# A Unified PCR/CE Carrier Screening Workflow for *CFTR*, *SMN1*, and *FMR1* Variants that Consolidates the Detection of SNVs, Indels, CNVs, and Triplet Repeat Expansions

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## Summary

- Broad access to carrier screening requires streamlined, accurate, and scalable assays that can be run in different lab settings using readily-available instrumentation.
- We developed a PCR/CE assay* using AmplideX® chemistry that identifies 23 *CFTR* variants [1] recommended by ACMG/ACOG.
- The *CFTR* assay design is compatible with co-injection of *SMN1* copy number and *FMR1* CGG repeat-primed PCR amplicons.
- This assay approach enables a simple, single-platform solution for the detection of multiple variant types across three of the most common carrier screening genes.

## Introduction

About 5% of Western populations are genetic carriers for one of three disorders: Fragile X Syndrome (FXS), Spinal Muscular Atrophy (SMA), or Cystic Fibrosis (CF). Reliable detection of the pathogenic mutations that cause these disorders requires molecular assays that accommodate multiple classes of sequence variants, namely CGG repeat expansion in *FMR1* (FXS), copy number variants in *SMN1* (SMA), and single nucleotide variants (SNVs) and indels in *CFTR* (CF). These variants are each technically challenging to resolve, and current screening assays often rely on distinct technologies, platforms and workflows. We describe a simple and accessible multiplex PCR approach that can report genetic variants in *FMR1*, *SMN1*, and *CFTR* by capillary electrophoresis (CE) in a single workflow.

## Materials and Methods

A prototype PCR/CE assay* was developed to detect and discriminate 23 mutations (SNVs and indels) in the *CFTR* gene. Multiplex primers were derived using a novel pipeline and designs that allowed detection of proximal variants. Primers were tagged with a NED dye and configured with different amplicon mobilities to optimize multiplex co-detection of *CFTR* mutations and resolve *SMN1* and *FMR1* PCR amplicons generated from AmplideX® PCR/CE *FMR1*** and *SMN1*** kits using a single CE injection on the Applied Biosystems™ 3500 Series Genetic Analyzer.

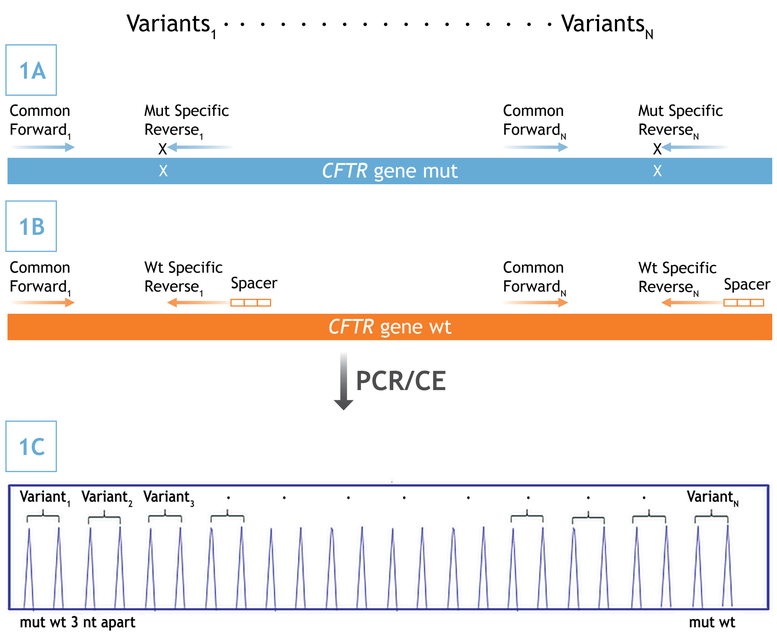


**Figure 1. PCR/CE Primer Design Strategy Enables Differentiation and Detection of Mutant (mut) and Wild-type (wt) Alleles.** The schematic shows the primer design strategy for PCR and a virtual electropherogram for an unspecified number of **A)** mutant and **B)** wild-type amplicon pairs. For the detection of multiple mutations (>50 in a single dye channel), the relative positions of the gene-specific (GS) common primer and allele-specific (AS) primers were adjusted to ensure complete separation between different variants on CE (panel **C**). Wt and mut peaks are separated by 3 nucleotides (spacer) on the electropherogram in our designs.

## Results

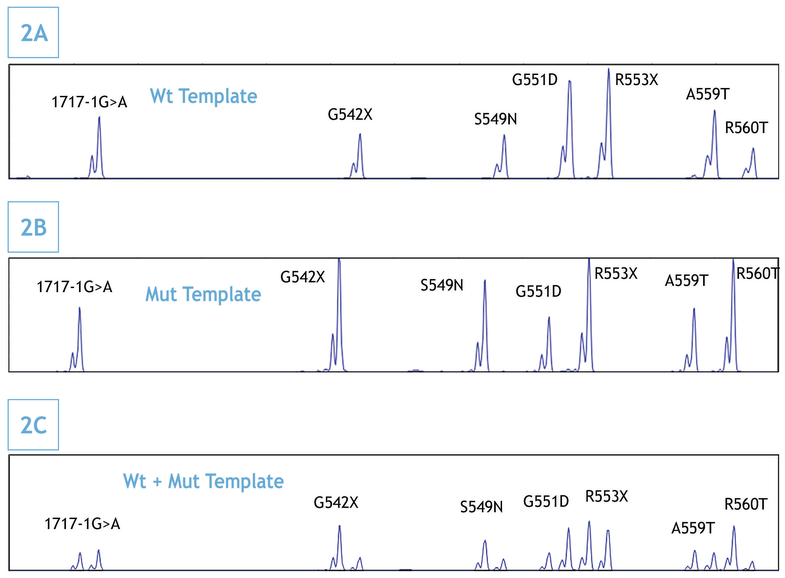


**Figure 2. Specific and Multiplexed Allelic Discrimination.** Seven SNPs are located within <100 bp *CFTR* genomic region. Using a proprietary primer design strategy, specific differentiation of wt and mut was achieved across all seven mutations. **A)** Cell line NA12878 from the Coriell Cell Repository (CCR) was used for wt alleles (top). **B)** A gBlock bearing all seven mutant alleles shows specific detection of mutant alleles. **C)** A mixture of gBlock and cell-line DNA demonstrates multiplexed detection in a single PCR reaction.

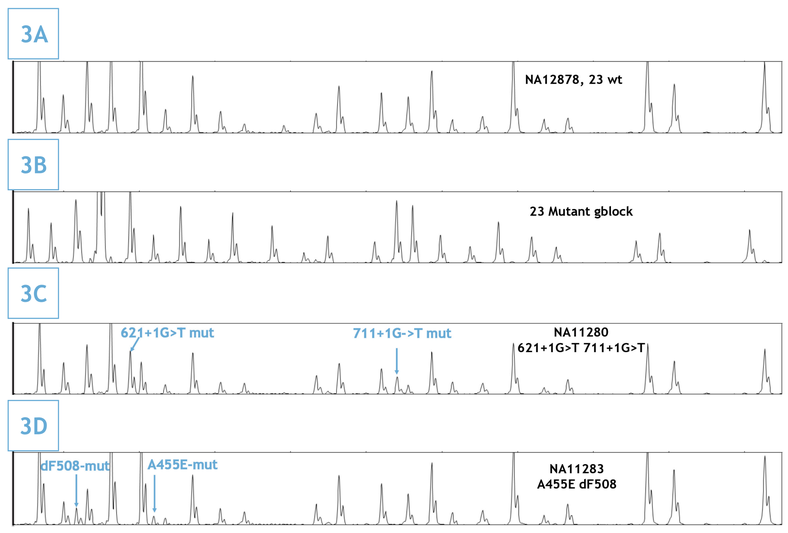


**Figure 3. Multiplex *CFTR* Assay Targeting 23 Mutations Resolved on the 3500xL CE. A)** NA12878 manifests 23 wt peaks at the expected locations. **B)** Mixture of 23 gBlocks with mutant alleles for each of the 23 mutations displays peaks shifted 3 bp shorter than the corresponding wt peak size per the design. **C)** Cell line NA11280 with heterozygous mutations 621+1G>T and 711+1G>T. Both mutant alleles were observed in addition to the wt allele. **D)** Cell line NA11283 with heterozygous mutations A455E and dF508. Both mutant alleles were observed in addition to the wt allele.

| CCR | *FMR1* CGG Repeat # | | *SMN1* Copies | *CFTR* Mut Detected |
|---|---|---|---|---|
| | Allele 1 | Allele 2 | | |
| NA12878 | 30 | | 2 | wt |
| CD00008 | 28 | 30 | 2 | 2184delA |
| NA00897 | 30 | | 2 | dF508 |
| NA00999 | 30 | 56 | 2 | 621+1G>T |
| NA07464 | 29 | | 2 | R553X |
| NA07732 | 29 | | 2 | dF508 |
| NA11277 | 32 | | 2 | dI507 |
| NA11280 | 31 | 41 | 2 | 621+1G>T 711+1G>T |
| NA11283 | 30 | | 2 | A455E, dF508 |
| NA11288 | 36 | | 2 | dF508 |
| NA11496 | 29 | 33 | 2 | G542X |
| NA11723 | 20 | 30 | 2 | W1282X |
| NA11859 | 20 | 29 | 2 | 2789+5G>A |
| NA12585 | 30 | | 2 | R1162X |
| NA12960 | 30 | | 2 | R334W |
| NA13423 | 29 | | 3 | G85E |
| NA20737 | 29 | 38 | 2 | R347H |
| NA21847 | 19 | 23 | 2 | dF508 |
| NA00232 | 55 | | 0 | wt |
| NA03815 | 20 | | 1 | wt |
| NA05756 | 30 | | 2 | wt |
| NA17117 | 21 | | 3 | wt |
| NA13664 | 30 | 52 | 2 | wt |
| CD00014 | 56 | | 2 | wt |
| NA20242 | 30 | 74 | 2 | wt |
| NA06891 | 119 | | 2 | wt |
| NA02039 | 20 | >200 | 2 | wt |
| NA04025 | >200 | | 2 | wt |

**Table 1. Single-platform, Combined Detection of *CFTR* Mutations, *SMN1* Copy Number, and *FMR1* Triplet Repeats.** Nineteen *CFTR* mutations (within the 23 panel) were detected in 17 mutant *CFTR* cell lines. All *FMR1* CGG repeat numbers and *SMN1* copy numbers were correctly called using AmplideX® PCR/CE Reporter software with *FMR1* and *SMN1* assay-specific modules. The co-detection results were also 100% concordant with separate CE injection for each individual assay.

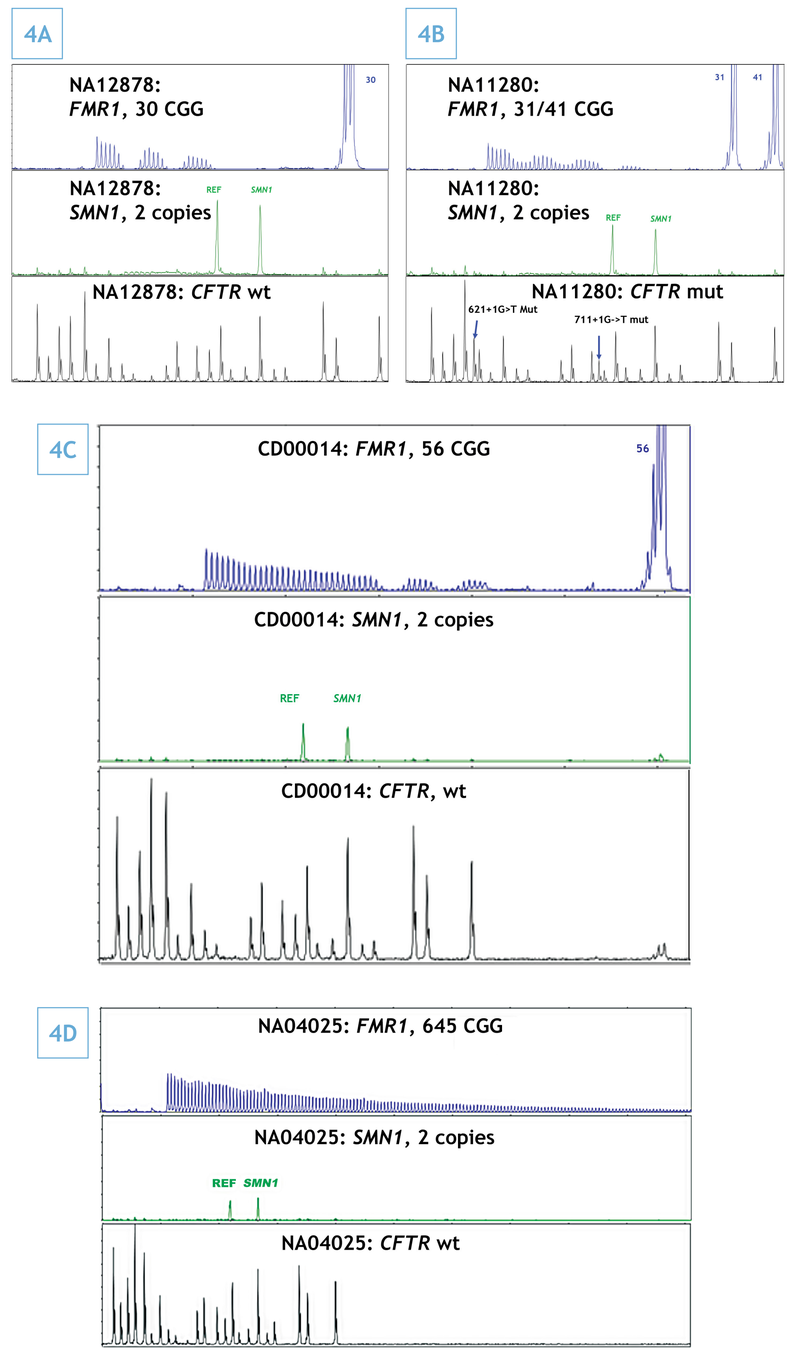


**Figure 4. Representative Electropherograms for Co-detected *FMR1* CGG Repeats, *SMN1* Copy Numbers and *CFTR* Mutations.** All amplicons were pooled and co-injected with the ROX 1000 ladder from Asuragen. **A)** Cell line NA12878 **B)** Cell line NA11280 **C)** Cell line CD00014. To simplify comparison of electropherograms, the full-mutation pile-up peak for the 645 CGG trace is not shown. **D)** Cell line NA04025.

## Conclusions

- We report a single-tube, PCR/CE assay that can resolve all 23 ACMG recommended *CFTR* variants.
- Our results demonstrate feasibility for a streamlined, single-platform, multi-modal, PCR-based assay that can unify the detection of *FMR1*, *SMN1*, and *CFTR* variants.
- This technology has the potential to address carrier screening in decentralized laboratory settings using standardized reagents, existing instrumentation, and fit-for-purpose analysis software.

**Reference**

1. Watson MS, Cutting GR, Desnick RJ, Driscoll DA, Klinger K, Mennuti M, Palomaki GE, Popovich BW, Pratt VM, Rohlfs EM, Strom CM, Richards CS, Witt DR, Grody WW: Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med 2004, 6:387e391.

*Product under development. Future availability and performance cannot be ensured.
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Presented at ASHG 2018 –2892W

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