CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
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Project Number
S0401

Project Title
Cloning a Human Gene in Genetically Recombinant Bacterial Colonies

Abstract

Objectives/Goals
How effective is cloning a human gene in a bacterial strain by transformation as genetic recombination in various bacterial strains?

Methods/Materials
1. After sterilizing all materials with the alcohol burner, use the micropipette to transfer 250 µL of calcium chloride into a test tube.
2. Mix the desired plasmid, restriction enzyme, and the human insulin gene and place in an ice bath for 15 minutes
3. Transfer a large (3 mm) colony of the desired bacteria using an inoculating loop into the calcium chloride.
4. Use the micropipette to place 10 µL of the desired plasmid solution in the test tube. Keep the tube on ice for 15 minutes.
5. Heat-shock the cells in the tube by holding the tube in the heated water bath for 90 seconds. It is essential that this is a sharp and distinct shock.
6. Immediately return the cells to the ice for two minutes.
7. Use the micropipette to add 250 µL of pure Luria Broth agar to the tube.
8. Place 100 µL of the solution on the desired agar plate (depending on plasmid solution used). All plates should contain an X-gal solution as well as the antibiotics.
9. Allow plates to set for at least 24 hours. Incubate at 37⁰C.

Results
The number of white colonies (with a disrupted lacZ gene) was more than that of the blue colonies. The human gene was successfully uptaken. The transformation efficiencies for each colony of bacteria varied if it was transformed with more than one plasmid. Some bacterial strains such as Bacillus megaterium did not have good transformation efficiencies no matter the plasmid. On the other hand, some had excellent values.

Conclusions/Discussion
Depending on the strain of bacteria and its ecological niche, genetics, and morphology, transformation efficiency varies from bacteria. Not very many bacterial strains are naturally competent to uptake naked DNA in the form of a plasmid. Those that do have need for the process in their niche, the genes to produce DNA-uptake proteins, and morphological traits such as a pilus.

Summary Statement
How transformation and genetic recombination is used to clone a human insulin gene in various bacterial colonies.

Help Received
Parents paid for materials and helped to set up the board