Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description:	Seraseq® ctDNA Reference Material v2 WT		
Material Number:	0710-0208	Batch Number:	10387336
Material Description:	ctDNA-like human genomic DNA from the reference cell line, GM24385.		
Concentration (Qubit dsDNA BR Assay):	32.4 ng/mL		
Volume:	5.0 mL		
Date of Manufacture:	14 Dec 2018	Expiration Date:	14 Dec 2021
Storage:	2-8°C		

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

Gene ID	COSMIC Identifier	AF%[1]
AKT1	COSM33765	0.03
APC	COSM13127	0.05
APC	COSM18561	0.00
ATM	COSM21924	0.00
BRAF	COSM476	0.00
CTNNB1	COSM5664	0.02
EGFR	COSM6224	0.02
EGFR	COSM12378	0.02
EGFR	COSM6225	0.00
EGFR	COSM6240	0.02
ERBB2	COSM20959	0.00
FGFR3	COSM715	0.00
FLT3	COSM783	0.00
FOXL2	COSM33661	0.00
GNA11	COSM52969	0.00
GNAQ	COSM28758	0.00
GNAS	COSM27887	0.06
IDH1	COSM28747	0.08
JAK2	COSM12600	0.00
KIT	COSM1314	0.01
KRAS	COSM521	0.02
MPL	COSM18918	0.00
NCOA4-RET	N/A	0.00
NPM1	COSM17559	0.00
NRAS	COSM584	0.09
PDGFRA	COSM736	0.03
PDGFRA	COSM28053	0.00
PIK3CA	COSM763	0.03
PIK3CA	COSM12464 [3]	0.00
PIK3CA	COSM775	0.10
PTEN	COSM4986	0.00
PTEN	COSM5809	0.00
RET	COSM965	0.01
SMAD4	COSM14105	0.01
TP53	COSM10648	0.05
TP53	COSM10660	0.03
TP53	COSM10662	0.07
TP53	COSM6530	0.00
TP53	COSM18610	Not Tested[2]
TPR-ALK	N/A	0.01

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

[2] NGS testing alone is used for QC of variant COSM18610.

[3] As of June 2019 this mutation is no longer listed in the COSMIC database.

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NGS was performed as an orthogonal verification step. Results confirm no variants were detected above 0.1%, except for the TP53 COSM10648 variant detected at 0.1% in the WT sample; the corresponding dPCR data was 0.05%. Thus, we associate the allele frequency for the TP53 COSM10648 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 = 2
# of total reads / sample = 8.2 M
Average read depth = 6000X
On-target reads = ~94%
Q30 score = ~93%
Analysis = Archer Analysis Suite v5.1.7

Default analysis settings except for the following:

- ERROR_CORRECTION is turned to ON (default=OFF; cfDNA pipeline)
- MAPQ_THRESHOLD_FOR_VARIANT_CALL is set to 10 (default=0)
- MIN_ALLELE_FRACTION_FOR_VARIANT_CALL is set to 0.00025 (default=0.001)
- MIN_BASEQUAL_FOR_VARIANT_CALL is set to 30 (default=20)
- NORMALIZATION_LEVEL is set to 10000000 (default is 3500000; cfDNA pipeline)
- VARIANT_DOWNSTREAM_ROI_SIZE is set to 150 (default=400; cfDNA pipeline)

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

Approval:

13 Jun 2019

Prepared By | Date