HIGH-RESOLUTION AMPLIFICATION AND GENOTYPING TECHNOLOGIES FOR PREIMPLANTATION GENETIC DIAGNOSIS OF FRAGILE X SYNDROME FROM SINGLE CELLS

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SUMMARY

- We report findings from a study assessing the performance and reproducibility of a new high-resolution method for fragile X preimplantation genetic diagnosis (PGD).
- The study utilized a set of well-characterized lymphoblastoid fragile X cell lines and novel methods that were tested by 2 different laboratories.
- A combination of whole genome amplification (WGA) and the AmplideX® PCR/CE *FMR1* Kit (Asuragen) can detect repeat expansions from 1-5 fragile X cells.

INTRODUCTION

PGD methods are increasingly used to detect chromosomal abnormalities and genetic disorders relevant to in vitro fertilization (IVF). One such disorder is fragile X syndrome (FXS), the most common form of inherited intellectual disability. An estimated 1.5 million women in the US are fragile X carriers yet most are unaware of their carrier status. Currently, the identification of FXS by PGD is mainly limited to low-resolution linkage analysis. The goal of this study was to assess the performance and reproducibility of a new high-resolution method for fragile X PGD. The study utilizes a set of well-characterized lymphoblastoid fragile X cell lines as a model for the low cell count biopsies used in PGD.

METHODS

Lymphoblastoid cells from 5 fragile X cell lines (one normal (NOR), two premutations (PM), and two full mutations (FM)), along with matching genomic DNA samples, were genotyped using whole genome amplification (WGA) and the AmplideX PCR/CE *FMR1* Kit. WGA typically generated 10-40 µg amplified gDNA. One, two or five intact cells were placed in PCR tubes under a dissecting microscope to monitor the release of the cell(s) into the tube; cell-line DNA was isolated using the DNeasy kit (Qiagen). Individually picked cell samples were tested in triplicate on three different days by two different laboratories and results were compared. Samples were also assessed using direct cell inputs into *FMR1* PCR without upstream WGA.

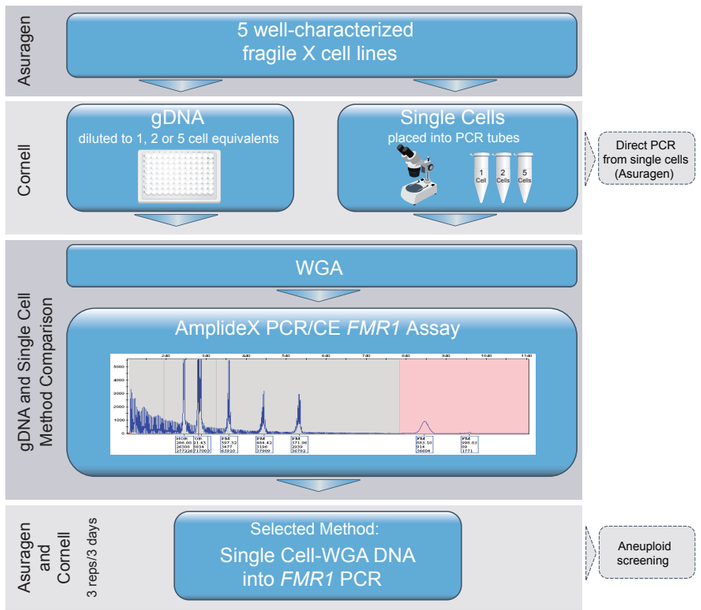

Figure 1. Overview of study design across 2 sites.

RESULTS

Samples (3 replicates per run)				% Detected from gDNA		% Detected from Cells					
					Cornell		Cornell			Asuragen		
Sample	Sex	Indication	CGG #	WGA Input (gDNA)	Run1	WGA Input (Cells)	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
RU009	F	NOR	30, 32	6 pg	0%†*	1 cell	100%	67%†	100%	100%	67%§	100%
				12 pg	0%†*	2 cells	100%	100%	67%†	100%	100%	100%
				30 pg	0%†	5 cells	100%	100%	100%	100%	100%	100%
RU011	F	PM	30, 56	6 pg	NT	1 cell	100%	100%	100%	67%§	67%†	67%§
				12 pg	NT	2 cells	100%	100%	100%	67%§	0%†	67%†
				30 pg	NT	5 cells	100%	67%†	100%	100%	100%	100%
RU007	F	PM	18, 116	6 pg	0%§	1 cell	100%	100%*	33%†	100%‡	33%‡†	33%†
				12 pg	67%†	2 cells	100%*	100%	100%	100%	100%	100%‡
				30 pg	100%	5 cells	100%	100%*	100%	100%	100%*	100%
RU010	F	FM	30, >200	6 pg	0%§	1 cell	67%†	67%§	33%†	33%†	100%‡	33%‡§
				12 pg	0%§	2 cells	100%	100%	100%	100%	67%†	33%‡†
				30 pg	100%	5 cells	100%	100%*	100%	100%	67%†	67%†
RU003	M	FM	>200	6 pg	0%§	1 cell	100%	67%§	100%	67%§	67%§	33%§
				12 pg	0%§	2 cells	100%	67%§	100%	67%§	67%§	67%§
				30 pg	0%§	5 cells	100%	67%§	100%	100%	100%	100%

NT, Not tested; *Unexpected peak detected; †Expanded allele not detected; ‡Normal allele dropped out, but expanded allele detected; §None of the expected alleles detected

Table 1. AmplideX *FMR1* PCR genotyping success rates across two sites for WGA DNA from individual fragile X cells tested in triplicate on 3 different days. Using an optimized WGA-based protocol, normal and expanded genotypes were detected from 1-5 cells in less than 48 hours. The genotyping assay was determined to be successful if the longest allele was detected. Results were much more consistent when intact cells rather than purified gDNA were used as the input into WGA.

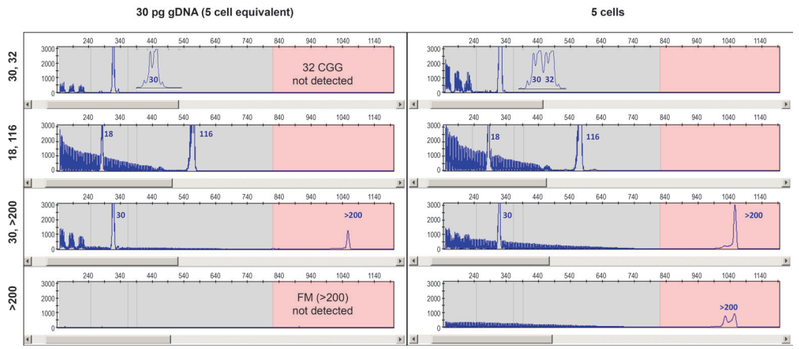

Figure 2. Comparison of intact cell inputs and cell-equivalent inputs of purified gDNA. WGA of intact cells demonstrated superior sensitivity, repeatability, and reproducibility after *FMR1* PCR compared to matched cell equivalents of purified gDNA.

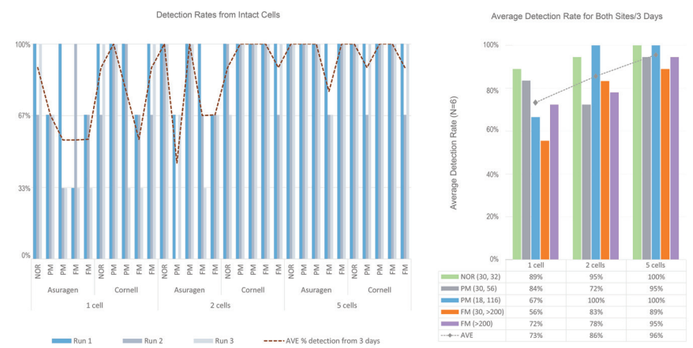

Figure 3. Summary of detection rates. FM alleles were accurately detected in 92% of replicate runs across the two sites when 5 cells were input into WGA. With 1 and 2 cell inputs into WGA, FM alleles were detected in at least 1/3 replicates, and in 5 cells at least 2/3 replicates were detected.

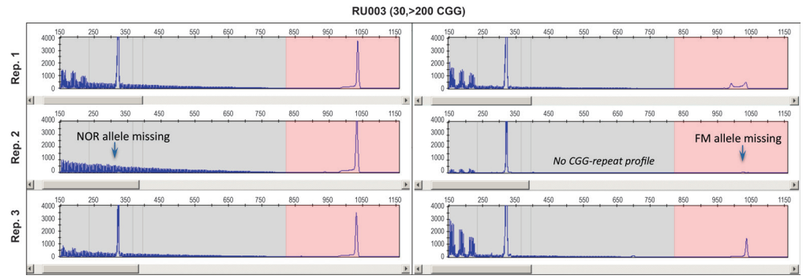

Figure 4. Example of sporadic random allele dropouts in WGA from intact cells. In a two-step amplification process, random allele dropouts occurs during the WGA step as confirmed by controls used in the PCR step and consistent with previous publications[1,2]. In addition, genotyping of a second WGA aliquot reproduced original dropout in PCR.

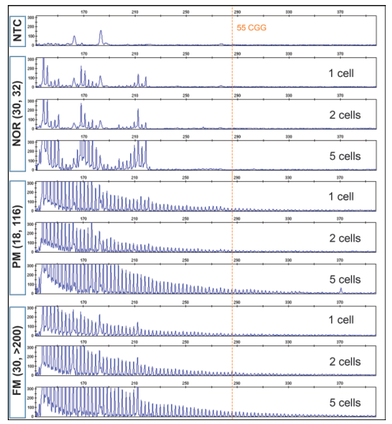

Figure 5. Feasibility of direct PCR (without WGA) to detect expanded *FMR1* alleles from single cells. Modified AmplideX PCR technology shows a repeat-primed profile extending to >55 CGGs for expanded samples with clear distinction from normal sample or reaction without template (NTC) and a turnaround time <6 hours. In total, 5 cell lines were tested and all expanded alleles were detected with no dropouts.

CONCLUSIONS

- A combination of WGA and the AmplideX PCR/CE *FMR1* Kit can genotype fragile X expansions from 1-5 fragile X cells.
- Across 2 laboratory sites, 92% of full-mutation cell lines with 5-cell inputs were accurately detected. This result augurs PGD applications for FXS using D5/6 trophectoderm clinical biopsies.
- A further benefit of the approach is that it generates microgram quantities of WGA DNA amenable for other IVF-related genetic tests.
- In preliminary studies, fragile X expansions were also detected using a modified AmplideX PCR technology from a single cell without WGA.

References

1. Chen L, Hadd A, Sah S, Filipovic-Sadic S, Krosting J, Sekinger E, Pan R, Hagerman PJ, Stenzel TT, Tassone F, Latham GJ. An information-rich CGG repeat primed PCR that detects the full range of fragile X expanded alleles and minimizes the need for Southern blot analysis. J Mol Diagn. 2010 Sep;12(5):589-600.
2. Filipovic-Sadic S, Sah S, Chen L, Krosting J, Sekinger E, Zhang W, Hagerman PJ, Stenzel TT, Hadd AG, Latham GJ, Tassone F. A novel *FMR1* PCR method for the routine detection of low abundance expanded alleles and full mutations in fragile X syndrome. Clin Chem 2010; 56: 399-408.

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