Toward elimination of Southern blot analysis for fragile X syndrome: A rapid and sensitive PCR-based assay for accurate sizing of FMR1 alleles, and the detection of full mutation CGG expansions. *C. Stolle, T. Tischler, A. Santani* Molecular Genetics Laboratory, Dept of Pathology & Lab Med, Children's Hosp Philadelphia, Philadelphia, PA.

In a pediatric setting, molecular diagnosis of fragile X syndrome is used to determine if a child has mental retardation or developmental delay due to an expanded, hypermethylated FMR1 allele. For this purpose, Southern blot analysis (SB) has been the only method capable of detecting both expanded alleles and methylation status. However, SB is labor intensive, time consuming, and unreliable for allele sizing. The exact size of alleles in the normal to premutation range is important for risk assessment and diagnosis of fragile X associated tremor and ataxia syndrome and premature ovarian failure. PCR methods may be used to size alleles, but some methods cannot detect expanded alleles and require SB for males with no amplified allele, females with a single allele, potential mosaics, and to determine the methylation status of large premutations. We describe validation testing of a commercially available PCR assay capable of detecting large alleles and accurately sizing alleles in the normal to premutation range. Validation samples consisting of normal, gray zone, premutation, and full mutation alleles from males, mosaics males, and females previously analyzed by SB, as well as DNA samples of known repeat size, were analyzed using the AmplideX™ Gene Specific PCR and/or CGG Repeat Primed PCR reagents (Asuragen) and resolved by capillary electrophoresis (ABI 3730). Allele sizing was performed using Gene Mapper. The observed allele size of DNAs with a known number of CGG repeats exactly matched expected values for alleles in the 20-80 repeat range. Heterozygous females with alleles differing by a single repeat were clearly resolved and accurately sized. One premutation allele exhibited a one repeat difference in size (91 vs 92), suggesting that this assay is capable of sizing alleles with an accuracy of +/- 1 repeat. Detection of full expansions was in 100% agreement with SB results in both male and female samples. As expected, the PCR method was superior to SB in sizing normal, gray zone, and premutation alleles. Differences in allele sizing resulted in reclassification of the allele designation in 6 out of 62 samples. Mosaic males, females with a full expansions, and homozygous females were also correctly identified. This assay enables detection of alleles in all size ranges and accurate allele sizing in the normal to premutation range. Reflex testing by SB may only be required to determine the methylation status of alleles in the high premutation range.