Fragile X: \textit{FMR1} Gene Sequencing

\textbf{Test Code:} SFMR1  
\textbf{Turnaround time:} 4 weeks  
\textbf{CPT Codes:} 81479 x1

\section*{Condition Description}

Expansion of a CGG triplet repeat leading to DNA methylation and silencing of the \textit{FMR1} gene is the most frequent cause of fragile X syndrome. However, other mutations within the \textit{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 \textit{FMR1} gene will only be done if the patient first tests negative for expansion of the CGG tract and \textit{FMR1} DNA methylation. The \textit{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 \textit{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, splicing 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 \textit{FMR1} DNA methylation alone does not rule out a diagnosis of fragile X syndrome or involvement of \textit{FMR1} in the patient's phenotype. Specialized consultation is available with Dr. Stephen Warren, an authority on \textit{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 \url{www.ThinkGenetic.com} for patient-friendly information on fragile X syndrome.

Reference:  
- GeneReviews Clinical Summary

\section*{Genes}

\textbf{FMR1}

\section*{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.

\section*{Methodology}

Sequencing of the \textit{FMR1} gene will only be done if the patient first tests negative for expansion of the CGG tract and \textit{FMR1} DNA methylation. The \textit{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 \textit{FMR1} promoter, including the four known transcription factor binding sites and the transcription initiation site, are assessed by DNA sequencing.

\section*{Detection}

This test uses sequence analysis of the coding region of the \textit{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.

\section*{Specimen Requirements}

Submit only 1 of the following specimen types  
\begin{itemize}  
\item Preferred specimen type: Whole Blood  
\end{itemize}

\subsection*{Type: Whole Blood}

Specimen Requirements:  

In EDTA (purple top) or ACD (yellow top) tube:  
- Infants (2 years): 3-5 ml  
- Older Children & Adults: 5-10 ml
Specimen Collection and Shipping: Refrigerate until time of shipment. Ship sample within 5 days of collection at room temperature with overnight delivery.

**Type: Saliva**

Specimen Requirements:

Oragene™ Saliva Collection kit (available through EGL) used according to manufacturer instructions.

Specimen Collection and Shipping: Store sample at room temperature. Ship sample within 5 days of collection 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.