from unfragmented, 100% tumor cell line gDNA analyzed via the Agilent SureSelectXT Human AllExon V6 + COSMIC panel with >600 characterized mutations*.

2. **dPCR Analysis**
   A subset of the synthetic variant pool from the blend of gDNA extracted from the matched normal cell line and biosynthetic constructs (at 0.5% tumor content equivalent = 0.25% VAF), pre-fragmentation, was analyzed by dPCR. The observed VAFs were matching expected (blended) VAFs (see Table 1).

3. **Targeted NGS Panel Analyses**
   After fragmentation to ctDNA-like size profiles, MRD variants at various tumor blends were analyzed by two targeted NGS panels – a custom Agilent SureSelect XT HS2 panel and the ArcherDx ctDNA Reveal-28 panel. The ArcherDx panel was used to analyze the synthetic variants in the MRD mix to confirm VAF concordance at ~0.25% (0.5% tumor blend) in pre- and post-fragmented samples at 0% and 0.5% VAF (see Table 2). The custom Agilent SureSelect XT HS2 panel was used to analyze selected 100 tumor-derived variants at all tumor blend dilution series: 0.5%, 0.05%, 0.005% and 0% (See Table 3). The respective VCFs are named *Archer_ctDNA_0_MRD_[batch#], Archer_ctDNA_P5_MRD_[batch#], P5_MRD_[batch#], P05_MRD_[batch#], P005_MRD_[batch#], and 0_MRD_[batch#].*

Table 1: Digital PCR (dPCR) analysis of a subset of synthetic variants (gDNA) in the Seraseq ctDNA MRD Panel Mix at 0.5% tumor blend (0.25% VAF).

Gene	NA Change	AA Change	VAF (%)
AKT1	c.49G>A	p.E17K	0.49
ALK	c.3522C>A	p.F1174L	0.60
BRCA1	c.1961delA	p.K654fs*47	0.25
EGFR	c.2369C>T	p.T790M	0.50
NRAS	c.182A>G	p.Q61R	0.40
CD74-ROS1	N/A	Translocation	0.35
		Average	**0.43**

Table 2: Analysis of ctDNA variants (DNA plasmids) in the Seraseq ctDNA MRD Panel Mix using the ArcherDx ctDNA Reveal-28 assay.

Gene	NA Change	AA Change	TF 0%	TF 0.5%
			VAF (%)	VAF (%)
AKT1	c.49G>A	p.E17K	0.00	0.27
ALK	c.3604G>A	p.G1202R	0.00	0.39
ALK	c.3522C>A	p.F1174L	0.00	0.62
BRAF	c.1799T>A	p.V600E	0.00	0.54
EGFR	c.2235_2249del	p.E746_A750del	0.00	0.22
EGFR	c.2240_2257del	p.L747_P753delinsS	0.00	0.36
EGFR	c.2254_2277del	p.S752_I759del	0.00	0.05
EGFR	c.2369C>T	p.T790M	0.00	0.27
EGFR	c.2573T>G	p.L858R	0.00	0.31
ERBB2	c.2313_2324dup	p.Y772_A775dup,p.A775_G776insYVMA	0.00	0.22
KIT	c.2447A>T	p.D816V	0.00	0.32

KRAS	c.183A>C	p.Q61H	0.00	0.40
KRAS	c.35G>A	p.G12D	0.00	0.38
KRAS	c.34G>T	p.G12C	0.00	0.47
NRAS	c.182A>G	p.Q61R	0.02	0.48
PIK3CA	c.3140A>G	p.H1047R	0.00	0.39
PIK3CA	c.3204_3205insA	p.*1069Mext*3,p.*1069fs	0.00	0.18
		Average	**0.00**	**0.34**

Table 3: MRD variant analysis# of 100 tumor-derived variants at different blends (0%, 0.5%, 0.05% and 0.005%) in the Seraseq ctDNA MRD Panel Mix.

Kit component	Measured concentration (ng/µL)	Number of tumor variants with at least 1 copy observed	Total number of observed tumor variant copies	Average VAF (%) with "not observed" set to zero**
Seraseq ctDNA MRD 0% Tumor	11.4	1	1	0.000%
Seraseq ctDNA MRD 0.5% Tumor	14.6	83	206	0.135%
Seraseq ctDNA MRD 0.05% Tumor	13.1	20	22	0.011%
Seraseq ctDNA MRD 0.005% Tumor	13.1	4	4	0.001%

#Analysis performed using a custom Agilent SureSelect XT HS Panel, on a NextSeq 2000 sequencer.
*MRD variant lists are available by contacting us at CDx-info@LGCgroup.com

The package insert for this panel can be found at www.seracare.com.

A printed copy of the package insert or data sheet may be requested by email at info@seracare.com or by phone at 508.244.6400.

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