INTRODUCTION

Fragile X (FX) syndrome is the most common cause of inherited mental retardation resulting from a dynamic expansion of a trinucleotide (CGG) repeat in the fragile X mental retardation gene (FMR1). Testing for FX and FX-related symptoms constitutes a significant workload in many genetic laboratories and requires a rapid and inexpensive test to identify normal, grey and premutation alleles and distinguish them from full mutations that will need additional testing for methylation status.

The triplet primed PCR (TP-PCR) is a chimeric PCR that generates a stuttering peak pattern used to screen for expanded alleles in FX sample. The stuttering pattern represents PCR products differing in size by one triplet CGG repeat and can be used for counting the number of CGG repeats present in a sample.

RESULTS

Thirty fragile X samples genotyped previously (ten normal alleles, ten intermediate alleles, and ten premutation alleles) were analyzed in this study. The genotypes ranged from 20-123 CGG repeats. PCR was performed according to the manufacturer’s recommendations. Six of the 30 samples representing two normal alleles, two intermediate alleles, and two premutation alleles were amplified in triplicate and assayed in three separate runs for within run and between run reproducibility studies, respectively. Precision results were used to create bins for automatic allele sizing.

Macros for automatic allele binning were created using the mean of each CGG repeat and 2 standard deviations for setting the upper and lower boundary of each CGG bin in Gene Marker software version 2.6 (Soft Genetics, State College, PA), such that the inclusion of ‘stray alleles’ into the wrong CGG bin interval is minimized.

Fig 1: Electropherogram of FX samples with 29/31 CGG repeats. The first trinucleotide stuttering starts at the 3rd CGG repeat and continues up to the size of the CGG repeats which makes allele calls easy and accurate. The prominent peaks are called.

RESULTS

This FX screening assay was found to be an efficient and rapid test for screening for expanded alleles and sizing the CGG repeats for FX samples in the normal, grey and premutation ranges. Since many of the FX testing is to “rule out” FX, this assay is an economically and reliable method in reducing the number of samples needing methylation analysis, yet still providing sizing information to clinicians.

The advantage of using CGG allele binning is that the stuttering peaks can accurately assist in estimating the bins of all possible CGG alleles. With stuttering always starting on the 3rd CGG repeat, it is very accurate to size any FX sample from 3 to 123 CGG repeats (based on this study).

The FX CGG stuttering can be used as an allelic ladder to generate CGG bins in Gene Mapper or Gene Marker software for correct automated CGG repeat sizing in FX samples on capillary electrophoresis instruments.

Most FX workflow can be streamlined by use of this TRP-TPX-PCR, where expanded alleles and apparent homoygous alleles are easily distinguished by this simplified methodology. Southern blotting is expensive, labor intensive, requires high concentrations of DNA, and can delay turnaround time.

CONCLUSION

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REFERENCES


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Table 1: Comparison of 30 samples between a FX triple primed PCR and regular FX assay

Table 1: All 30 fragile samples were 100% concordant (within 1 or 2 CGG repeats) with previously obtained results from PCR designed for sizing by amplifying over the CGG repeat. Sizing was reproducible with no variation other within or between runs.