Fragile X: FMR1 Gene Sequencing

**Test Code:** SFMR1  
**Turnaround time:** 4 weeks  
**CPT Codes:** 81479 x1

### Condition Description

Expansion of a CGG triplet repeat leading to DNA methylation and silencing of the FMR1 gene is the most frequent cause of fragile X syndrome. However, other mutations within the FMR1 gene have also been identified that cause fragile X syndrome. These include deletions, point mutations that disrupt RNA splicing and a missense mutation. EGL Genetics offers full gene sequencing to detect mutations other than CGG expansion as a cause of fragile X syndrome.

Sequencing of the FMR1 gene will only be done if the patient first tests negative for expansion of the CGG tract and FMR1 DNA methylation. The FMR1 gene consists of 17 exons. These coding exons, as well as the immediate flanking regions, are PCR amplified and sequenced in both forward and reverse strands. In addition, the entire FMR1 promoter, including the four known transcription factor binding sites and the transcription initiation site, are assessed by DNA sequencing. This analysis will therefore detect coding sequence changes, splice donor and acceptor site mutations and changes in the promoter sequence. In addition, both small and large deletions will be detected in males. Small deletions will also be detected in females, although larger deletions of the entire gene potentially could escape detection.

It is important to note that testing for expansion of the CGG tract and FMR1 DNA methylation alone does not rule out a diagnosis of fragile X syndrome or involvement of FMR1 in the patient's phenotype. Specialized consultation is available with Dr. Stephen Warren, an authority on FMR1, on the interpretation of missense mutations.

For patients with mutations not identified by full gene sequencing, a separate deletion/duplication assay is available using a targeted CGH array KQ. Visit [www.ThinkGenetic.com](http://www.ThinkGenetic.com) for patient-friendly information on fragile X syndrome.

**Reference:**  
- GeneReviews Clinical Summary

### Genes

**FMR1**

### Indications

This test is indicated for:

- Confirmation of a clinical diagnosis of fragile X syndrome who have tested negative by CGG repeat analysis.  
- Carrier testing in adults with a family history of fragile X syndrome who have tested negative by CGG repeat analysis.

### Methodology

Sequencing of the FMR1 gene will only be done if the patient first tests negative for expansion of the CGG tract and FMR1 DNA methylation. The FMR1 gene consists of 17 exons. These coding exons, as well as the immediate flanking regions, are PCR amplified and sequenced in both forward and reverse strands. In addition, the entire FMR1 promoter, including the four known transcription factor binding sites and the transcription initiation site, are assessed by DNA sequencing.

### Detection

This test uses sequence analysis of the coding region of the FMR1 gene which is estimated to identify 90-95% of mutations. Mutations in the promoter region, some mutations in the introns, and other regulatory elements cannot be detected by this analysis. Large deletion and insertion mutations will not be detected by this assay. It is possible that some patients with a typical presentation may not carry a mutation detected by this analysis. This analysis may detect novel variants of unclear effect, which may require further studies.

### Specimen Requirements

**Submit only 1 of the following specimen types**

**Type:** Whole Blood (EDTA)

**Specimen Requirements:**  
- EDTA (Purple Top)  
- Infants and Young Children (2 years of age to 10 years old): 3-5 ml  
- Older Children & Adults: 5-10 ml  
- Autopsy: 2-3 ml unclotted cord or cardiac blood

**Specimen Collection and Shipping:**  
Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.
Type: Saliva

Specimen Requirements:
Oragene™ Saliva Collection Kit
Orangene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

Specimen Collection and Shipping:
Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

Type: DNA, Isolated

Specimen Requirements:
Microtainer
8µg
Isolation using the Perkin Elmer™Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

Specimen Collection and Shipping:
Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

Special Instructions
Submit copies of diagnostic biochemical test results with the sample. Sequence analysis is required before deletion/duplication analysis by targeted CGH array. If sequencing is performed outside EGL Genetics, please submit a copy of the sequencing report with the test requisition. Contact the laboratory if further information is needed.

Related Tests
- For Fragile X testing, CGG repeat analysis is the recommended first tier test. Sequencing and deletion/duplication analysis are also available and should follow CGG repeat analysis.