

Abstract 38

DEVELOPMENT OF A HIGH-THROUGHPUT ASSAY FOR DETECTING FRAGILE X EXPANDED ALLELES

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Population screening has been proposed to identify Fragile X pre-mutation carrier females and affected newborns. Effective screening requires a rapid and robust method suitable for high-throughput analysis. Testing from dried blood spots (DBS) offers a facile path for newborn screening. Here we describe a PCR-based method coupled with capillary electrophoresis to quickly identify samples with expanded FMR1 alleles. **Methods:** We tested 205 samples that were previously genotyped for FX at ARUP using a lab-developed PCR assay and Southern Blot analysis using the prototype assay. The prototype assay uses Celera's General Purpose Reagents (High GC PCR buffer, TR Enzyme Mix and ROX 1000 Size Standard) and a new Analyte Specific Reagent (FMR1-Primers-2). Fragment analysis was performed on an Applied Biosystems 3100 Genetic Analyzer. Additionally, DBS samples were extracted both manually, and with a Qiagen BioSprint96 instrument prior to being tested with the prototype assay. To assess sensitivity to full-mutation mosaic samples, a "mock" mosaic sample was generated by diluting a full mutation DNA sample with a normal DNA sample. A threshold of 55 repeats was set as the basis for determining an expanded allele. **Results:** All 205 samples showed 100% concordance with previous results. There were 132 samples with expanded alleles (59 pre-mutation, 71 full-mutation, 2 mosaics) and 73 normal samples. The DBS samples showed successful amplification with the prototype assay and 100% concordance with results from the lab-based genotyping test. The "mock" mosaic study showed that the prototype assay is capable of detecting an expansion when only 12.5% of the sample contains an expanded allele. **Conclusions:** We have developed a rapid and sensitive prototype assay suitable for the detection of expanded FX alleles from either whole blood or DBS extracted DNA. Coupled with a sample preparation instrument the prototype assay could be suitable for high-throughput applications such as population screening.