Development of Plasmid Vectors of Neurodevelopmental Proteins

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Abstract

Throughout this semester, work has been done to generate copies of the human genes for *protocadherin-19* (PCDH-19), *neural cadherin* (NCAD), *receptor-like tyrosine kinase* (RYK), and a recombinant protein of *Green Fluorescent Protein* (GFP) with a Protocadherin-19 signal peptide that would create a form of GFP secreted from the cell. These cloned vectors would then be able to be used to study whether disruptions of interactions between these proteins lead to a female-specific form of epilepsy. These plasmids containing cloned human genes will allow for neurodevelopmental protein interactions to be tested in a mammalian cell culture system in future experiments.

Methods

Samples were prepared using the Platinum Taq DNA Polymerase High Fidelity system from Thermo Fisher. Two agarose gels were run to isolate the target DNA sequence via weight. A 0.8% for the heavier PCDH-19 and NCAD and a 1.5% for the signal peptide of PCDH-19 and GFP which were then run through PCR again together with primers in order to produce a recombinant gene. Each gene (PCDH-19, NCAD, RYK and the recombinant) was then put through a restriction digest using Nhe 1 for all and not 1 for the recombinant gene and a CMV strep-N1 vector, all other sequences were cut with BamH1. Target sequences were again isolated and purified using gel electrophoresis and Gel/PCR Cleanup kit. Ligation was then performed using T4 DNA ligase to add PCHD-19 and the recombinant gene into a CMV GFP-N1 plasmid and the NCAD and RYK genes into a CMV Strep-N1 plasmid vector. Successful ligations were found via gel electrophoresis and used to transform DH5***α*** Competent cells. The plasmid contained a kanamycin resistance gene which was used to identify successfully transformed cells. The plasmids were then isolated using the NucleoSpin Plasmid Kit.

Preliminary Testing

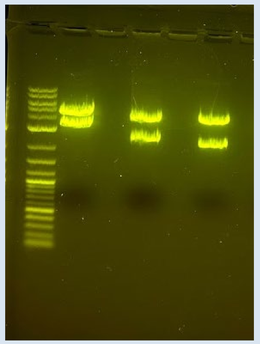

Figure 1. Agarose gel of the plasmid vectors demonstrating a successful ligation of PCHD-19 (lane 2), NCAD (lane 4), and RYK (lane 6). DNA ladder in lane 1 (1 kb plus ladder)

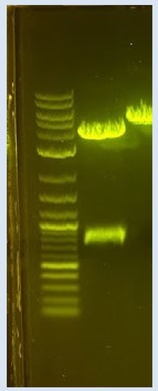

Figure 2. Agarose gel of the plasmid vectors demonstrating a successful ligation of the recombinant protein (PCDH-19 signal peptide ~70 bp and GFP ~700bp) in lane 2. DNA ladder in lane 2 (1 kb plus ladder).

Classical Sequencing Results

- Successful ligation of PCDH-19 with a 100% match to the database
- Successful ligation of NCAD with a 98.5% match to the data base (5 nucleotide mismatches)
- Successful recombination of the signal peptide of PCDH-19 with GFP as well as successful ligation of the new sequence.
- The RYK sequencing returned an unsuccessful experiment result and will need to be repeated.

Confirmation via Sequencing

Once fully generated and isolated via miniprep, plasmids were then prepared for classical sequencing. Sequencing was performed at Ohio State university and data processing was performed using BioEdit software and NCBI BLAST to evaluate what DNA sequences were present in the samples.

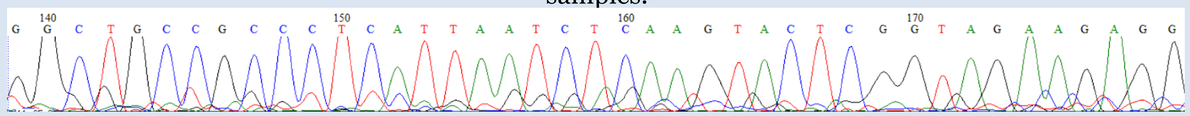

Figure 3. Example of DNA sequence in BioEdit

Future Directions

These cloned genes contained in plasmid vectors will be used in future research to investigate the interactions between the neurodevelopmental proteins. They can be used to transfect mammalian cells for experimentation, or they can be used to transform bacteria in order to produce many copies of the proteins. This project's goal was achieved in generating a stock of human gene-containing plasmids for use in future experimentation.

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