

Technical Product Report

For Research Use Only; Not for use in Diagnostic Procedures

Product Description: Seraseq[®] ctDNA Reference Material v2 AF0.125%

Material Number: 0710-0207 Batch Number: 10518773

Material Description: A ctDNA-like mixture of human genomic DNA from the reference cell line, GM24385, and synthetic DNA constructs

Concentration (Qubit dsDNA BR Assay): 26.8 ng/mL

Volume: 5.0 mL

Date of Manufacture: 17 Aug 2017 Expiration Date: 16 Aug 2021

Storage: 2-8°C

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

Gene ID	COSMIC Identifier	AF%
AKT1	COSM33765	0.16
APC	COSM13127	0.18
APC	COSM18561	0.11
ATM	COSM21924	0.16
BRAF	COSM476	0.14
CTNNB1	COSM5664	0.17
EGFR	COSM6224	0.17
EGFR	COSM12378	0.18
EGFR	COSM6225	0.12
EGFR	COSM6240	0.18
ERBB2	COSM20959	0.14
FGFR3	COSM715	0.12
FLT3	COSM783	0.13
FOXL2	COSM33661	0.14
GNA11	COSM52969	0.15
GNAQ	COSM28758	0.20
GNAS	COSM27887	0.20
IDH1	COSM28747	0.19
JAK2	COSM12600	0.12
KIT	COSM1314	0.13
KRAS	COSM521	0.15
MPL	COSM18918	0.14
NCOA4-RET	N/A	0.13
NPM1	COSM17559	0.10
NRAS	COSM584	0.21
PDGFRA	COSM736	0.14
PDGFRA	COSM28053	0.14
PIK3CA	COSM763	0.13
PIK3CA	COSM12464 ²	0.13
PIK3CA	COSM775	0.17
PTEN	COSM4986	0.10
PTEN	COSM5809	0.10
RET	COSM965	0.14
SMAD4	COSM14105	0.18
TP53	COSM10648	0.20
TP53	COSM10660	0.16
TP53	COSM10662	0.20
TP53	COSM6530	0.14
TP53	COSM18610	Not Tested ¹
TPR-ALK	N/A	0.13

¹ NGS testing alone is used for QC of variant COSM18610.

² As of June 2019 this mutation is no longer listed in the COSMIC database

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

Gene	COSMIC Identifier	AF%
AKT1	COSM33765	0.12
BRAF	COSM476	0.11
CTNNB1	COSM5664	0.04
EGFR	COSM6225	0.08
EGFR	COSM12378	0.11
EGFR	COSM6240	0.17
EGFR	COSM6224	0.05
ERBB2	COSM20959	0.18
IDH1	COSM28747	0.19
KIT	COSM1314	0.11
KRAS	COSM521	0.08
NRAS	COSM584	0.17
PDGFRA	COSM28053	0.06
PDGFRA	COSM736	0.09
PIK3CA	COSM763	NMD
PIK3CA	COSM775	0.04
PIK3CA	COSM12464 ¹	NMD
RET	COSM965	NMD
TP53	COSM10660	0.14
TP53	COSM10662	0.14
TP53	COSM6530	0.07
TP53	COSM10648	0.05
TP53	COSM18610	0.18

¹ As of June 2019 this mutation was no longer listed in the COSMIC database.

NMD= No Mutation Detected. Results presented in this table represent a single QC run. Variants listed as NMD were successfully detected in other rounds of QC testing. Stochastic failures are expected for this 0.125% allele frequency tier under the run conditions that were used for QC testing of the Seraseq ctDNA Mutation Mix v2.

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NGS was performed as an orthogonal verification step. Parameters used:

NGS Parameters:

DNA input = 50 ng

of samples / flow cell = 3

of total reads / sample = 5.1 M

Average read depth = 3444X

On-target reads = ~97%

Q30 score = ~89%

Analysis = Archer Analysis Suite v5.1.2

Default analysis settings except for the following:

- ERROR_CORRECTION is turned to ON (default=OFF; cfDNA pipeline)
- NORMALIZATION_LEVEL is set to 10000000 (default is 3500000; cfDNA pipeline)
- VARIANT_DOWNSTREAM_ROI_SIZE is set to 150 (default=400; cfDNA pipeline)
- BARCODE_HAMMING_DISTANCE=1 (default=2)

Please see the poster from NIST for more information about assay sensitivity:

<https://digital.seracare.com/multilab-assessment-reference-materials-ctdna-poster2018>

Approval:

A handwritten signature in blue ink, appearing to read "Aimee Tarkenton".

03-Sep-2020

Prepared By

Date