Gene expression is very strictly regulated under normal circumstances. However, in some pathological cases such as tumors, the expression of some genes gets out of hand, especially oncogenic genes and tumor suppressor genes. Translocation is one of many processes that can lead to this. As shown below, when a gene translocation occurs between the Igh locus and the c-myc locus, the expression of c-myc is under the control of the Igh promoter, thus inducing oncogenic c-myc expression, which may cause Burkitt lymphoma.

In our experiment, we transformed a plasmid, which is composed of a retroviral vector and the c-myc gene insert, into competent DH5α bacteria. As there is ampicillin-resistance fragment (ampR) within the plasmid, only ampicillin-resistant bacteria will grow on an LB agar plate containing ampicillin. This enables us to pick up single colonies and prepare for later mini prep.

After mini prep, it is necessary to ensure that the colonies we picked are all exact clones. To achieve this goal, we used restricted endonucleases BamHI and BstBI to digest the plasmid DNA. Then a gel electrophoresis was performed to compare undigested DNA to digested DNA.

Testing the Concentration and Purity of DNA

Once a sample consists of only the DNA and H2O, a spectrophotometer can be used to measure the quality and concentration of the DNA while disregarding the aqua solution. The spectrophotometer measures the amount of light absorption at a wavelength of 260 nm vs 280 nm. For pure DNA, the ratio of light absorption (A260/280) should be above 1.8.

Enriching Plasmid DNA via Maxi Prep

To isolate plasmid DNA from the bacteria, we used an alkaline solution to lyse the bacterial cells and denature the double-stranded (ds) DNA into single strands (ss). Then, we renatured the ssDNA into dsDNA (this process will only renature the plasmid DNA; chromosomal DNA is too big to get renatured) and precipitated protein by SDS. After we roughly obtained DNA through isopropanol precipitation, we dissolved them in a CsCl solution. The CsCl dissociates in the water into Cs+ and Cl-. Being more dense than water, the Cs+ will sink, thus creating a concentration gradient throughout the centrifuge tube. After high speed centrifugation, DNA will finally concentrate at the place where its density equals the local Cs+ density. As we added ethidium bromide (EB) to DNA, it gave an orange color to the plasmid band.

INFECTION OF THE RETROVIRUS

The graph to the left shows the results of a spin infection. It visually demonstrates the number of cells infected with the retrovirus. By the second round of infection, 58.5% of the cells had been infected with hCD25 which is a human cell surface reporter molecule.

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