Gene therapy: lab report

Health & Medicine

The LIEU gene Is a linear fragment that does not notation an Autonomous Replication Sequence, so it could not replicate on its own and needed to be integrated by homologous recombination. The TRIP gene was a circular plasmid that contained an EARS, which allowed for it to act as an extra chromosome In the gene. The objective was to insert a " wild gene" and replace the defective genes and then grow them on a medium that does not contain TRIP or LIEU to prove that the genes had been cured.

To help determine if recombination took place in the LIEU gene, and to compliment negative data from the 431 LIEU drop out medium, the " cured" LIEU gene was compared to the " diseased" ELISE gene. The expectation was that the " cured" LIEU gene would be a different size from that of the " diseased," which would be proven through a PC run of the two DNA strands after they were replicated under the same in vitro conditions. The purpose of the PC was to show what kind of mutation occurred in the mutant to cause It to lose Its LIEU function.

Methods Yeast Transformation Procedure Both hands and bench tops were sterilized by 10% ethyl alcohol and were continually wiped down at various times throughout the lab. Gloves were also worn for the duration of the lab to help prevent contamination. The first step was to obtain doth strains of yeast, AY 235 and AY 431, with the fat end of a sterile tooth pick from an augur plate and place them into two separate Offender tubes.

The Offender tubes were filled with poll of solution 1 (50 ml sterile water) before the yeast was added to them. The tubes were then spun in a centrifuge for four seconds to separate the excess water from the pellet that

formed from the yeast. The supernatant were discarded and the pellets were suspended in poll of solution 2 (0. MM Lilac; 0. 01 M Tries, 8. 0; 0. MOM DEED). The solution was once again spun for four seconds in the centrifuge and the supernatant were discarded. The pellets were re- suspended in 1 Pool of solution 2.