Experiment #2
DNA isolation, Restriction Digest, and Electrophoresis

Introduction

Recombinant DNA technology involves the biochemical manipulation of genes by using enzymes that normally participate in DNA or RNA metabolism. These enzymes are isolated from a variety of organisms and can be commercially obtained. Genes are manipulated for a variety of reasons, not limited to the following: to facilitate the study of gene expression and physiological regulation, to identify the product of a gene and/or bring about the overexpression of the gene product, to study the structure/function relationships in proteins, or to identify cellular components that interact with particular nucleic acid sequences or protein domains.

The genetic information of all living organisms is contained within the sequence of the nucleic acids adenine, thymine, cytosine and guanine. For most organisms, the genetic information is contained in deoxyribonucleic acid (DNA). In certain viruses, the genetic information is contained in ribonucleic acid (RNA). The complete set of nucleic acids containing the genes for an organism is referred to as the genome. The term chromosome is used to describe particular individual DNA or RNA molecules that are part of the genome. The genome of the bacterium *E. coli* consists of a single, double-stranded DNA molecule; thus this organism is said to contain a single chromosome. Eukaryotic organisms and some bacteria contain genomes that consist of more than one molecule of DNA, and each of these DNA molecules is referred to as a chromosome.

The maintenance, storage, repairs, replication, and expression, of the genome, be it RNA or DNA, require a large number of different enzymes and other proteins as well as nucleic acids. The study of such protein-nucleic acid and nucleic acid-nucleic acid interactions is important for developing an understanding of the physiological processes central to life. Furthermore, understanding these central physiological processes may permit the identification of the cause of various disease states and facilitate the development of clinical strategies to cure or ameliorate the disease state. In these sets of experiments, you will purify plasmid DNA from bacterial cells, use restriction enzymes to

cleave DNA, determine the concentration of DNA, and utilize gel electrophoresis to visualize your DNA bands.

Plasmid Preparation

In the incubator/shaker will be a culture tube with 5ml of *E. coli* culture grown for 15 hours. Take one culture tube that has been labeled for your section and follow the protocol:

1. Transfer the culture to the centrifuge tubes provided by your TA, and harvest the cells at 4,000 rpm for 5 minutes. When finished spinning the supernatant should be clear and a distinct pellet should be evident on the bottom wall of the tube.
2. Discard the supernatant and resuspend the pellet in 250 μL of buffer P1. **Make sure RNase A has been added to the buffer P1 you use.** Transfer the resuspended cells to a microfuge tube.
3. Add 250 μL of buffer P2 to the microfuge tube and mix by gently inverting 4-6 times. This procedure will break the cells, **do not allow this to proceed for more than 5 minutes.**
4. Add 350 μL of buffer N3 and invert the tube immediately but gently 4-6 times. Centrifuge for 10 minutes in the table-top centrifuge at top speed. During centrifugation, place a QIAprep column in a 2 mL collection tube.
5. Apply the supernatant from previous step to the QIAprep column by decanting or pipetting. Centrifuge for 1 minute and discard the flow-through.
6. Wash the spin column by adding 0.5 ml of buffer PB and centrifuging for 1 minute. Discard the flow-through.
7. Wash the spin column by adding 0.75 ml of buffer PE and centrifuging for 1 minute. Discard the flow-through.
8. Centrifuge the empty tube for 1 additional minute to remove residual wash buffer. Unless the flow-through is discarded from the previous step, residual wash buffer may not be removed.
9. Place the spin column in a sterile 1.5 mL-microfuge tube. To elute DNA, add 30 μL of buffer EB to the center of the spin column. Be sure not to disrupt active resin with the pipette tip. Let stand for at least 1 minute before centrifuging for 1 minute.

DNA determination

For this procedure you will need the quartz cuvet, which is very expensive and fragile, so proceed with caution. To the quartz cuvet add 0.5 ml of buffer EB and zero the Ultrospec 1000 at 260 nm. Now add 10 μL of your purified plasmid DNA, cover the cuvet with parafilm or the cuvet lid, and mix the contents

by inverting cuvet several times. Read the absorbance at 260nm and record it in your notebook.

The concentration of DNA can be calculated using following conversion factor:

> 1 absorbance unit at 260 nm = 50 μg/ml of double stranded DNA

For example, if absorbance at 260 nm= 0.5833, then 0.5833 x 50 = 29.17 mg/ml. Note that 10 μL of original DNA stock was diluted into 510 μL, therefore 29.17 μg/ml x 510/10= 1.487 μg/μL.

Restriction Digest

You will use the restriction enzyme *Hind* III to cut your vector and then observe the bands on an agarose gel. *Hind* III recognizes the sequence 5'-AAGCTT-3' and cleaves between the two adenines. Your vector has four *Hind* III restriction sites and thus four bands will be observed. They should be of the following sizes: 4253 bp, 3330 bp, 1036 bp and 928 bp.

In order to cleave your plasmid mix following in the Eppendorf tube:

> ? μL of miniprep DNA (use appropriate volume to deliver 3 μg of DNA)
> 5 μL of 10x BSA
> 5 μL of 10x Buffer 2
> ? μL of ddH_2O (use appropriate amount to get total volume of 50 μL)
> 1 μL of *Hind* III
> _____
> 50 μL total volume

Mix the contents gently and incubate at 37^oC in the table top incubator for one hour. After this time you will run the sample on an agarose gel.

Gel Electrophoresis

In a 125 ml flask mix 50 ml of distilled water, 1.5 ml of 50x

TAE, and 0.4 grams of agarose. Heat on a hot plate while stirring until all of the agarose dissolves. At this time allow the flask to cool. You can run the flask under cool water, but take precaution because the flask will be hot. Once the flask cools to luke warm add 3 μL of 10 mg/ml ethidium bromide, mix and pour the gel. **Always wear gloves when working with ethidium bromide!!!** After pouring the gel, push any air bubbles to one side with the comb and then insert the comb. It will take about 20 minutes for the gel to polymerize.

After the restriction digest is complete, add 4 μL of loading dye, and load your sample onto the gel, in addition to this you should run a standard and a ladder provided by your TA. The gel will be run for 40 minutes at 80 volts. Once completed, you can view your gel under the UV lamp in the safe viewing box. Additionally, your TA will provide you with a picture of your gel.