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3.034 FALL 2005
ORGANIC AND BIOMATERIALS CHEMISTRY

LABORATORY EXPERIMENT 3

Engineering DNA via Restriction Enzymes

PreLab Questions:

1. What is the definition of an enzyme? Describe the function of restriction enzymes?
2. What is a plasmid?

I. Introduction

Enzymes are proteins that serve a specific function: They catalyze chemical reactions, meaning that they initiate / facilitate reactions without being consumed or altered significantly in the process. Enzymes are typically named by the following convention: Substrate + ase. For example, an enzyme that catalyzes degradation of another protein is called a proteinase or protease. An enzyme that digests milk sugar (lactose) is called lactase.

In this laboratory, you will learn how to visualize the effects of an enzyme that ligates (cuts) DNA into small fragments through gel electrophoresis. Restriction enzymes are DNA-cutting enzymes found in bacteria (and harvested from them for use). Because they cut within the molecule, they are often called restriction endonucleases. A restriction enzyme recognizes and cuts DNA only at a particular sequence of nucleotides, or DNA bases A, T, G and C. For example, *E. coli* bacteria that carry the HindIII gene from the bacterium *Haemophilus influenzae* Rd can be used to produce an enzyme named **HindIII** (“HIND THREE”) that cuts double-stranded DNA into double-stranded DNA fragments wherever it encounters the sequence:



The cut is made between the adjacent A and T, as shown by the arrows. This sequence is found in many DNA, including linear, double-stranded DNA isolated from the bacteriophage lambda (Lambda DNA) which has 48,502 base pairs. Treatment of Lambda DNA with this enzyme produces exactly 7 fragments, each with a precise length and nucleotide sequence.

This fragmentation of the DNA via the restriction enzymes is called restriction enzyme digestion, and occurs during incubation of the enzyme with the double-stranded DNA molecules in a NaCl buffer of concentration and temperature optimized for the enzyme activity. These fragments can be separated from one another through gel electrophoresis, a technique that separates charged molecules under the influence of an electric field. The sequence of each fragment is determined by the molecular weight of each fragment and *a priori* knowledge of the cutting site of the particular restriction enzyme used.

You will demonstrate the effect of this restriction enzyme digestion for a plasmid (circular rings of double-stranded DNA) called pBPZ, analyzed via gel electrophoresis. Since the lengths of the Lambda/HindIII digest are well known, you will use the fragment lengths (“bands”) from the Lambda digest as a marker lane or “ladder”, to determine the segment lengths of the pBPZ digest. In gel electrophoresis, the gel is made from agarose dissolved in a gel stain solution [SYBR safe DNA gel stain]. The gel stain binds to nucleic acids and fluoresces under UV illumination, facilitating the visualization of the DNA fragments.

II. Objectives

The goal of this laboratory experiment is to:

- Analyze through agarose gel electrophoresis the effects of a DNA restriction enzyme on the size of two types of virus DNA (λ -phage and pBPZ).
- Construct a new plasmid to produce the protein Green Fluorescent Protein (GFP).