



Seraseq™ circulating tumor DNA Reference Materials

**The most patient-like ctDNA reference
materials on the market**

Ruth Mayes,
Business Development Manager,
EMEA

Overview:

Circulating Cell Free DNA

- Reference Materials
 - Purpose and utility
- Current challenges for labs bringing on ctDNA assays
- Seraseq™ ctDNA 2.0 technology and product design
- Preliminary data
- Collaborations to advance ctDNA validation

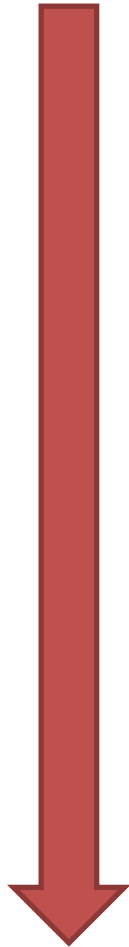
SeraCare Provides Clinical Genomics Standards and Reference Materials for NGS-based Assays

Technology Evaluation

Technology Optimization

Assay Validation

Lab QC Monitoring and Proficiency



1. Highly multiplexed 'truth sets' labs can use through all assay development stages from Technical Evaluation through Analytical Validation
 - Widest variety of important and difficult to source somatic variant types
 - Patient-like materials
2. Faster validation/verification by your customers
3. Quantitative, precise and reproducible 'in-kit controls' for ongoing monitoring of assay performance
4. Easily track and report all your labs NGS QC metrics
5. Measure inter-lab performance through innovative proficiency programs

Why are reference materials so important?

- Reagents designed and manufactured to assess
 - Random error
 - Trueness or bias
 - Measurement accuracy
- Improved and standardized ctDNA measurements will lead to better discrimination in diagnosis and personalized treatment
- Testing with reference materials is the only “effective” way to evaluate performance across the many variables, assays, platforms and laboratories

Clinical labs developing and running ctDNA assays face several challenges

1. What is 'truth'? How can I use a 'truth set'?
2. How does my assay perform across different variants? Different variant types?
3. Where can I find specimens with a wide variety of variant types?
4. How can I assess the sensitivity AND specificity of my assay?
5. How commutable are commercially-derived reference materials?

NGS Guideline from Association for Molecular Pathologists / College of American Pathologists

SPECIAL ARTICLE

Guidelines for Validation of Next-Generation Sequencing—Based Oncology Panels



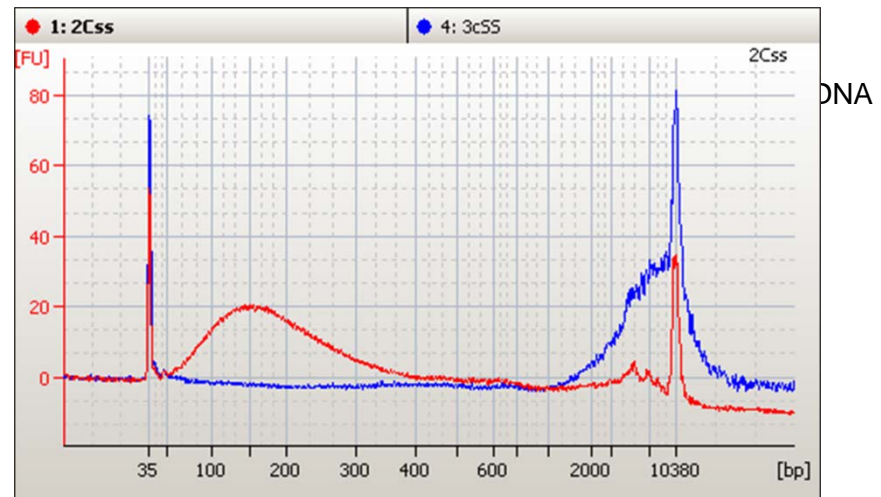
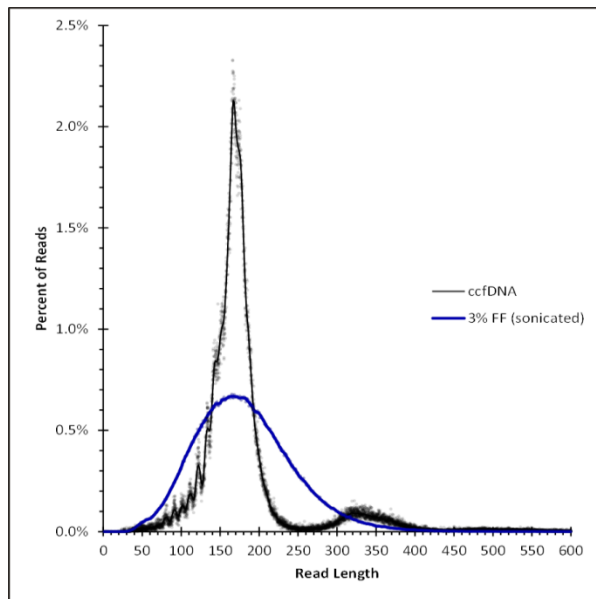
A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists

Lawrence J. Jennings,^{*†} Maria E. Arcila,^{*‡} Christopher Corless,^{*§} Suzanne Kamel-Reid,^{*¶||} Ira M. Lubin,^{*•**} John Pfeifer,^{*††} Robyn L. Temple-Smolkin,^{‡‡} Karl V. Voelkerding,^{*§§¶¶} and Marina N. Nikiforova^{*|||}

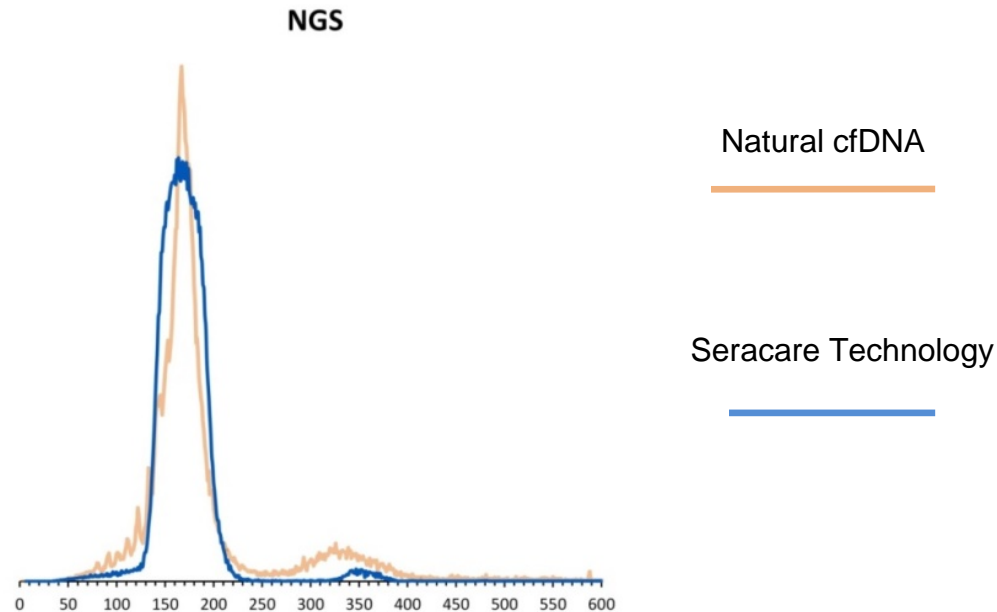
Required sample volumes far exceed available donor material and thus the most viable options are biosynthetic reference materials

Current reference materials have limitations

- Ultrasonication of cell line DNA has low performance compared to native cfDNA
 - Library prep efficiencies and diversity can be compromised—resulting in variable performance
- DNA in synthetic plasma matrices without stabilization can aggregate
 - Size-appropriate DNA in plasma-like matrices should be stable over time to enable consistent performance trending over time



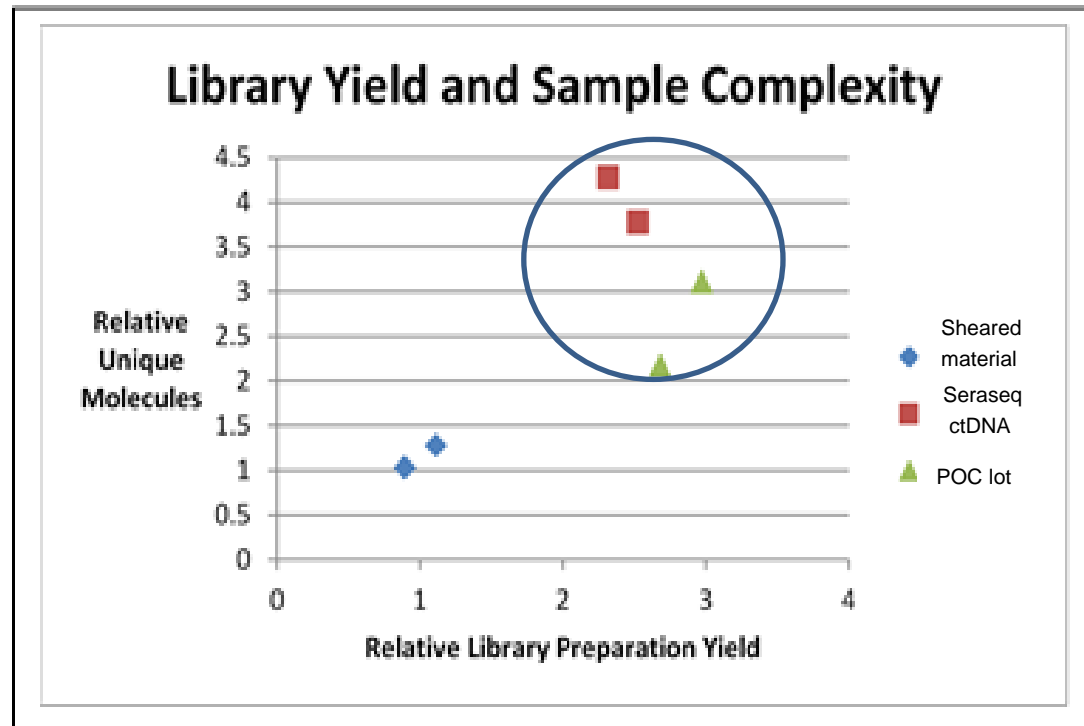
More 'patient-like' cell free DNA: Seraseq ctDNA v2 has improved size distribution



Very similar in bp-size and form to patient samples

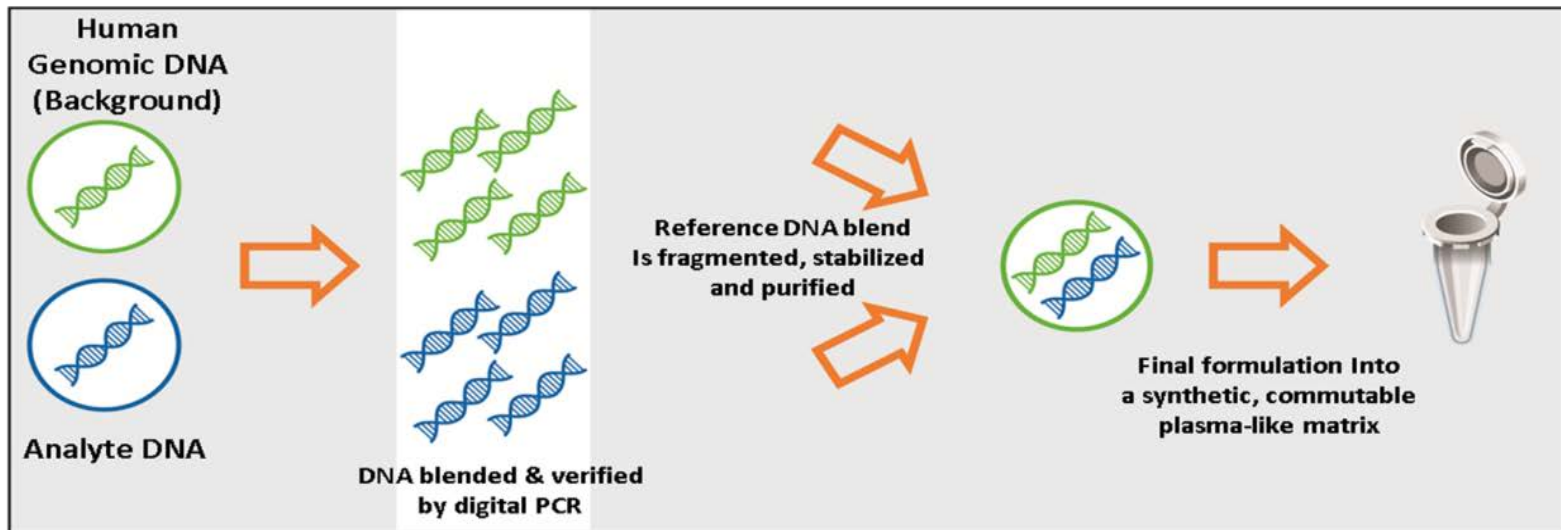
More 'patient-like' cell free DNA:

Seraseq ctDNA v2 has superior library yield and complexity



- Greatly superior library yield and complexity compared to ultrasonicated material

Full process ctDNA reference materials



1 – 40 variants

dPCR
validated
assay for
each
variant

Blended with
GM24385
background
genome and
stabilized

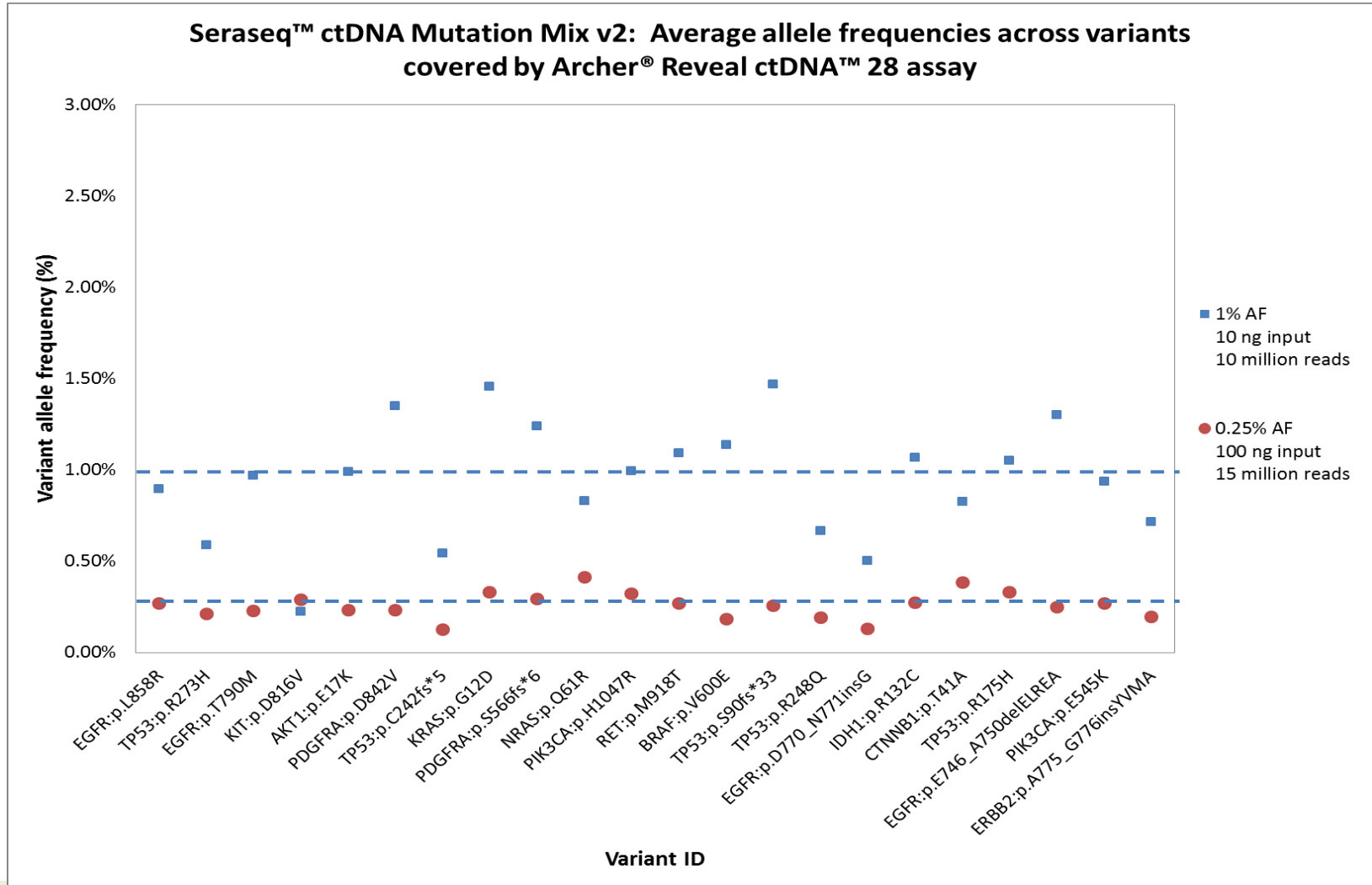
- 5ml Plasma matrix (0.1-2% allelic frequency ranges)
- Validated dPCR and NGS for each mix
- (Matrix has 2 years stability)

Compatible across a wide-range of DNA extraction systems

Extraction/Quant (n)	HIGH Conc: Efficiency at 50ng/ul	LOW Conc: Efficiency at 25ng/ul
MagMAX manual/ Qubit HS (n=3)	79%	74%
Maxwell RSC/ Quantifluor dsDNA (n=6)	108%	125%
Maxwell RSC/ Qubit HS (n=2)	81%	66%
QIAamp manual/ Quantifluor dsDNA (n=3)	118%	124%
QIAamp manual/ Qubit HS (n=2)	92%	88%
QIAamp manual/ Tape Station HS D5000 (n=3)	98%	119%
QIAamp MinElute/ Qubit HS (n=2)	68%	65%
QIAsymphony/ Qubit HS (hi n=8; lo n=4)	64%	69%
QIAsymphony/ TapeStation (n=3)	81%	78%
Average Yield	88%	90%

- Study showing recovered yields (%) for two input concentrations (50ng/ul and 25ng/ul) of the plasma-like ctDNA materials across several extraction platforms
- Suitable for evaluation and monitoring of pre-analytical performance factors
- Consistent extraction efficiencies across platforms and chemistries

Seraseq™ ctDNA v2 mutation mix performance on Archer Reveal™ ctDNA 28 assay at 2 allele frequencies (1% and 0.25%)



Seraseq™ ctDNA v2:

Very broad array of rare and important variants and variant types for more comprehensive validations

Gene ID	Mutation Type	Amino Acid Change
AKT1	Substitution	p.E17K
APC	Substitution	p.R1450*
APC	Insertion in HP 7N	p.T1556fs*3
ATM	Deletion	p.C353fs*5
BRAF	Substitution	p.V600E
CTNNB1	Substitution	p.T41A
EGFR	SNV in 3N	p.L858R
EGFR	Insertion	p.D770_N771insG
EGFR	Deletion	p.E746_A750delELREA
EGFR	Substitution	p.T790M
ERBB2	Insertion	p.A775_G776insYVMA
FGFR3	Substitution	p.S249C
FLT3	Substitution	p.D835Y
FOXL2	Substitution	p.C134W
GNA11	Substitution	p.Q209L
GNAQ	SNV in HP 3N	p.Q209P
GNAS	Substitution	p.R201C
IDH1	Substitution	p.R132C
JAK2	SNV in HP 3N	p.V617F

Gene ID	Mutation Type	Amino Acid Change
KIT	Substitution	p.D816V
KRAS	Substitution	p.G12D
MPL	Substitution	p.W515L
NCOA4-RET	Gene Fusion (DNA)	N/A
NPM1	Insertion	p.W288fs*12
NRAS/CSDE 1	Substitution	p.Q61R
PDGFRA	Substitution	p.D842V
PDGFRA	Insertion	p.S566fs*6
PIK3CA	Substitution	p.E545K
PIK3CA	Insertion	p.N1068fs*4
PIK3CA	Substitution	p.H1047R
PTEN	Insertion	p.P248fs*5
PTEN	Deletion 6N > 5N	p.K267fs*9
RET	Substitution	p.M918T
SMAD4	Insertion	p.A466fs*28
TP53	Substitution	p.R175H
TP53	Substitution	p.R273H
TP53	Substitution	p.R248Q
TP53	Deletion	p.C242fs*5
TP53	Deletion 5N >4N	p.S90fs*33
TPR-ALK	Gene Fusion (DNA)	N/A

Product Configurations: Available Soon

Format	Catalog Number	Frequency	Concentration	Volume	Total mass
ctDNA Reference Material v2 (Full-process in synthetic plasma)	0710-0203	2.0%	25 ng/mL	5 mL	125 ng
	0710-0204	1.0%	25 ng/mL	5 mL	125 ng
	0710-0205	0.50%	25 ng/mL	5 mL	125 ng
	0710-0206	0.25%	25 ng/mL	5 mL	125 ng
	0710-0207	0.125%	25 ng/mL	5 mL	125 ng
	0710-0208	WT (0%)	25 ng/mL	5 mL	125 ng
ctDNA Mutation Mix v2 (Purified DNA)	0710-0139	2.0%	10 ng/uL	25 uL	250 ng
	0710-0140	1.0%	10 ng/uL	25 uL	250 ng
	0710-0141	0.50%	10 ng/uL	25 uL	250 ng
	0710-0142	0.25%	10 ng/uL	25 uL	250 ng
	0710-0143	0.125%	10 ng/uL	25 uL	250 ng
	0710-0144	WT (0%)	10 ng/uL	25 uL	250 ng

Contact us for exclusive first access to these products as they become available

<https://www.seracare.com/about-us/contact-us/>

Thank You
