

## Abstract 104

### NOVEL PCR REAGENTS THAT REPRODUCIBLY AMPLIFY FMR1 EXPANDED ALLELES IN MALES AND FEMALES

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Current FMR1 PCR methods are limited to the detection of alleles of ~100 CGG repeats and are unable to reconcile female homozygous samples from those with one unamplifiable longer allele. We have recently evaluated a PCR methodology capable of distinguishing full mutation alleles and normal alleles in females. However, there is a compelling need to improve the assessment of FMR1 genotypes without reliance on the labor intensive and DNA quantity requirements of Southern Blot. Here, we describe the application of two novel PCR strategies (FMR1 gene-specific and CGG Repeat Primed PCR) for the detection of full mutations and resolution of female homozygous samples in whole blood and blood spot card samples. In addition, we describe the testing of novel PCR reagents that can accurately assess FMR1 methylation status. Cell line DNA samples from the Coriell Cell Repositories or clinical DNA specimens from the M.I.N.D. Institute at UC Davis were tested with gene-specific and CGG Repeat Primed PCR. Blood spot samples were also evaluated. The results of testing over 400 clinical and cell line samples with the novel FMR1 PCR reagents (Asuragen) were concordant with the results obtained by Southern Blot analysis. Full mutation alleles with up to 1300 CGG were detected. All female homozygous samples were correctly resolved. These PCR reagents were able to detect large full mutations even in blood spot samples. The combination of Asuragen PCR research reagents that reproducibly detect Fragile X full mutations with reagents that can determine the methylation status of the FMR1 gene suggests the possibility of a PCR-only workflow for FMR1 molecular diagnostic testing.