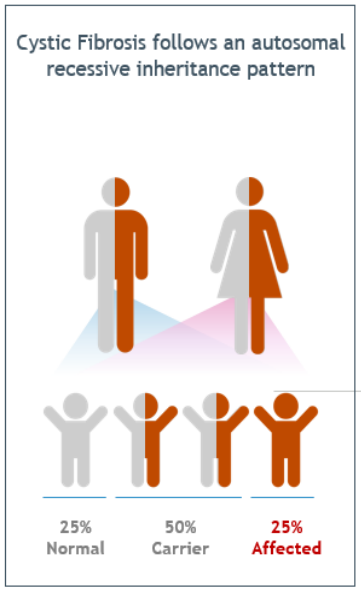
AmplideX PCR/CE *CFTR* Prototype Assay has Broad Coverage for Diverse Populations

- Cystic Fibrosis is an autosomal recessive condition caused by mutations in the *CFTR* gene.
- More than 2000 *CFTR* variants have been identified. Disease varies by severity of the mutations, genetic modifiers, and environmental factors. The mutation profiles varies greatly with different population groups.
- Reliable detection of *CFTR* mutations is crucial to inform disease diagnosis, therapy decisions, carrier screening and pre-natal testing.
- AmplideX PCR/CE *CFTR* Prototype addresses both European (ECFS guidelines) & US recommendations (ACMG/ACOG guidelines) for *CFTR* mutation detection.

Asuragen's kit is designed to detect more at-risk couples[3] than any other commercial Kit



	% COVERAGE[1]	At-Risk Couple Detection Rate[2]	For every 20 ARCs[3] detected by Asuragen, # missed by others
Asuragen	93.0%	86.5%	--
Illumina	87.8%	77.0%	-2
Luminex*	87.0%	75.7%	2-3
Agena	86.9%*	75.5%*	2-3
Elucigen	86.2%	74.3%	2-3
GenMark ACMG 23	78.9%	62.3%	5

*Luminex xTAG® Cystic Fibrosis (CFTR) 71 kit v2

This product is under development. Performance specifications have not been finalized

1 Based on US population demographics
2 % coverage squared for carrier alleles in both parents
3 Predicted at-risk couples (ARC) is calculated from *CFTR* mutation coverage assuming fully pathogenic alleles

Contemporary View of *CFTR* Variants Emphasizes Quality Over Quantity

"…the number of variants should not be used as a comparator; instead, the focus should always be on sensitivity and specificity (either analytical or clinical) of known pathogenic variants."

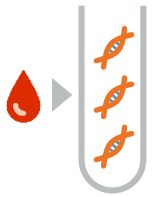
Beauchamp et al. (2019)

For additional information about this poster and others from Asuragen, visit https://asuragen.com/eshg-20/

AmplideX PCR/CE *CFTR* Prototype System includes all necessary Reagents and Software to go from Sample to Results in ~ 5 hours

AmplideX®

Pre-Analytics → PCR → CE → Data Analysis



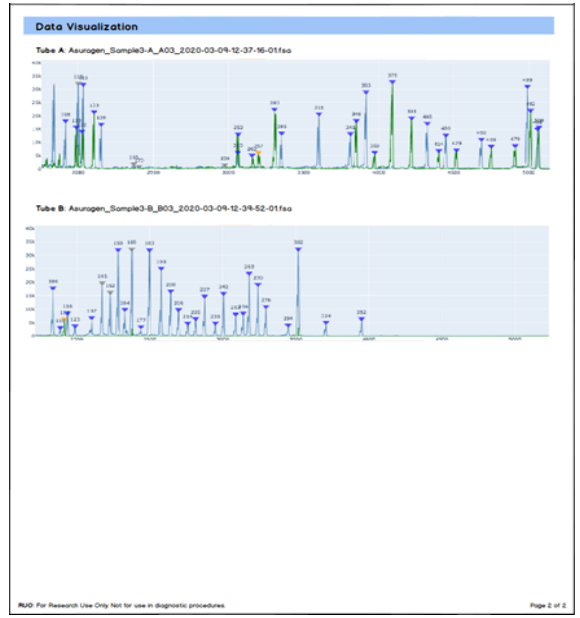
Agnostic DNA isolation approach with conservative sample input requirements

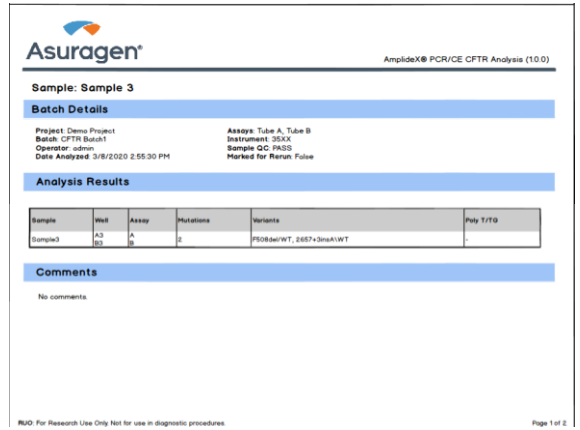
Kit include necessary reagents for both PCR and CE steps and assay is compatible with widely-established instruments

Automated interpretation of results is enabled through push-button analytics modules

DNA to Data Within One Day

Software Report





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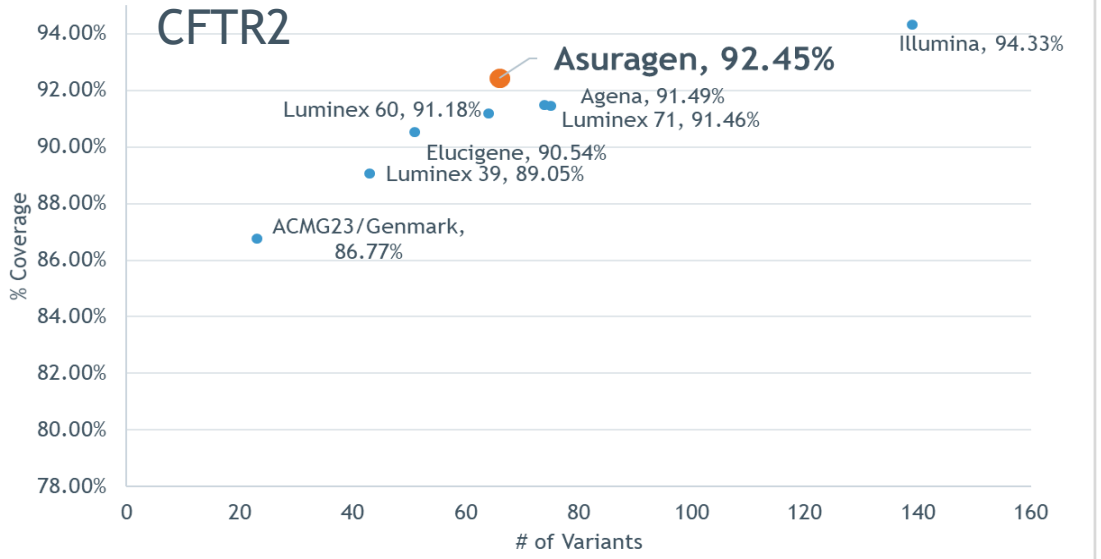
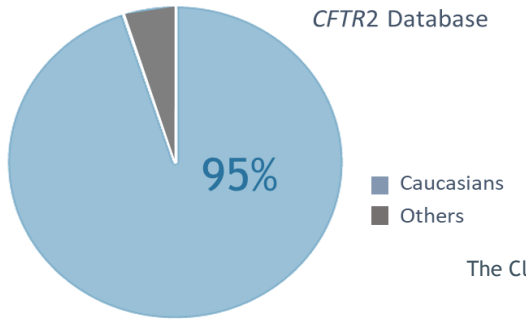
A Rapid Prototype Multiplex PCR/CE Assay and Software System has Broad Coverage of Pathogenic *CFTR* Mutations for Diverse Populations

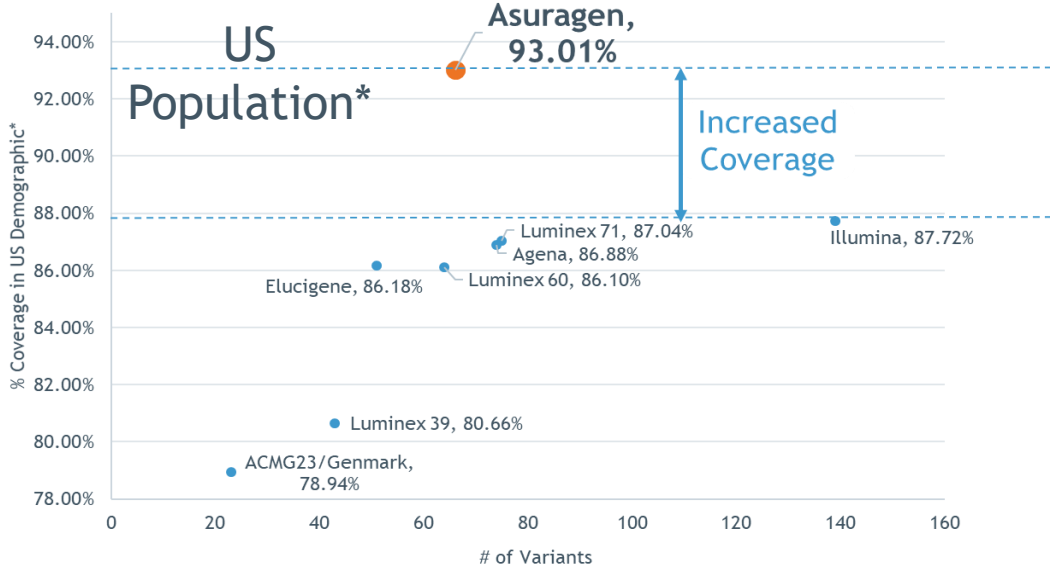
- The AmplideX PCR/CE *CFTR* Prototype assay genotypes 66 pathogenic variants that address >92% mutant prevalence in an ethnically diverse US demographic and *CFTR*2.org
- The Prototype addresses both European and US recommendations for *CFTR* mutation detection
  - Targets at least one pathogenic mutation in >96% of CF patients as per ECFS guidelines.
  - Includes all 23 ACMG/ACOG minimum list recommended *CFTR* mutations.

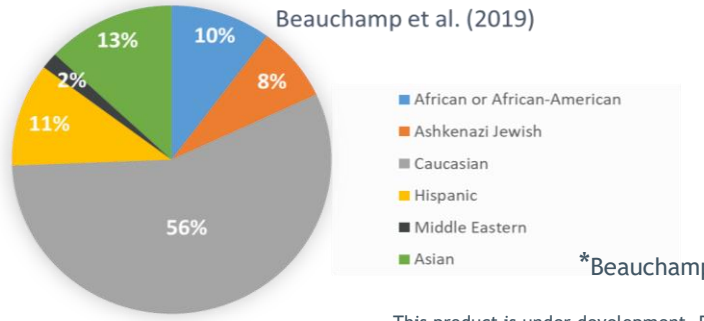




CFTR2 database lacks ethnic diversity

The Clinical and Functional Translation of CFTR (CFTR2); http://cftr2.org





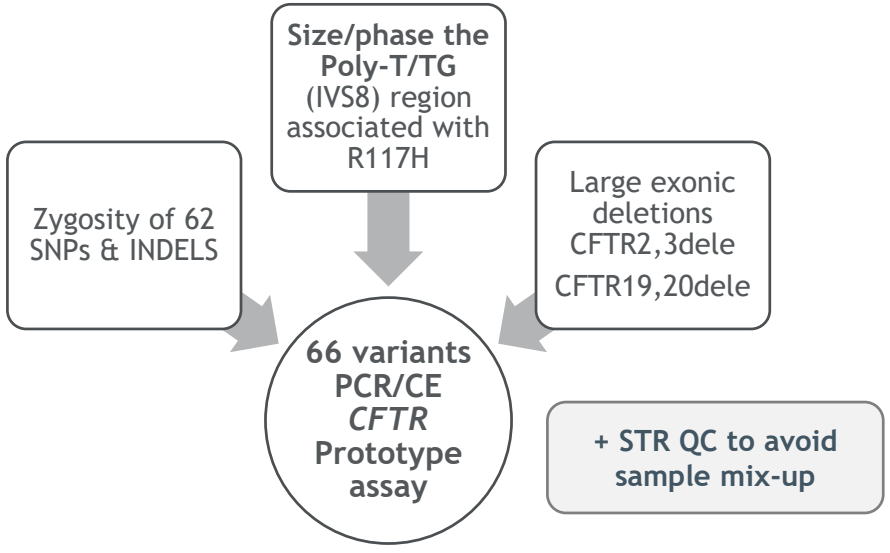
More actionable information for the diverse US population

*Beauchamp K. et al. (2019) Genet in Med 21:1948-1957

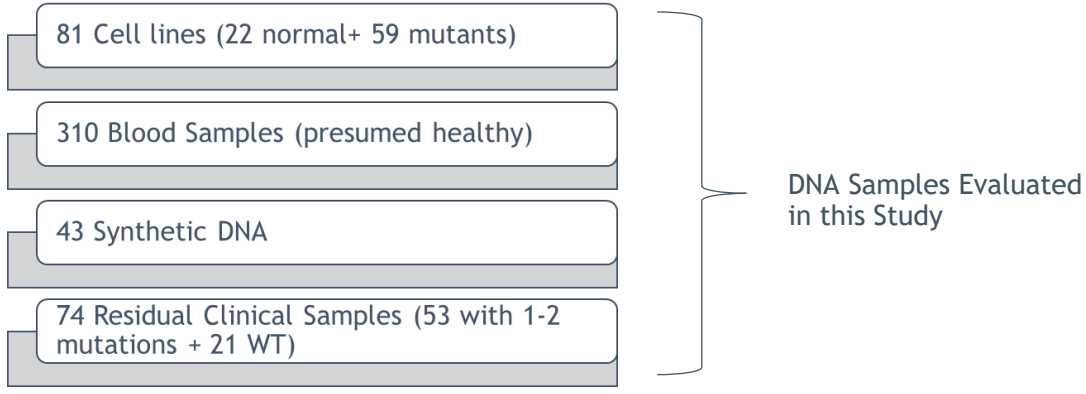
This product is under development. Performance specifications have not been finalized

A Two-Tube PCR/CE Assay was Designed to Detect Multiple Classes of Variants using a Streamlined Workflow, and Evaluated across 508 Samples

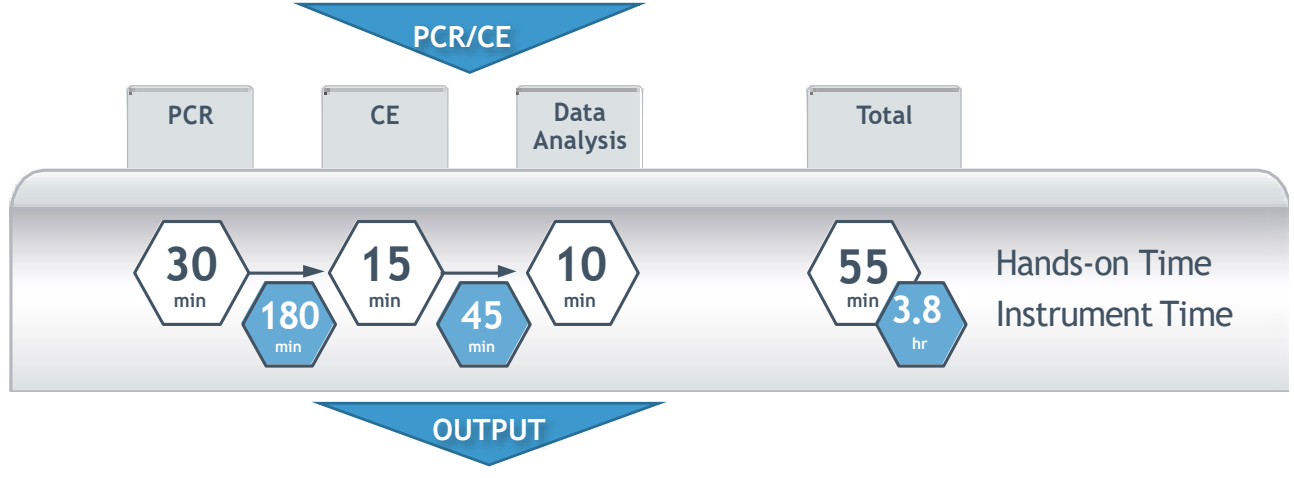
Detects SNPs, INDELs, CNVs and IVS8 poly-T/TG modifier



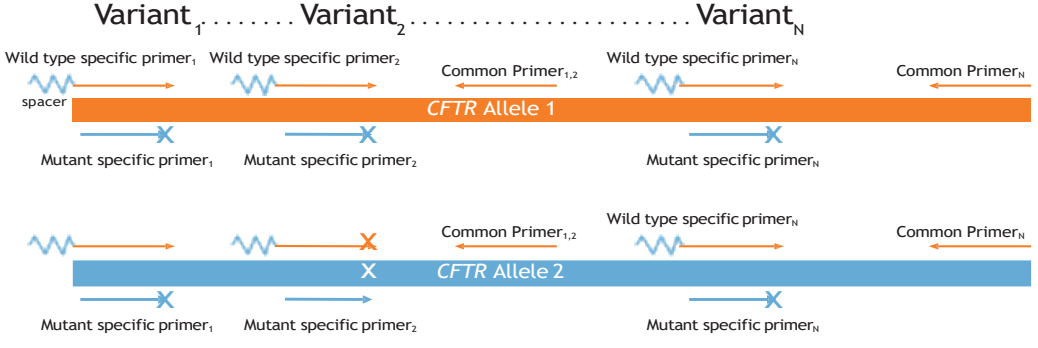
Cell line, synthetic, presumed normal blood and residual Clinical samples were evaluated



Streamlined Workflow ~ 5 hours from Sample to Results



Allele-specific primers amplify wild-type or mutant allele variants with a set of common primers



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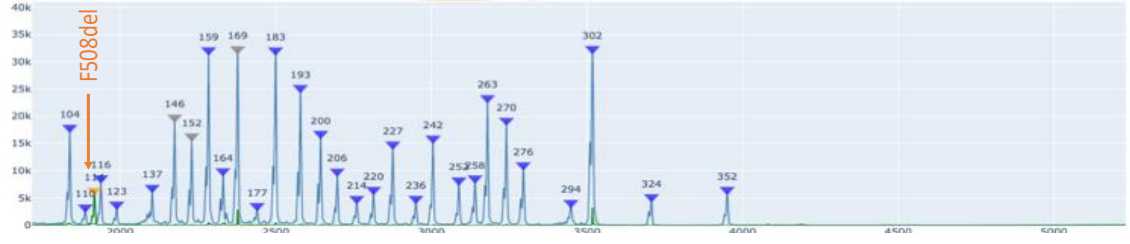
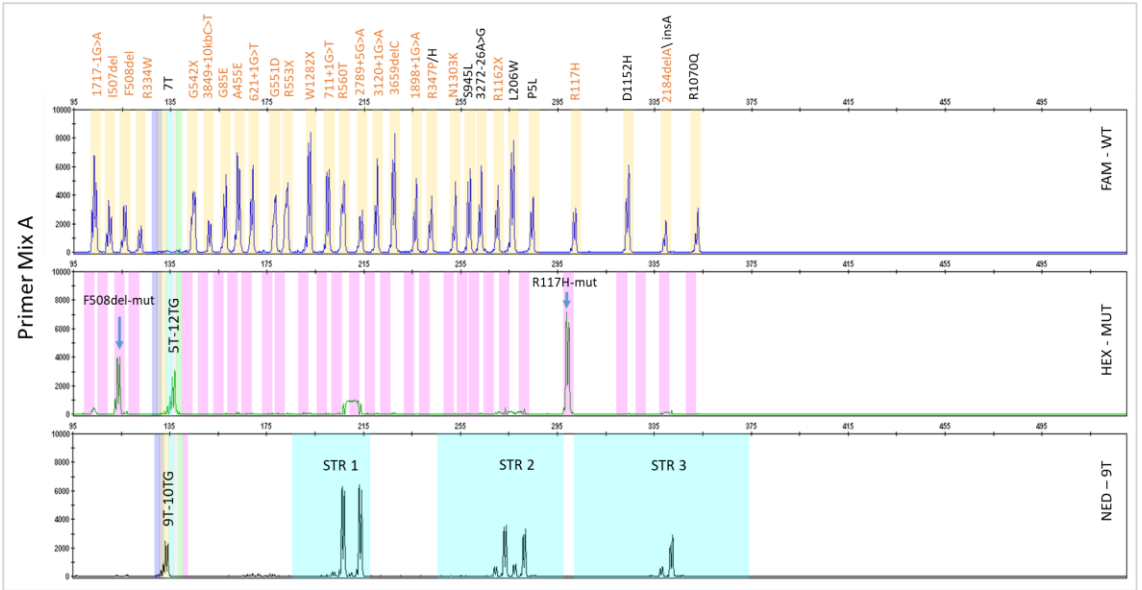
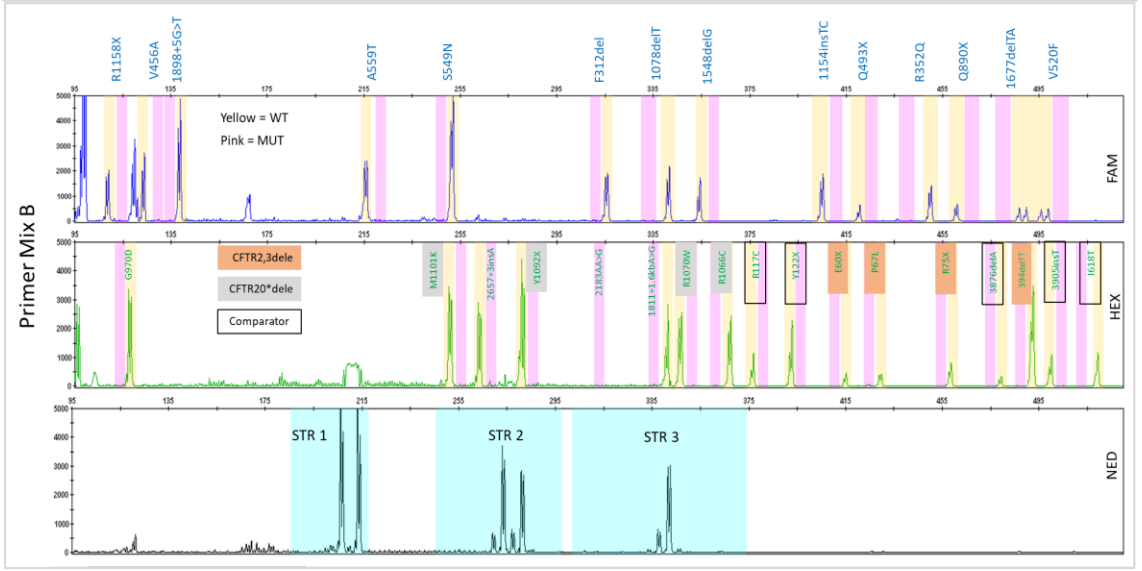


The Prototype Accurately Determined Zygosity for 81 cell lines and >10,500 alleles with 100% Agreement to Reference Results

Prototype PCR/CE *CFTR* Assay Profile for Two-Tube PCR (Primer Mix A,B) with NA13591, a Compound Heterozygote Mutant F508del & R117H with 5T allele





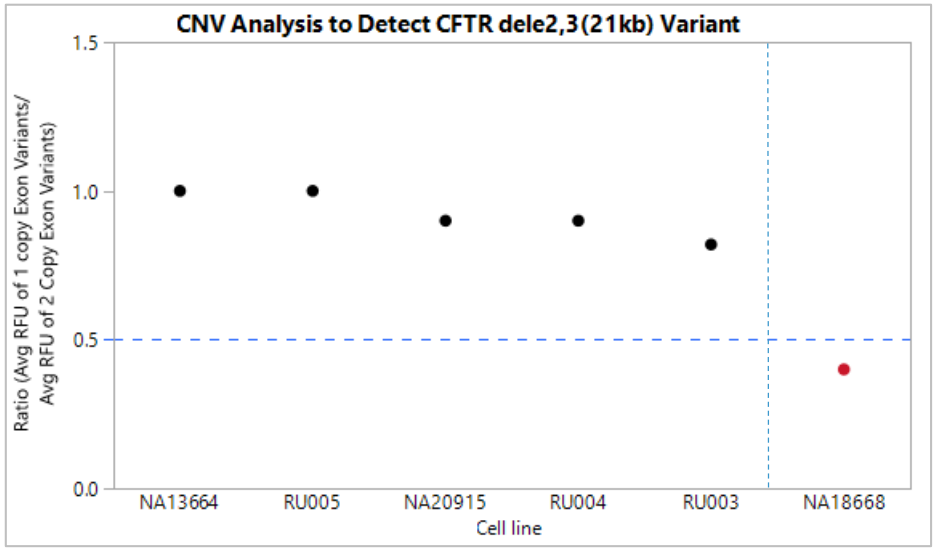
100% Agreement of Prototype Assay with the Comparator Method for Zygosity and IVS8 Sizing-Phasing

IVS8 Size Distribution and Agreement	10 TG	11 TG	12 TG	Totals
5T	-	2	2	4/4 (100%)
7T	39	72	10	121/121 (100%)
9T	35	2	-	37/37 (100%)
	74/74 (100%)	76/76 (100%)	12/12 (100%)	162/162 (100%)

Agreement with comparator assay for observed allele peak			Sanger Sequencing: Homozygous wt/wt	Sanger Sequencing: Homozygous mut/mut	Sanger Sequencing: Heterozygous wt/mut	Variant Agreement
Prototype PCR/CE *CFTR* Assay	Homozygous	wt/wt	5114	-	-	5114/5114 (100%)
	Homozygous	mut/mut	-	5 4 var. - Tube A 1 var. - Tube B	-	5/5 (100%)
	Heterozygous	wt/mut	-	-	65 45 var. - Tube A 20 var. - Tube B	65/65 (100%)

Variants R347H/R347P are detected by the same allele specific primers and cannot be differentiated.
2184delA, 2184insA and 2183AA>G are detected by the same allele specific wild type primer but with different mutant primers.

CNV analysis accurately determined *CFTR*dele2,3 HET variant in cell line NA18668



Ratio of Exon 3 variants (averaged) to Exon 04, 14, 23 variants (averaged) was ~0.4 in this cell line vs 0.8-1 in other wt cell lines shown here.

This product is under development. Performance specifications have not been finalized

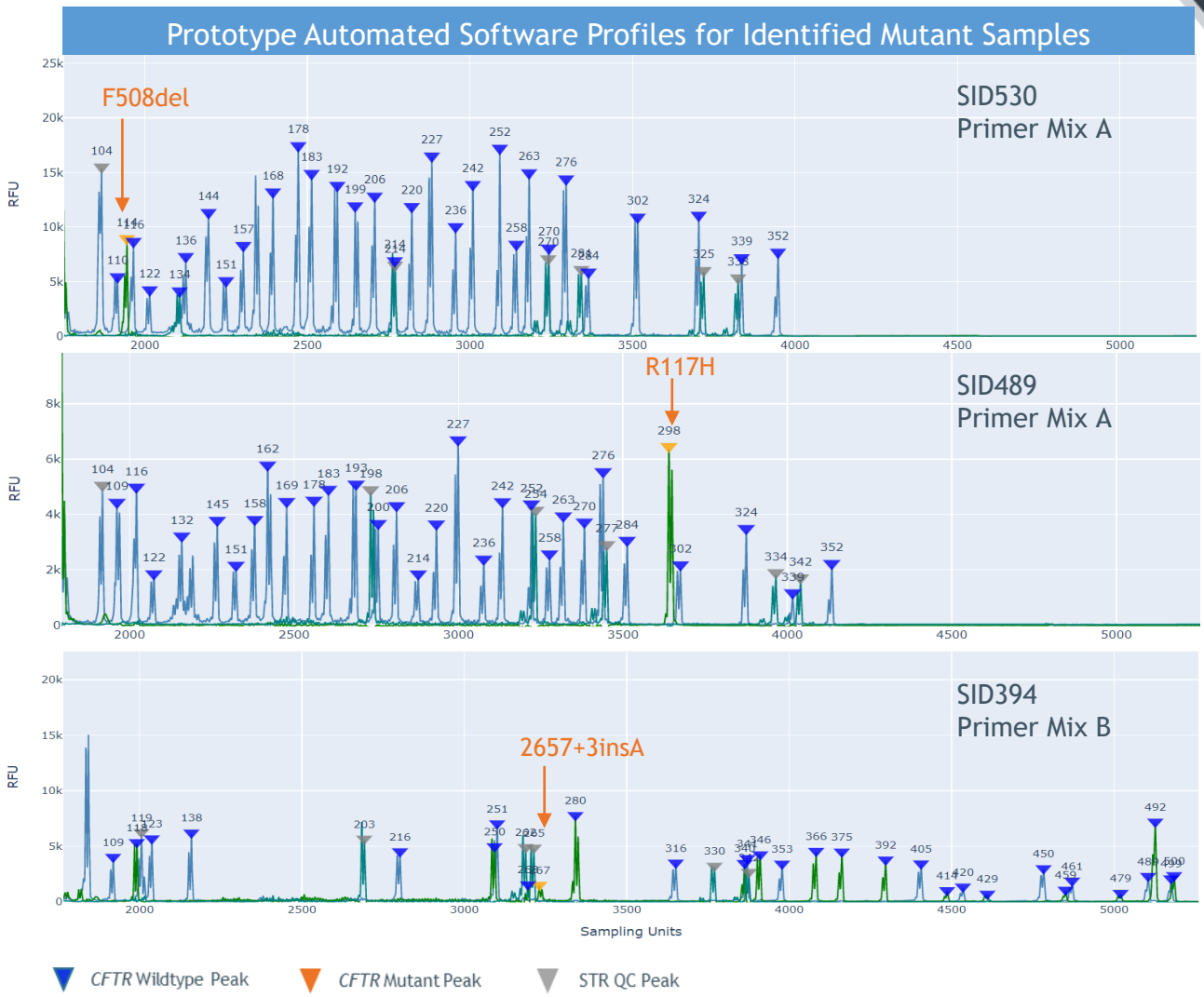
The Prototype PCR/CE *CFTR* assay Identified 12 *CFTR* Carriers from 310 Presumed Normal WB Donors

- The Prototype assay showed 100% agreement of all 12 mutant alleles and IVS8 sizing/phasing with Comparator (Sanger Sequencing).
- Prototype Automated Software accurately identified all 12 mutant variants that were called manually on GeneMapper® software.

Sample ID	Mutant Allele & Zygosity	Allele 1 (T-TG)	Allele 2 (T-TG)	Sanger Sequencing Agreement
SID530	F508del Het	7T-12TG	9T-10TG	✓
SID637	F508del Het	7T-10TG	9T-10TG	✓
SID278	F508del Het	9T-10TG	9T-10TG	✓
SID388	F508del Het	7T-11TG	9T-10TG	✓
SID396	F508del Het	7T-11TG	9T-10TG	✓
SID481	F508del Het	7T-10TG	9T-10TG	✓
SID562	F508del Het	7T-11TG	9T-10TG	✓
SID569	F508del Het	7T-11TG	9T-10TG	✓
SID489	R117H Het	7T-10TG	7T-12TG	✓
SID302	D1152H Het	7T-11TG	7T-11TG	✓
SID301	D1152H Het	7T-11TG	7T-11TG	✓
SID394	2657+3insA Het	7T-10TG	7T-10TG	✓

Observed carrier frequency across reactions was similar to the recently published† carrier rate observed in the US population (1 in 26)

	Estimated	Actual
Primer Mix A	1/26*86% = 3.3%, 10-11	11/310 (3.54%)
Primer Mix B	1/26*7.01% = 0.27%, 0-1	1/310 (0.32%)



†Westemeyer M et al. Genet Med. 2020, PMID: 32366966

The Prototype PCR/CE *CFTR* assay Accurately Determined Zygosity for 74 Residual Clinical Samples with 100% Agreement to an Orthogonal Assay

Sample Agreement with Reference Assay			xTAG® Cystic Fibrosis (CFTR) 60 Kit v2			Overall Sample Agreement
			Homozygous or Compound Heterozygous		Heterozygous	
			WT/WT	MUT/MUT	MUT/WT	
Prototype CFTR PCR/CE Assay	Homozygous or Compound Het	WT/WT	21	0	0	21/21 (100%)
		MUT/MUT	0	18	0	18/18 (100%)
	Heterozygous	MUT/WT	0	0	35	35/35 (100%)

- A total of 21 distinct variants were represented in 53 mutated Clinical samples
- All mutations and their zygosity agreed with the reference assay calls

Variants Identified	No. of Samples
F508del	24
D1152H	5
R117H	5
G542X	4
G551D	4
621+1G>T	3
1717-1G>A	2
2789+5G>A	2
3120+1G>A	2
3659delC	2
711+1G>T	2
R1162X	2
W1282X	2
1898+1G>A	1
3849+10kbC>T	1
394delTT	1
E60X	1
G85E	1
L206W	1
M1101K	1
R347H or P	1

Note: 13 samples were compound heterozygotes with > 1 mutation

For additional information about this poster and others from Asuragen, visit https://asuragen.com/eshg-20/