CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
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Project Number
J0509

Project Title
Developing a Tool for Studying Alzheimer's: A Bacterial Expression Vector for the M3-M4 Fragment of the nAChR alpha-7

Abstract

Objectives/Goals
The purpose of the Bacterial Expression Vector containing the M3-M4 fragment of the Nicotinic Acetylcholine Receptor (nAChR) Alpha-7 is to study Alzheimer's disease. I hypothesize that Gprin-1 (G-protein Regulator of Induced Neurite Outgrowth 1), as a key component of neural growth cones (developing neurons throughout the brain), combined with this expression vector, can potentially allow for the faster development and re-growth of neurons possessing the alpha-7 receptor.

Methods/Materials
PCR Template: Plasmid including Human nAChR (pcDNA3.1-CHRNA7 Addgene), Primers: Home designed, PCR Kit: Taq Kit (NEB), DNA electrophoresis: Agarose gel, Ligation: plasmid pGEX-KG, Ligation Kit, Competent Bacteria/Media, Selection Plates, Growth and Extraction: Miniprep Kit

Using miniprep, plasmids were extracted from 1.2mL bacterial culture. The M3-M4 part of the nAChR alpha-7 plasmid was replicated using PCR and primers F3-F4. Thereafter, the empty expression vector was cut at a specific part to allow for the insertion of a specific piece of the nAChR alpha-7, during digestion. In ligation, the nAChR alpha-7 PCR product was placed into the empty plasmid. The ligation product was used to enable the E.coli to absorb the expression vector. Bacteria were tested in selection plates to ensure these have plasmid inside of them. Working plasmid was taken out of bacteria through miniprep. The existence of the M3-M4 loop in the expression vector was verified using PCR and PCR products were visualized in gel.

Results
1. Verified size and confirmed that bacterial cells contained expression vectors
2. Verified correct ligated fragment in the bacterial expression vector
3. Final Plasmids: Average purity for HB-101 = 1.65; Average purity for BL-21 = 1.62; Average ng/ul of HB-101 = 12.33, BL-21 = 21.26 (HB-101 and BL-21 are E. Coli strains)

Conclusions/Discussion
The nAChR alpha-7 M3-M4 Loop Bacterial Expression Vectors (pASNGST and pASCGST) met production goals of quantity and quality. The plasmids were visualized in agarose gel and the nanodrop machine provided quantity in ng/ul. The nanodrop machine also provided the A260/A280 ratio that confirmed high purity of the vectors with values close to 1.8. These Expression Vectors are available to produce and purify the protein segment and to test intracellular interactions of the receptor nAChR alpha-7, including the potential interaction between nAChR alpha-7 and Gprin-1.

Summary Statement
I developed a bacterial expression vector for the M3-M4 fragment of the nAChR alpha-7, to study Alzheimer's disease.

Help Received
I designed and built the expression vector by myself. I got help in understanding specifically why I was performing the protocol (why it would work) and how the expression vector would function from Dr. Sonia Cuellar, my mentor. I used Schmahl Science Workshop's labs to conduct my experiment.