Cloning of Ubiqilin-1 Gene

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Abstract

Background:

Ubiqilin-1 is a protein in humans and functions as a ubiquitin receptor in eukaryotes. Ubiquilin-1 is a protein that brings proteins to the proteasome for degradation. Mutations in ubiquilin-encoding genes have been linked to the origin of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. High levels of ubiquilin-1 in the brain decreased formation of the Amyloid precursor protein molecule, that plays a role in triggering Alzheimer's disease. Lower levels of ubiquilin-1 in the brain will increased formation of Amyloid precursor protein. Ubiquilin-1 interacts with various transmembrane proteins, but we haven't yet characterized the specific interactions

Objective:

The objective is to ready the Ubiquilin-1 DNA fragments for gene expression and protein purification. Ubiqilin-1 gene is encoded in the vector PCS2 (Figure 1.) To express ubiqilin-1 domains as a protein, I need to isolate the DNA sequences that encodes my target domains. The first insert will encode domains STI-1 to STI-2, the second UBA to STI-2, the third UBA to STI-4 and the full construct UBA to UBL. To do this I will be using the methods of molecular cloning. Molecular cloning are methods that can be used to collect recombinant DNA molecules and put their replication in a new vector. Fragments of Ubiqlin-1 will be taken from the vector PCS2, then treated with enzymes in the test tube to generate smaller DNA fragments. These fragments are then combined with PET28a vector DNA to generate recombinant DNA molecules.

Methods:

PCR/ Polymerase Chain Reaction is a method of amplifying DNA. Which means millions of copies being made from a single DNA Template (Figure 2). PCR has three stages denaturing 94 C annealing 60 extension 72.

Gel electrophoresis will be used to separate inserts based on its size (Figure 3).

Gel Purification is used to isolate the inserts from the PCS2 vector and purified to have just the inserts.

Enzyme Restriction Digest is a process to cut the insert of interest and the PET28a vector. I will use enzymes XhoI and EcoRI to cut the ends of the inserts and PET28a vector. (Figure 4)

Ligation is a process by which the enzyme DNA Ligase joins together the sugar/phosphate backbones of two strands of DNA. DNA ligase is an enzyme which can connect two strands of DNA together by forming a bond between the phosphate group of one strand and the deoxyribose group on another.(Figure 4.)

Bacterial Transformation- Transformation is the process of introducing the PET28a with inserts into bacteria on an agar kanamycin plate. After being ligated the inserts were placed through transformation. . PET28a has a kanamycin resistant gene. Once PET28a has been introduced into the bacteria, any plasmid with the PET28a vector will survive in the bacteria. The plasmids that survive will cure large cultures of colonies. The colonies will have the PET28a vector with the inserts, the colonies will then be picked and placed into LB KAN. This is an antibiotic that will allow the bacteria to grow (Figure 5).

Results

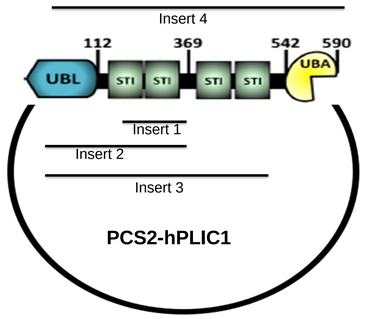


Figure 1. Ubiqilin-1 is encoded in the PCS2 vector. h stands for human. PLIC1 is another name for ubiqilin-1. The PCS2 vector encodes the whole ubiqlin-1 contains the entire gene of ubiqilin-1. I will be expressing different domains. To do that I will have to clone and isolate the DNA sequences that encode the domains of interest. The first insert encode domains STI-1 to STI-2, the second encodes domains UBA to STI-2, the third insert encodes domains UBA to STI-4 and the fourth insert encodes the full construct UBA to UBL.

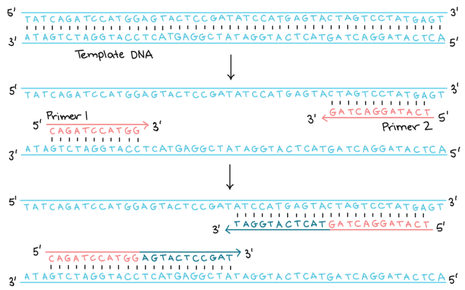


Figure 2

Denaturing 94 C-The high temperature causes the hydrogen bonds between the bases in two strands of the PSC2 template DNA to break and causes the two strands to separate. These two single strands of DNA, will become templates to produce the new strands of DNA.

Annealing 60° C -In Annealing temp will be lowered to 60 degrees. when the temperature is lowered it enables the DNA primers to bind to the single stranded PCS2 template DNA. The primers run in opposite directions on from the 5' end and from the 3' end. The primers are used because vent polymerase cannot bind to a single strand DNA.

Extension 72° C - The Vent polymerase attaches to the end of the primers and extends. It adds bases to the single strand one-by-one in the 5' to 3' direction.

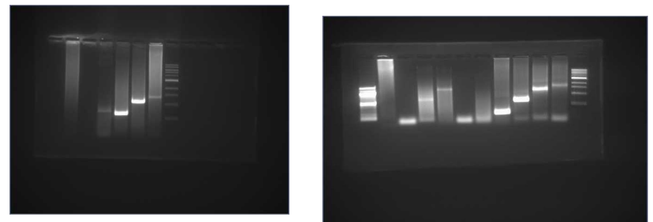


Figure 3a.

Figure 3b.

Sample size with primers

Insert 1: 252 bp
Insert 2: 774 bp
Insert 3: 1430 bp
Insert 4: 1770 bp

Figure 3a.

This PCR was done with a different polymerases. Instead of Vent polymerase I used Taq hot start. This polymerase works the same as vent, the difference is vent has higher fidelity than Taq. This means Taq is more prone to make mistakes in coping the single stranded DNA in the extension phase of the PCR. If vent makes a mistake it can go back and correct its mistake. I used Taq to experiment with primers. I wanted to make sure the primers and my template was working correctly. Lane 1 is empty, Lane 2 is negative, Lane 3 is empty, lane 4 is insert 1, lane 5 is insert 2, lane 3 is insert 3, lane 6 is insert 4, lane 7 is 1kb ladder.

Figure 3b.

This PCR was done with vent polymerase and was able to move forward with the project. The last five lanes are the inserts that are at the correct height. In the far left lane is the 100bp ladder the far right is the 1kb lane. The 100bp and 1kb measure the lengths of inserts. Lane 7 is a negative, to make sure there is not any contamination in any of the inserts. Lane 8 is insert 1 and is at 252bp. Lane 9 is at 774bp. Lane 10 is at 1430. Lane 11 is at 1770bp. Since these inserts are at the correct height, they were cut using UV box and moved on to purification. They were labeled 1, 2, 3,and 4. these four were moved on to purification and ligation phase.

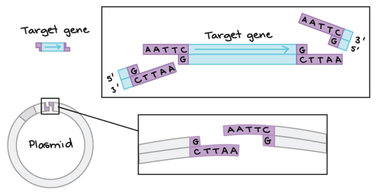


Figure 4.

The inserts and the PET28a vector will be cut using enzymes Ecorl and Xhorl. When EcoRI and XhoI recognizes the complimentary site, it cuts it into a certain pattern that produces ends with single-stranded DNA. When it cuts into the insert it is cuts into "underhangs". When it cuts into the vector it is cut into "overhangs". I cut my inserts labeled 1, 2, 3, and 4.

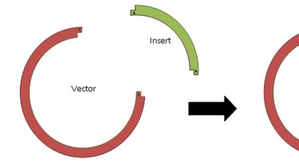
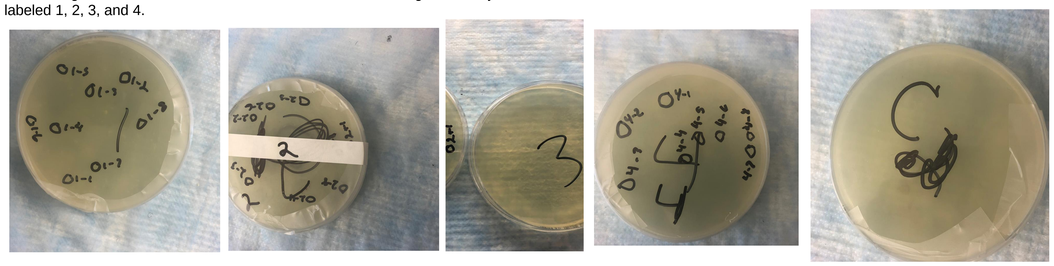


Figure 5.

In ligation, this is were the inserts and the PET28a vector are brought together. The T4 ligase will bind the vector and the inserts together. Once the inserts labeled 1, 2, 3 and 4 where cut I ligated them with PET28a. These inserts were then transferred to bacteria.



These are the colonies created during bacterial transformation. Plate labeled C is the controlled. This is to make sure the vector does not ligate back on itself. Plate 1 contains insert 1, plate 2 contain insert 2 , plate 3 contains insert 3, and plate 4 contains insert 4. Plates 1,2, and 4 created colonies. Eight colonies from each plate were picked. They were then placed in a rocker at 37 degrees over night, so the bacteria may grow.

Conclusions

During this project DNA fragments of interest in the ubiqilin-1 gene were amplified and then purified by gel electrophoresis. Once purified the inserts and PET28a is cut with the enzymes XhoI and EcoRI. They were then ligated using T4 ligase to the expression vector PET28a. Once ligated the plasmid was transformed to Ecoli bacteria. The Ecoli bacteria is placed on an agar plate that contained kanamycin. The bacteria is then set in a 37 C incubator to grow over night. The bacteria grow large colonies that has the PET28a vector. PET28a has a kanamycin resistant gene, the colonies with the PET28a vector was able to survive. Eight colonies from each plate were picked. The colonies were placed in 2ml of luria broth with kanamycin over night and allow to grow over night. The next stage is to mini prep the plasmids. This is the purification of the DNA with the inserts from the bacteria. The DNA will then be sent for sequencing to make sure the sequence is correct. I want to continue on after sequencing for protein expression. the proteins will then be used to study how ubiqilin-1 interacts with other protein.

Acknowledgements

Thank you to the NCI R25 grant support University of Louisville Cancer Education Program NIH/NCI (R25- CA134283) , Cory Kucera and Lavona Casson.