AUTOMATED FRAGMENT SIZE ANALYSIS OF AMPLIDEX® *FMR1* PCR PRODUCTS IMPROVES INTERPRETIVE ACCURACY AND REDUCES TURN-AROUND TIME

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SUMMARY

- Manual analysis of fragment-sizing data from AmplideX PCR/CE *FMR1* Reagents can be time-consuming and lead to both interpretation errors and inconsistencies in peak calling and genotype assignments.
- Here we present AmplideX PCR/CE *FMR1* Reporter, a stand-alone software tool developed to analyze capillary electrophoresis results and to perform highly accurate genotyping of the *FMR1* CGG repeat locus in a fraction of the time required for manual analysis.
- A signal processing algorithm was developed and trained on a diverse set of 167 clinical samples previously analyzed with AmplideX PCR/CE *FMR1* Reagents, and then tested on 1106 manually annotated residual clinical samples, including 172 normals 315 intermediates, 569 premutations, and 50 full mutations.
- The automated software provided reliable QC checks of electropherogram data, correctly genotyped all 1106 test samples, and consistently identified the presence of low-abundance alleles (<5%). Results were produced in less than 1% of the time required for manual analysis.

INTRODUCTION

In recent years, significant advancements in PCR technology have enabled the use of a PCR-only workflow for size analysis of the CGG-repeat region in the X-linked *FMR1* gene. This genetic locus is directly associated with the transmission and manifestation of fragile X syndrome and a number of related disorders. To date, interpretation of fragment sizes from *FMR1* PCR products can require significant hands-on analysis by trained operators, which is particularly laborious for large sample sets. To improve this workflow, an algorithm was developed to enable push-button analysis of capillary electrophoresis (CE) results from Asuragen's AmplideX PCR/CE *FMR1* Reagents workflow.

METHODS

The *FMR1* CGG repeat region was amplified for 1273 residual clinical samples using AmplideX PCR/CE *FMR1* Reagents. These PCR fragments were then run on an ABI 3500 Genetic Analyzer to generate CE profiles for analysis. Manual annotation was performed by trained operators for all residual clinical samples using GeneMapper® 4.1 software (ABI). An algorithm was developed using 167 residual clinical samples representing the full spectrum of *FMR1* genotypes to automatically produce genotype results. Testing was then performed on an independent cohort of 1106 clinical samples spanning full *FMR1* genotype range.

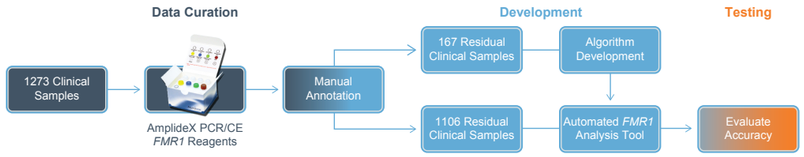

Figure 1. Schematic of experimental design.

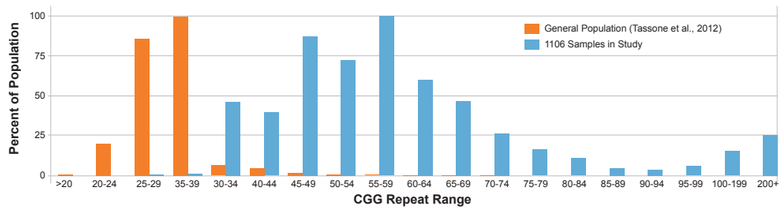

Figure 2. Distribution of *FMR1* genotypes in the general population (Tassone et al., 2012) compared to the the distribution of genotypes in the 1106-sample validation dataset.

Research Use Only – Not For Use In Diagnostic Procedures
Preliminary research data. The performance characteristics of this assay have not yet been established.
Presented at AMP 2015

RESULTS

All clinical samples passing QC criteria (n=1035) were correctly genotyped according to ACMG sizing guidelines (Monaghan et al., 2013). All 71 samples failing automated QC were manually confirmed to have poor/no amplification. A quantitative analysis on associated time-to-result showed a >100 fold decrease for samples analyzed using the algorithm. Finally, the AmplideX PCR/CE *FMR1* Reporter software platform was built to provide an intuitive and easy-to-use user interface to the algorithm, and to dramatically improve analysis time for lab technicians using AmplideX PCR reagents.

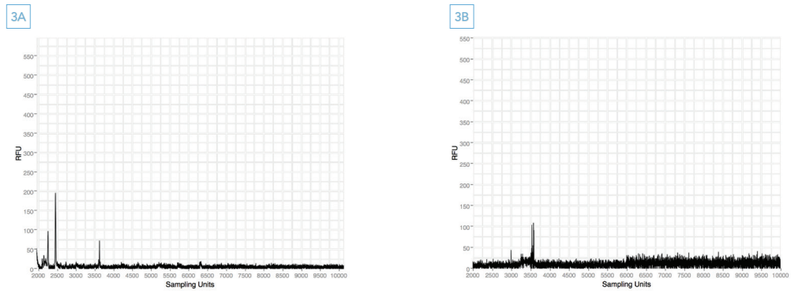

Figure 3. Examples of poor-quality sample data that is automatically flagged by the AmplideX PCR/CE *FMR1* Reporter software. A) Representative sample flagged as a QC failure due to no amplification. **B)** Representative sample flagged as QC failure due to undetectable ROX ladder.

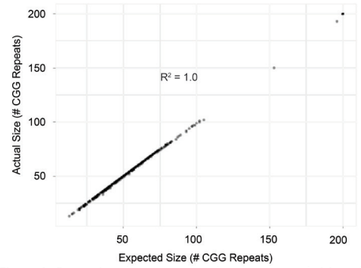

Figure 4. Comparison between manual and automated sizing of all major alleles reveals a quantitative correlation.

Automated Analysis	MANUAL ANALYSIS			
	Normal (0-44)	Intermediate (45-54)	Premutation (55-200)	Full Mutation (>200)
Normal (0-44)	162	0	0	0
Intermediate (45-54)	*6	295	*1	0
Premutation (55-200)	0	*9	527	0
Full Mutation (>200)	0	0	0	35

*Within ± 1 CGG of the manually-annotated size.

Table 1. Accuracy of automated genotyping compared to manual genotyping by expansion mutation category.

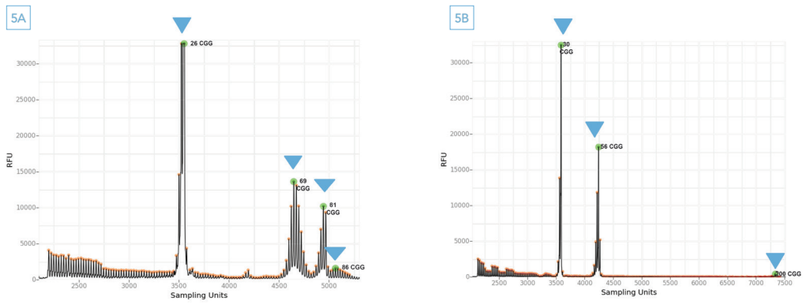

Figure 5. Accurate annotation of mosaic and low-abundance gene-specific products by the AmplideX PCR/CE *FMR1* Reporter software. Blue arrows indicate calls made by the algorithm. **A)** Detection of primary and mosaic gene-specific products. **B)** Detection of low-abundance expanded allele.

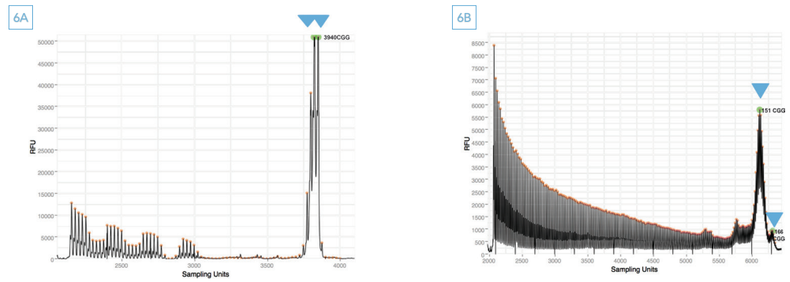

Figure 6. Examples highlighting annotation of similarly-sized gene-specific products and expanded minor alleles by the AmplideX PCR/CE *FMR1* Reporter. Blue arrows indicate calls made by the algorithm. **A)** Normal similarly-sized (n/n+1) sample. **B)** Premutation sample with minor allele.

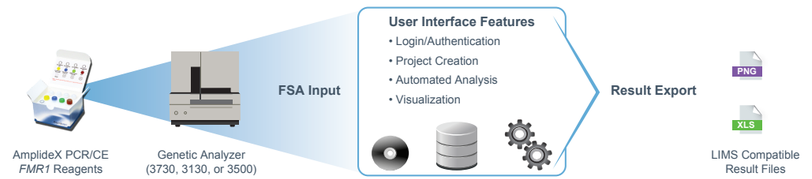

Figure 7. Diagram of AmplideX PCR/CE *FMR1* Reporter software components.

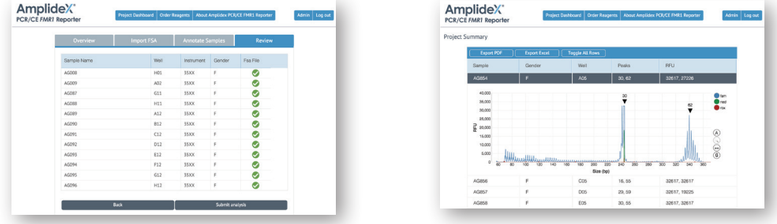

Figure 8. Screenshots of the application interface. A) Project submission. **B)** Results visualization.

CONCLUSIONS

- The AmplideX PCR/CE *FMR1* Reporter, a highly accurate, automated *FMR1* analysis engine and software interface improves the efficiency and consistency of capillary electrophoresis-based assays targeting the CGG-repeat region of the *FMR1* gene.
- The software was tested on >1000 residual clinical samples processed using Asuragen's AmplideX PCR/CE *FMR1* Reagents and demonstrated 100% agreement with manual genotyping (within suggested ACMG sizing guidelines).
- The software also demonstrated high sensitivity to detect similarly-sized, mosaic, and low-abundance gene-specific products, while accurately flagging sample failures. Failure modes included identification of poor amplification and various ROX ladder issues seen in practice.
- This software can accelerate analysis times for *FMR1* assay workflows by >1 order of magnitude, provide automated QC checks of electropherogram data, and has the potential to improve inter-operator consistency in resolving CE profile ambiguities.

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