Enabling Single-Platform Testing and Carrier Screening of the *FMR1*, *SMN1/2*, and *CFTR* Gene Trio

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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 harmonized electrophoresis conditions across 2 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 can provide valuable information for couples to help guide their reproductive decision-making. Screening studies have shown that as many as 1 in 20 individuals has pathogenic variants associated with at least 1 of 3 conditions: spinal muscular atrophy (SMA), fragile X syndrome (FXS), and cystic fibrosis (CF). Many of these conditions cause severe symptoms, and SMA and CF can lead to premature death. The three genes associated with SMA, FXS and CF (*SMN1*, *FMR1* and *CFTR*, respectively) each present unique technical challenges and currently require distinct molecular diagnostic methods and analysis platforms. Here we demonstrate the feasibility of a common, scalable workflow for analyzing combinations of PCR products from all 3 genes on a single capillary electrophoresis (CE) instrument.

Materials and Methods

DNA samples were PCR amplified using AmplideX PCR/CE *SMN1/2* Plus*, *FMR1**, and *CFTR** kits (Asuragen) and electrophoresed on the Applied Biosystems 3500 and 3730 Series Genetic Analyzers (Thermo Fisher Scientific). PCR products from a collection of 48 unique genomic DNA samples isolated from human cell lines or whole blood, covering the breadth of possible genotypes for all three assays, were tested in singleton with each assay and combined onto a single CE plate for injection using the recommended conditions for the *FMR1* kit on each Genetic Analyzer. Data were processed using AmplideX Reporter Software. Samples were analyzed using a single CE plate for all 3 assays injected with a single set of injection conditions, then compared to the same amplicons injected on separate CE plates for each stand-alone assay using the recommended conditions for each assay.

*Research use only. Not for use in diagnostic procedures.
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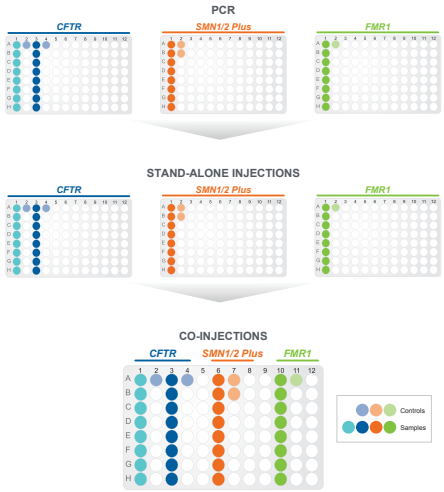


Figure 1. Example Plate Maps for Assay-Specific PCRs, Stand-Alone Injections, and Co-Injections. Samples and assay-specific controls were amplified using each kit with 1 reaction per sample for the *FMR1* and *SMN1/2* Plus kits and 2 reactions per sample for the *CFTR* kit. Example shows 8 samples.

Table 1. CE Co-Injection Parameters.

CE Parameters	3500 (50cm)	3730 (36cm)
Oven Temp	60	63
Injection Voltage (kV)	2.5	2.5
Injection Time (s)	20	20
Run Voltage (kV)	19.5	15
Run Time (s)	2400	2400

Results

Table 2. Percent Agreement Between Stand-Alone Injection and Co-Injection by Assay and CE Instrument. Percent concordance and number correct over total results reported for each assay, excluding QC failures. *SMN* copy numbers reported as 0, 1, 2, 3, or ≥4. *FMR1* genotypes reported as normal, intermediate, premutation, or full mutation based on CGG repeat length. *CFTR* genotypes reported as wild type, heterozygous, homozygous, or compound heterozygous.

Assay	Reported Result	3500 (50cm)	3730 (36cm)
FMR1	Genotype	98% (47/48)	100% (46/46)
CFTR	Genotype	100% (48/48)	98% (47/48)
SMN1/2 Plus	*SMN1* Copy Number	100% (48/48)	100% (48/48)
	SMN2 Copy Number	100% (48/48)	100% (48/48)
	Variants	100% (48/48)	100% (48/48)

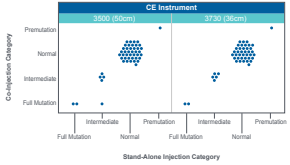


Figure 2. *FMR1* Category Agreement Between Stand-Alone Injection and Co-Injection by CE Instrument. Percent agreement was 98% percent on the 3500 (50cm) due to one false call of a full mutation peak in a single sample caused by spectral pullup from an unexpected peak in the HEX channel. Percent agreement was 100% on the 3730 (36cm), excluding two QC failures.

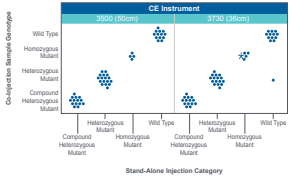
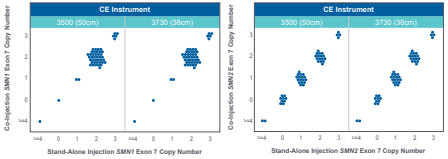


Figure 3. *CFTR* Sample Genotype Agreement Between Stand-Alone Injection and Co-Injection by CE Instrument. Percent agreement was 100% on the 3500 (50cm) and 98% on the 3730 (36cm). A false variant peak for 1717-1G>A was called in 1 sample on the 3730 (36cm) due to an erroneous peak in the HEX channel. The sample noted with an asterisk (*) was the only instance in which sample genotype differed across the 2 CE instruments. This discordance was due to dropout of the wild-type peak for 2184delA on the 3730 (36cm), caused by decreased peak heights compared to the 3500 (50cm).

4A ***SMN1* COPY NUMBER** **4B** ***SMN2* COPY NUMBER**



4C GENE DUPLICATION AND DISEASE MODIFIER VARIANTS

Variant	3500 (50cm)	3730 (36cm)
c.*3+80T>G	100% (48/48)	100% (48/48)
c.*211_*212del	100% (48/48)	100% (48/48)
c.859G>C	100% (48/48)	100% (48/48)

Figure 4. *SMN1/2* Plus Copy Number and Silent Carrier Agreement Between Stand-Alone Injection and Co-Injection by CE Instrument. A) *SMN1* exon 7 copy number was 100% across both CE instruments for all samples tested. **B)** *SMN2* exon 7 copy number was 100% across both CE instruments for all samples. **C)** All three variants were 100% concordant across both CE instruments for all samples. Of the 48 samples, 6 were identified to be positive for the variants c.*3+80T>G and c.*211_*212del associated with silent carrier risk. Although not relevant for carrier screening, 1 known positive sample for the disease modifying variant c.859G>C was also identified correctly with co-injection on both instruments.

Conclusions

- We demonstrated the feasibility of co-injecting amplicons from AmplideX® PCR/CE *FMR1*, *SMN1/2* Plus and *CFTR* kits using existing PCR workflows and harmonized CE injection conditions.
- For *FMR1*, *SMN1/2* Plus, and *CFTR* co-injection, ≥98% genotype concordance with stand-alone injections was observed in all three assays using 3500 and 3730 CE instruments.

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