Testing AAV Vector Based Gene Therapy for Fragile X Syndrome *In Vitro*

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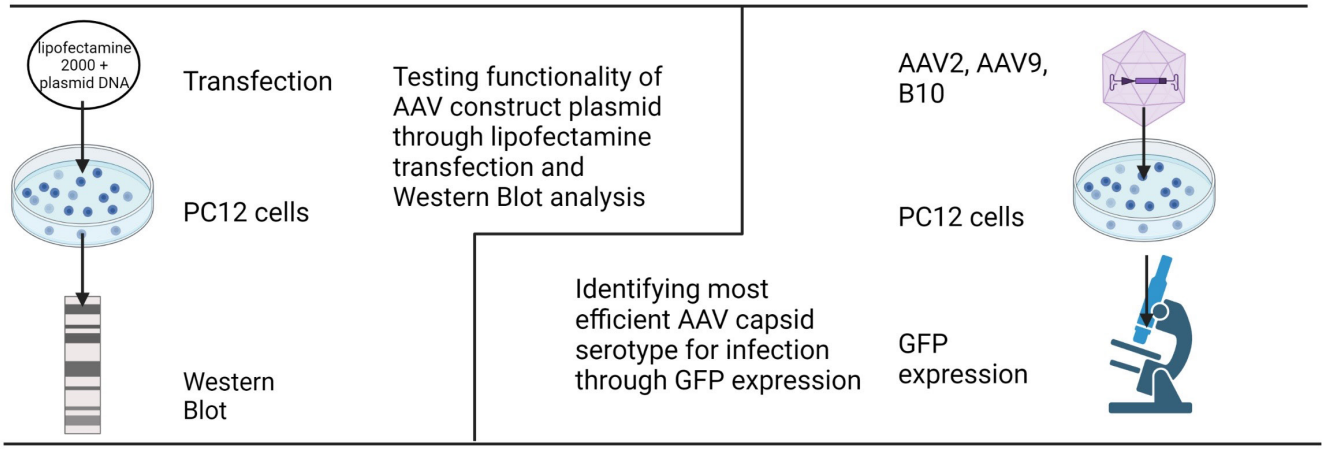
Fragile X syndrome (FXS) is an X linked dominant condition where the FMR1 gene that encodes fragile X mental retardation protein (FMRP) is silenced. The mutated FMR1 gene has an increased number of CGG repeats, leading to methylation and silencing of its promoter. Due to its monogenic nature, FXS is an excellent target for treatment using gene therapy. This study aims to evaluate the effectiveness of using Adeno Associated Viruses (AAV) as a delivery mechanism for a functional copy of the FMR1 gene in in vitro models. First, the recombinant AAV vector construct's functionality in expressing FMRP was determined through two different methods. PC12 cells were transfected with the construct plasmid DNA using lipofectamine 2000 and a Western Blot was performed to detect FMRP expression. The second method was to generate recombinant AAV virus to infect PC12 cells. They were produced through transfecting 293T cells with a helper, rep, and cap plasmid. To identify the most efficient capsid serotype for direct infection, PC12 cells were infected with AAV2, B10, and AAV9. AAV2 was determined to be most efficient. When testing the capsid serotypes on undifferentiated IPSCs, cell death occurred. Literature suggests that differentiated IPSCs tolerate infection better. Future testing will test infecting differentiated IPSCs with AAV to examine infection efficiency and FMRP expression. Supported by NIH R25ES020721.



Goal: Use AAV to deliver a functional copy of the FMR1 gene to patient derived IPSCs with a mutated FMR1 gene