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 amplimers.
- 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.

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 mixture of gBlocks 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 4. Representative Electropherograms for Co-detected FMR1 CGG Repeats, SMN1 Copy Numbers and CFTR Mutations. All amplimers were pooled and co-injected with the ROX 1000 ladder from Asuragen. A) Cell line NA12878. B) Cell line NA11280 C) Cell line NA11283. D) to simplify comparison of electropherograms, the full multiplex gBlock peak for the 645 CGG trace is not shown. D) Cell line NA11280.

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

*Product under development. Future availability and performance cannot be ensured.
**Research Use Only. Not for use in diagnostic procedures.

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