CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

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Project Number

S0510

Project Title

Recoding M13 for Improved Phage Display

Abstract

Objectives/Goals

There are many technologies to create proteins that bind well to specific antigens (chemicals or proteins), one of which is phage display. Phage display is a technology using the non-lysogenic helical M13 bacteriophage. Proteins or amino acid sequences of interest are fused to pIII, the outer coat protein, and washed against a specific antigen. The ones that bind are then resuspended and used to infect new bacteria. Then you repeat. Phage display is sometimes uses unnatural amino acids that are recoded for the amber codon (TAG). This is fairly efficient, albeit not perfect because translation is still occasionally terminated. However, George Church#s lab has rec ently used a new technology, MAGE (Multiplex genome engineering) to remove all TAG codons from an E coli genome as proof of concept and plans to do this with 13 other codons. The aim of this study is to develop phage display vectors that can be used in the #TAG-less# E coli with increased efficiency as well as create phage display vectors with the 13 forbidden codons removed, effectively recoding and refactoring an entire bacteriophage.

Methods/Materials

Growth media Antibiotics (Kanamycin, Tetracycline, and Ampicillin) PCR machine Gel box Ethidium Bromide Agarose Miniprep supplies DNA purification supplies Restriction enzymes (Namely XhoI, EcoRI, SpeI and BamHI) PCR supplies (Q5 polymerase ect) Pipettes Pipette tips Primers DNA M13KO7 Helper Phage E coli strains (SS320, TOP10, and TAG-less E coli) Centrifuge tubes Other normal lab equipment

Results

The M13 origin in M13KO7 cannot be PCRed

67 additional amino acids onto the pIV's C terminus only has minor effects on phage fitness

The 2 M13 origin fragments can delete themselves

Conclusions/Discussion

This project is continuing: However I have discovered that the M13 origin in M13KO7 (a helper phage) cannot be PCRed completely, through the use of several gel and sequencing runs.

This is likely because the kanamycin resistance and p15A origin are wedged inside of the M13 origin, and because of the phages ssDNA replication intermediate, this region could be getting deleted.

Summary Statement

Recoding M13 to improve unnatural amino acid incorporation for a better phage display

Help Received

Prof. Chang Liu allowed me to do this project in his lab