#P14.039C

Generalization of an Assay System using Repeat-primed PCR and Capillary Electrophoresis to Resolve Multiple AT- and GC-rich Short Tandem Repeats Associated with Neurological Diseases

Gary J Latham[1], Charles Redmond[1], Kim Nicholson[1], Errin Lagow[1], Jeff Wallace[2], Edgar Schreiber[2], Scott Higdon[2], and Eran Bram[1]

[1]Asuragen, Inc., Austin, TX USA
[2]Genetic Sciences Division, Thermo Fisher Scientific, Inc., South San Francisco, CA USA

Summary

- Short tandem repeats (STRs) impact human disease through variable length effects on gene expression, splicing, and translation.
- STR expansions are linked to >30 human disorders, but their routine analysis has often been thwarted by the lack of fast, simple, and accurate assay systems.
- We show that STRs in *FMR1*, *C9orf72*, *TOMM40*, *DMPK*, and *HTT* can be readily and reliably genotyped using on-market and prototype AmplideX® PCR/CE reagents with the SeqStudio™ Genetic Analyzer* (Thermo Fisher).

Introduction

DNA repeat sequences constitute roughly 50% of the human genome. Short tandem repeats (STRs) are a significant subclass of repeats that are associated with >30 known Mendelian disorders. The properties of these expanded repetitive sequences, however, challenge conventional PCR and associated analysis methods. The SeqStudio Genetic Analyzer* (Thermo Fisher) enables flexible, streamlined, and economical DNA fragment analysis using a universal polymer and cartridge-based system. Here, we describe the analytical performance of a suite of repeat-primed (RP) PCR assays on the SeqStudio Genetic Analyzer*. We also demonstrate the flexibility of the SeqStudio* to support copy number quantification using prototype AmplideX® PCR/CE *SMN1* reagents**.

Materials and Methods

Mono-, tri-, and hexa- nucleotide repeat expansions from STR disease markers, including *TOMM40*, *FMR1*, *DMPK*, *HTT* and *C9orf72*, as well as the AT-rich SMA-associated *SMN1* exon 7 locus, were amplified from clinically-derived genomic DNA samples and cell-line materials (n=55) using the AmplideX® PCR/CE *FMR1* kit (CE-IVD), or other corresponding AmplideX® PCR/CE kits (*C9orf72*, *TOMM40, DMPK*)* and prototype (*HTT*, *SMN1*)** reagents (Asuragen). FAM-labeled amplicons were resolved on the SeqStudio Genetic Analyzer* (Thermo Fisher) and compared with the 3500 Genetic Analyzer* (Thermo Fisher). SeqStudio* run settings were optimized across a range of injection and run conditions while assessing STR sizing accuracy, resolution, and sensitivity, along with copy number quantification for *SMN1*.

Results

Table 1. Comparison of General and AmplideX®-specific Platform Specifications for the 3500* and SeqStudio Genetic Analyzers*.

Feature	3500* Series	SeqStudio*
Capillary array	8/24	4
Capillary length	36/50	28 (Onboard click-in cartridge)
Polymer type	POP4/POP6/POP7	POP1 (Onboard click-in cartridge)
Buffer system	Separate	Onboard click-in cartridge
UI	Ancillary computer	Touchscreen/Cloud/Ancillary computer
Footprint	Large	Small
AmplideX® Kit Recommended Run Settings		
Injection voltage	2.5 kV	6 kV
Injection time	20 sec	2 sec
Run voltage	19.5 kV	6 kV
Run time	2400 sec	3300 sec

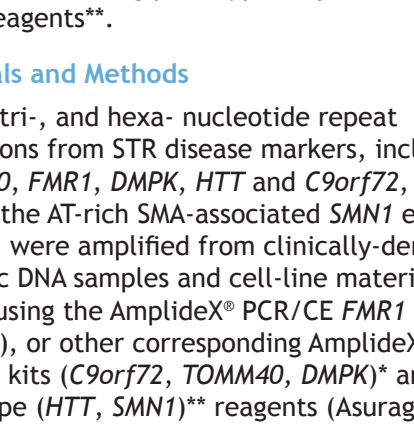

Figure 1. Schematic Representation of Single-tube, Long-range, 3-primer RP-PCR Assay Designs. Gene-specific (GS; blue) and repeat-primed (RP; red) primers create a multiplicity of FAM-labeled (star) PCR products from repetitive DNA (orange sawtooth). When separated by CE, these products manifest distinct full-length GS peaks and a complementary RP peak profile with single-repeat frequency and resolution.

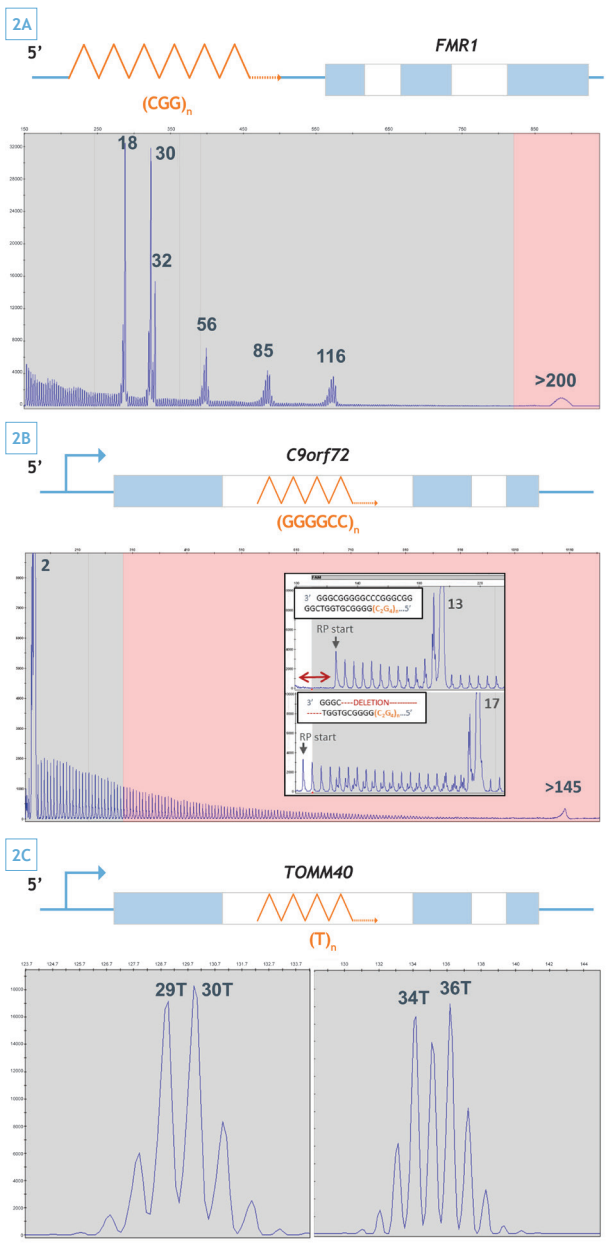

Figure 2. Representative SeqStudio* CE Profiles Generated using Commercially-available AmplideX® PCR/CE Kits. A) *FMR1* CGG repeat alleles ranging from 18 to >200 were amplified from the AmplideX® *FMR1* Control. **B)** A *C9orf72* G_4C_2 expansion in Coriell sample ND11081. The assay* also telegraphs 3' sequence variations (inset: Compare top sample ND06769 vs. lower ND12947). **C)** AmplideX® PCR/CE *TOMM40* reagents* show single bp size resolution of gene-specific PCR products (Coriell samples NG09714 and ASGN005). Run times could be reduced to 20 min for *TOMM40* polyT PCR products. Of note, different PCR/CE assays could be analyzed on the same CE plate using discrete or shared run conditions to harmonize and simplify batch run efficiency.

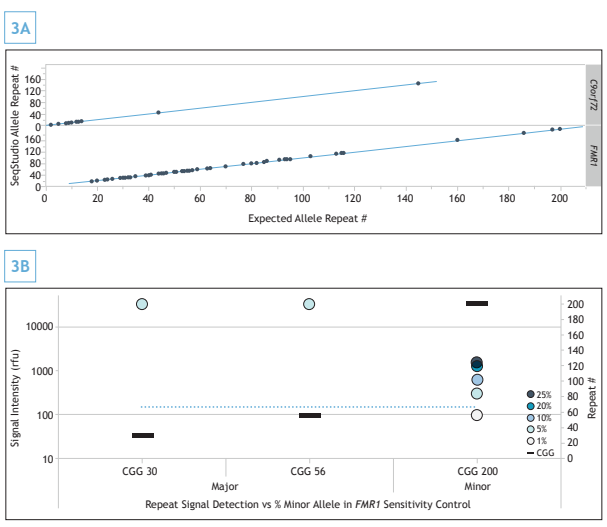

Figure 3. Samples Amplified using AmplideX® PCR/CE *FMR1* and *C9orf72 Reagents can be Sensitively and Accurately Resolved on the SeqStudio*, Consistent with Results on the 3500* CE.** PCR of 126 *C9orf72* and 448 *FMR1* alleles from clinically-derived gDNA samples were evaluated on both the SeqStudio* and the 3500* CE. **A)** Single-repeat concordance between SeqStudio* and 3500* platforms. **B)** Detection of a full mutation *FMR1* minor allele down to at least 5% mass fraction. **C)** All alleles in the AmplideX® *FMR1* Control were detected down to 1 ng of gDNA input.

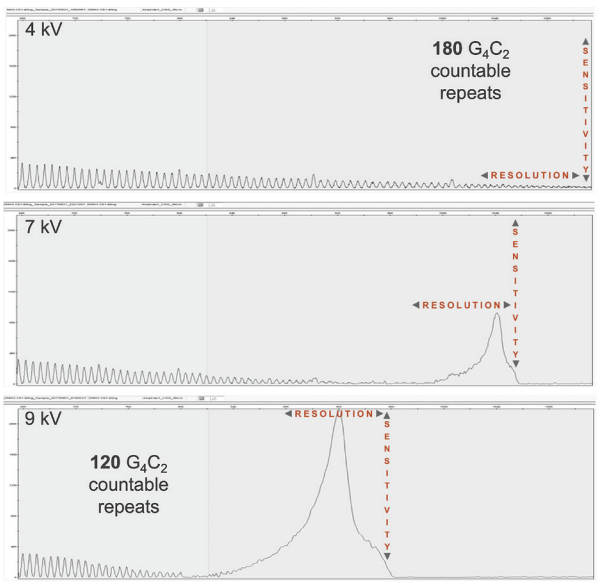

Figure 4. Tunable Resolution and Sensitivity Demonstrated with Expanded *C9orf72* Hexanucleotide Repeats. Run voltage on the SeqStudio* is a "tunable knob" for optimizing assay-specific variables such as resolution and peak detection sensitivity. Lower voltages extend the resolution of large fragments (top), whereas higher voltage reduces resolution and compounds the "peak pile-up" to enhance assay sensitivity (bottom). Optimized run conditions could extend the repeat resolution range by as much as 50%.

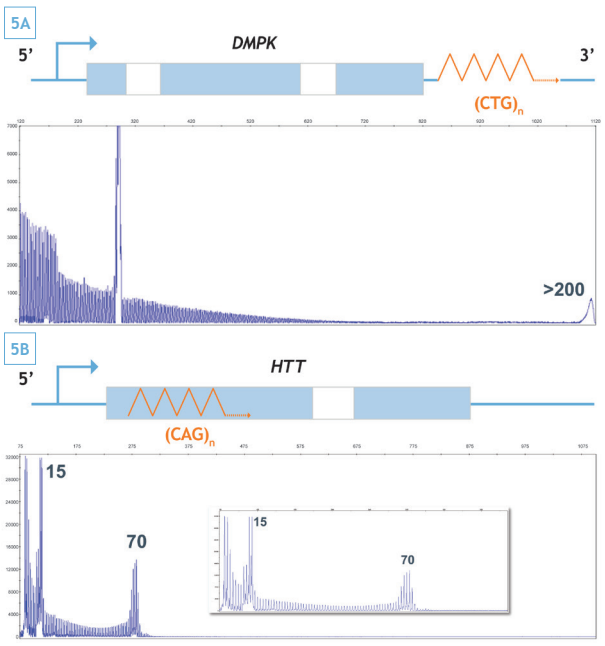

Figure 5. Representative SeqStudio* Electropherograms Generated using using AmplideX® PCR/CE DMPK* and prototype HTT Reagents. A)** Identification of a 25/~1100 CTG expansion in *DMPK*. Sample courtesy of Greenwood Genetics Center. **B)** A 15/70 CAG repeat expansion in *HTT* (Coriell sample NA13509) detected using a prototype 2-primer RP-PCR/CE assay.

Figure 6. Quantitative Analysis of *SMN1* Copy Number using Prototype AmplideX® PCR/CE *SMN1* Reagents on the SeqStudio* Platform.** Representative results from four gDNA samples reflecting 0-3 *SMN1* copies were accurately genotyped across a 5-fold input dilution series using SeqStudio* relative fluorescence levels.

Conclusions

- When coupled with AmplideX® PCR chemistries, the SeqStudio* platform provides versatile, sensitive, and accurate sizing of mono-, tri-, and hexa- nucleotide repeat expansions.
- The flexibility of both the PCR technology and the SeqStudio* CE to resolve multiple AT- and GC-rich STRs, as well as CNVs such as *SMN1*, suggests that a broad range of markers and gene targets can be routinely analyzed using these methods.
- These technologies can accelerate the development and adoption of STR-based assays for clinical research and future molecular diagnostics.

*For Research Use Only, Not for Use in Diagnostic Procedures
**This product is under development. Future availability and performance to be determined.

Disclosures: CR, KN, EL, EB, and GJL have stock/stock options in and/or are employees of Asuragen, Inc. JW, ES, and SH have stock/stock options in and/or are employees of Thermo Fisher Scientific, Inc.
Presented at ESHG 2018 - P14.039C

Asuragen®
www.asuragen.com