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**Fragile X Mutation Analysis**

**↑CPT**

PCR: 83891; 84311; 83898; 83909; 83912

Southern Blot: 83892 (x2); 83896; 83897; 83894; 83912

**↑Synonyms**

FMR-1; Fragile X Syndrome

**↑Test Includes**

Detection and sizing of CGG repeat expansions in the FMR-1 gene.

**↑Laboratory**

Molecular Pathology

**↑Availability**

Monday-Friday, 0700-1700

**↑Turnaround Time**

PCR: 3-14 days, Southern Blot: 14-21 days

**↑Specimen**

Whole blood or amniotic fluid

**↑Volume**

4 mL blood, 10 mL amniotic fluid

## **↑ Container**

Blood: lavender top (EDTA) tube; amniotic fluid: 10 mL container with no additives

## **↑ Storage Instructions**

Blood and amniotic fluid should never be frozen.

## **↑ Causes for Rejection**

Samples >48 hours old

## **↑ Reference Range**

<44 CGG repeats

## **↑ Use**

To detect CGG repeat expansions in the FMR-1 gene.

## **↑ Limitations**

PCR test can only detect CGG repeat lengths up to the mid-premutation range (about 115 repeats). The Southern Blot method detects both moderate and high premutations and full mutation CGG repeat expansions, but cannot accurately size them. Sizing is not required for full mutations, but it is useful for premutations: the latter is accomplished by a PCR assay.

## **↑ Methodology**

Polymerase Chain Reaction (PCR) amplification with a fluorescent labeled primer and detection of product(s) by electrophoresis on an ABI 3130xl genetic analyzer.

Southern Blot: Digestion with methylation sensitive and methylation insensitive enzymes, electrophoresis and detection with a chemiluminescent probe.

## **↑ Additional Information**

PCR: Detection and accurate sizing of FMR-1 premutations is useful for carrier screening and genetic counseling in individuals with a family history of mental retardation with features of fragile X syndrome. Detection of full FMR-1 repeat expansions for diagnostic purposes or high premutations requires Southern blot analysis, and this method will be employed automatically on any case in which a premutation or full mutation allele cannot be visualized by PCR.

Southern blot: This test is used primarily to diagnose or rule out fragile X syndrome in males or females with mental retardation, developmental delay, or other stigmata of fragile X syndrome. It is performed automatically on any case giving indeterminate results by the PCR method. Methylation analysis is useful to distinguish the two FMR-1 genes in females (since one of them lies on the inactive, methylated X-chromosome) and to determine the pathologic potential of a full mutation (since the fragile X phenotype is caused by both CGG repeat expansion and methylation of the cytosines in the repeat).

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