Proof-of-Concept for Single-Platform Trio Carrier Screening of *FMR1, SMN1/2*, and *CFTR* Variants using PCR and Capillary Electrophoresis with Consolidated Workflows

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Summary

- Carrier screening for fragile X syndrome, cystic fibrosis and spinal muscular atrophy often requires distinct molecular diagnostic methods and analysis platforms for each gene.
- We demonstrate the feasibility of trio carrier screening of *FMR1*, *SMN1/2* and *CFTR* using existing workflows and a single analysis platform with electrophoresis conditions harmonized across multiple CE instrument models.
- This approach reduces hands-on and instrument time and simplifies required instrumentation and consumables, providing a cost-effective option for laboratories who are interested in trio carrier screening.

Introduction

Carrier screening provides valuable information for couples to help guide their reproductive decision-making. Screening studies have shown that as many as 1 in 20 individuals is a carrier for one of the three most common hereditary genetic conditions: spinal muscular atrophy, fragile X syndrome, and cystic fibrosis. The genes causing these disorders each present a unique technical challenge, and each usually requires a distinct molecular diagnostic method and analysis platform. As a result, a simple, cost-effective, and unified screening system is not yet available for this trio of carrier genes. Here we demonstrate the feasibility of analyzing combinations of PCR products from these genes on a single capillary electrophoresis (CE) instrument using a co-injection strategy to demonstrate feasibility on multiple CE instrument models.

Methods

DNA samples were PCR amplified using AmplideX® PCR/CE *FMR1**, and *SMN1/2* Plus* kits (Asuragen) and electrophoresed on the Applied Biosystems™ 3500, 3730 and SeqStudio Genetic Analyzers (Thermo Fisher Scientific). *FMR1* amplicons (FAM labelled) were combined with *SMN1/2* Plus products (HEX labelled) in a single CE formulation to create different genotype combinations, including 0 to ≥4 copies of *SMN1* or *SMN2* and all *FMR1* categories, with 86 sample combinations total. Data were processed using AmplideX® Reporter Software without optimization of electropherogram characteristics for co-injection. QC failures were excluded from analysis. Proof-of-concept for co-injection of *CFTR* and *SMN1/2* Plus amplicons was demonstrated on a 3500 CE instrument using 94 combinations of AmplideX® PCR/CE *CFTR*‡ amplicons (created from prototype reagents) and *SMN1/2* amplicons of different genotypes.

CE Parameters	3500 (50cm)	SeqStudio (28cm)	3730 (50cm)
Oven Temperature	60	60	63
Injection Voltage (kV)	2.5	6	2.5
Injection Time (s)	20	2	20
Run Voltage (kV)	19.5	6	15
Run Time (s)	2400	3300	4200
Formulation Ratio HiDi:Ladder:*FMR1:SMN1/2* Plus	10:2:1:2[a]		

[a]For *CFTR+SMN1/2* Plus, 9:2:2:2 ratio was used

Figure 1. CE co-injection conditions.

Results

2A ***FMR1*: GM20231**
59,76 CGG

2B ***SMN1/2* Plus: NA19360;** 4 total *SMN1*, 0 *SMN2* copies; Variants: c.*3+80T>G; c*211_212del

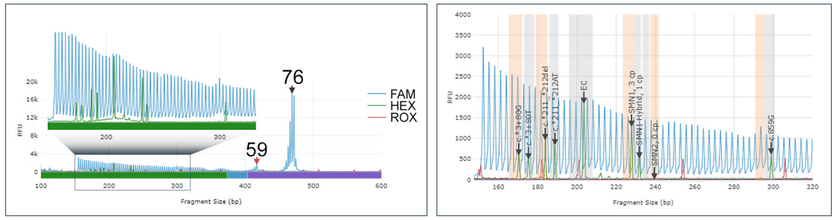
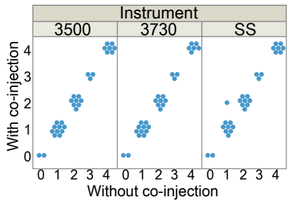
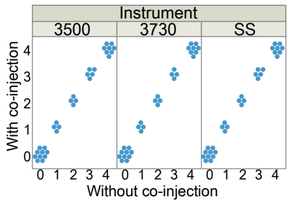


Figure 2. Example of electropherograms from *FMR1* (A, blue trace) and *SMN1/2* Plus (B, green trace) co-injection of PCR amplicons on 3500 Genetic Analyzer. Analysis modules, cell line DNA used, and expected genotype are indicated.

3A ***SMN1* Copy Number with and without Co-injection**



3B ***SMN2* Copy Number with and without Co-injection**



3C ***SMN1/2* Variant Percent Agreement**

Variant	3500	3730	SS
c.*3+80T>G	93.5% (29/31)	100% (31/31)	96.8% (30/31)
c*211_212del	96.8% (30/31)	100% (31/31)	100% (31/31)
c.859G>C	71.0% (22/31)	87.1% (27/31)	71.0% (22/31)

3D ***FMR1* Genotype Category with and without Co-injection**

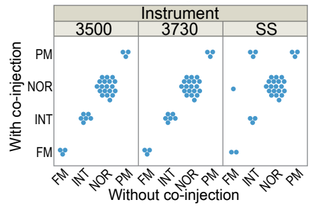
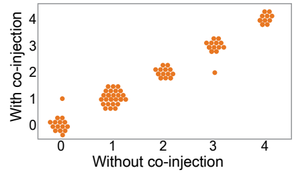
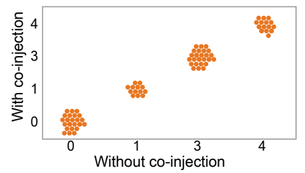


Figure 3. Agreement between separate and co-injection of *FMR1* and *SMN1/2* Plus Amplicons. **A)** Copy number agreement for *SMN1* was 100% on 3500 (30/30), 100% on 3730 (29/29) and 96.6% on SeqStudio (28/29). **B)** Copy number agreement for *SMN2* was 100% on 3500 (31/31), 3730 (30/30) and SeqStudio (31/31). **C)** Percent agreement of three SNPs/INDEL between separate and co-injection for each Genetic Analyzer. *SMN1* gene duplication variants associated with silent carriers (c.*3+80T>G, c.*211+212del) were occasionally not detected by the software but present in CE trace; visual review of CE traces is recommended during analysis. *SMN2* c.859G>C concordance impacted by FAM to HEX bleed-over of *FMR1* peaks ~20-21 CGG repeats, but not relevant for carrier status. **D)** Agreement of *FMR1* repeat length categories was 100% on 3500 (31/31), 100% on 3700 (31/31) and 87.1% on SeqStudio (27/31). Full and intermediate mutation dropouts were observed on SeqStudio, suggesting that further optimization is needed. Note: 4 = ≥4; SS=SeqStudio; PM=premutation; NOR=normal; INT=intermediate; FM=full mutation

4A ***SMN1* Copy Number with and without Co-injection**



4B ***SMN2* Copy Number with and without Co-injection**



4C ***CFTR*, NA20745, Heterozygote, S549N**

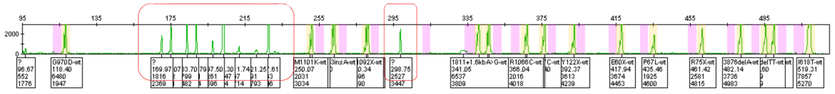


Figure 4. Agreement between separate and co-injection of *CFTR* and *SMN1/2* Plus Amplicons. PCR amplicons were combined and resolved on 3500. **A)** Copy number agreement for *SMN1* was 97.4% (74/76). **B)** Copy number agreement for *SMN2* was 100% (76/76). Percent agreement of three SNPs/INDEL between separate and co-injection were 100% (76/76) for c.*3+80T>G, 100% (76/76) for c*211_212del, 98.7% (75/76) for c.859G>C. **C)** Example of electropherogram from *CFTR* and *SMN1/2* Plus co-injection. Only HEX Channel is pictured. *SMN1/2* Plus peaks are circled in red. Other peaks, *CFTR* PCR amplicons, are annotated by variant. Analysis tool, cell line DNA used, and expected genotype are indicated. Agreement of *CFTR* results between separate and co-injection was 100% (94/94) for cell line and blood-derived gDNA samples and synthetic mixes covering all 34 variants detected in this reaction. The *CFTR* assay requires two tubes to detect all 67 variants covered by the complete panel. Note: 4 = ≥4.

Conclusions

- We demonstrated the feasibility of co-injecting PCR products from AmplideX® *FMR1+SMN1/2* Plus and *CFTR* prototype+*SMN1/2* Plus kits using existing PCR workflows and harmonized CE injection conditions.
- For *FMR1+SMN1/2* Plus co-injection, >95% genotype concordance was observed in both assays with stand-alone injections using 3500 and 3730 CE instruments.
- For *CFTR+SMN1/2* Plus co-injection, >97% genotype concordance was observed in both assays with stand-alone injections using the 3500 CE instrument.
- Additional optimization may expand the potential of this single-platform "Trio" PCR workflow across reagents, instruments and/or software.

**Research use only. Not for use in diagnostic procedures.*
‡Product in development. Specifications not finalized.

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