Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description:	Seraseq™ ctDNA Complete Reference Material WT		
Material No:	0710-0674	Batch No:	10346910
Material Description:	Human genomic DNA from the reference cell line, GM24385		
Date of Manufacture:	17May2018	Expiration Date:	17May2020
Concentration (Qubit dsDNA BR Assay):	Nominal value: 25 ng/mL; Average measured value after extraction using Qiagen QIAamp Circulating Nucleic Acid Kit: 31.8 ng/mL		
Volume:	5 mL		
Storage:	4 °C		

Digital PCR testing using BioRad QX200™ Droplet Digital™ PCR System:

Gene ID	COSMIC Identifier	Amino Acid Change	Average AF%[1]
AKT1	COSM33765	p.E17K	0.005
BRAF	COSM476	p.V600E	0.000
EGFR	COSM6224	p.L858R	0.001
EGFR	COSM6240	p.T790M	0.009
ERBB2	COSM682/20959	p.A775_G776insYVMA	0.007
KIT	COSM1314	p.D816V	0.002
KRAS	COSM521	p.G12D	0.012
NCOA4/RET	NA	Translocation	0.000
NRAS	COSM584	p.Q61R	0.006
PIK3CA	COSM775	p.H1047R	0.006
PIK3CA	COSM12464	p.N1068fs*4	0.006
EML4-ALK	NA	Translocation	0.001
ALK	COSM144250	p.G1202R	0.000
ALK	COSM28055	p.F1174L	0.000
BRCA1	COSM1383519	p.K654fs*47	0.001
BRCA2	COSM1738242	p.R2645fs*3	0.000
EGFR	COSM12370	p.L747_P753>S	0.000
EGFR	COSM6256	p.S752_I759delSPKANKEI	0.001
EGFR	COSM6223	p.E746_A750delELREA	0.008
KRAS	COSM516	p.G12C	0.001
CD74/ROS1	NA	Translocation	0.000
KRAS	COSM554	p.Q61H	0.004
		Average AF%	**0.003**

NA = not applicable

[1]Variant allele frequencies > 0.00% for this wild-type negative control are within the expected range for stochastic positive dPCR reactions.

NGS was performed as an orthogonal verification step. Results confirm no variants were detected above 0.1%, except for EGFR T790M variant detected at 0.13% in the WT sample; the corresponding dPCR data was 0.009%. We observed similar background in other Seraseq ctDNA Complete™ reference materials using this NGS assay, which were not observed in dPCR QC testing. Thus, we associate the allele frequency for the T790M variant with the background from the assay[1] using the conditions listed below.

Next Generation Sequencing testing using Archer® Reveal ctDNA™ 28 Kit run on an Illumina® MiSeq™ using v2 (2x150 bp) PE chemistry reagents:

NGS Parameters:
DNA input = 50 ng
# of samples / flow cell = 3-4
# of total reads / sample = 4-5M
Average read depth = 5000-10000X
On-target reads = ~94%
Q30 score = ~95%
Analysis = Archer Analysis Suite v5.1.7 (with error correction set to "ON")

[1]Please see the poster from NIST for more information about assay sensitivity:
https://digital.seracare.com/multilab-assessment-reference-materials-ctdna-poster2018

Note: Copy numbers of ERBB2 and MET were assayed by dPCR and found to be normal. They were not assayed by NGS as the wild-type sample is used as a normal control for determining copy number of genes in other samples.

Approval:

Prepared By

03/21/19
Date