Experiment 6
(Lab periods 5 and 6)

Restriction Enzymes and Restriction Fragments

Restriction enzymes (or restriction endonucleases) are bacterial proteins that act as defense mechanisms in these organisms. Restriction endonucleases cleave double stranded DNA internally, acting essentially like a pair of scissors to cut both strands of the molecule. When foreign DNA (such as viral DNA) is introduced into a bacterium it is the job of the restriction endonucleases to cut the foreign DNA so that they act like a simple immune system. The bacteria protect their own DNA by chemically modifying it – a job performed by a special DNA methylase enzyme.

In molecular biology we take advantage of the natural function of restriction enzymes by using them to perform a number of useful procedures. Two very common uses are 1) to generate DNA molecules that can be used to create recombinant DNA and 2) to get an estimate of the similarity in nucleotide sequence of different pieces of DNA. In this lab we will use restriction enzymes for the second purpose.

Different species of bacteria have different restriction enzymes. The enzymes are referred to by a designation of the species and strain from which they are isolated. For example, the enzyme EcoRI is isolated from *Escherichia coli* strain R and SacI is isolated from *Streptomyces achromogenes*. All restriction enzymes act in the same general manner but differ in an important detail. A restriction enzyme does not cut a DNA molecule randomly but, instead, each restriction enzyme recognizes a specific nucleotide sequence in the DNA molecule. This sequence is referred to as the recognition sequence for the enzyme. The difference between different restriction enzymes is that each has its own recognition sequence. For example the following are the recognition sequences for EcoRI and SacI:

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                     ?                                ?
EcoRI              GAATTC              SacI        GAGCTC
                   CTTAAG                          CTCGAG
                         ?                          ?
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(Notice that for both enzymes, the recognition site has the same DNA sequence, from 5' to 3', on both strands.) If you expose a double-stranded DNA molecule to EcoRI, the enzyme will cut the DNA at every location where the sequence GAATTC occurs, breaking the phosphodiester bond at the point indicated. This procedure is known as Digestion and the result is that the original DNA molecule is cut into smaller fragments.

In this lab we will use restriction enzyme digestion to estimate the similarity in nucleotide sequence between different DNA molecules. This can be accomplished by comparing the DNA fragments that result from digestion of each molecule. Let's assume that you have a molecule of DNA that is 10,000 bases (10 kilobases, or 10kb) in length that you are working with. If that molecule has the sequence GAATTC 4kb from one end then digestion with EcoRI will generate two fragments: one that is 4kb in length and one that is 6kb in length. Using gel electrophoresis following digestion you will be able to visualize the result. Any DNA molecule with the same sequence will always yield these two fragments when digested by EcoRI but DNA molecules with different sequences will be cut into fragments of different sizes. Therefore, the basic idea behind this technique is quite simple. To compare two different DNA samples you digest them with the same restriction enzyme and use gel electrophoresis to examine

the fragments generated. If the fragments are different then the two samples contain DNA molecules with different nucleotide sequences. Notice, however, that the opposite is not necessarily true, if the two samples yield the same fragments they may still have different sequences. Therefore, we repeat this for a number of different restriction enzymes. If the samples always yield the same fragments then they are either very similar or identical in nucleotide sequences. The more enzymes we use, the more likely we are to detect any difference in sequence between the two molecules. This is essentially what is done in DNA fingerprinting, which involves this type of comparison in areas of the human genome that vary substantially in sequence from one individual to the next. Obviously, sequencing the molecules would be conclusive but the technique you will follow here is quicker and much cheaper so it is still very common.

In the lab this week you will perform something similar to a very simple DNA fingerprint analysis. You will be given a DNA sample that represents the sample taken from a crime scene (the criminals sample). You will compare to DNA samples from three different suspects in order to determine which of the three matches the criminals. You will digest each of the three suspects as well as the crime scene DNA with 2 different restriction enzymes. The resulting fragments will be analyzed by gel electrophoresis the following week and by comparing the fragments generated you should be able to determine which of the three suspects is the criminal. (Of course, in real fingerprinting, many more enzymes are used to be much more precise than our example.) In addition, during the gel electrophoresis portion of the experiment you will be provided a diagram of a gel that shows the fragments generated by restriction digest of a DNA molecule with various restriction enzymes. You will use the data from this gel to determine the ‘Restriction Map’ of the DNA molecule that was digested; that is, a diagram showing the relative locations of all recognition sites along the molecule.

Procedure

Week 1 – Restriction enzyme digestion

1. Obtain the crime scene sample and the three suspects.

2. Label 8 different 1.5ml centrifuge tubes as EC, ES1, ES2, ES3, HC, HS1, HS2, HS3.

3. Add 5?l of DNA to the appropriate tube. To EC and HC add the crime scene DNA, to ES1 and HS1 add 'suspect' 1, to ES2 and HS2 add 'suspect' 2 and to ES3 and HS3 add 'suspect' 3.

4. Add 2?l of buffer and 12?l water to each tube. (Make sure that you use the deionized, autoclaved water that is supplied!)

5. To each of the tubes labeled with an E add 1?l of the restriction enzyme EcoRI. To each of the tubes with an H add 1?l of the enzyme HindIII. **(Keep restriction enzymes on ice at all times. If they warm up the protein degrades and loses activity.)**

6. Once the enzyme has been added to a tube, place that tube at 37C.

7. Allow the reaction to proceed for 1 hour at 37C. At the end of 1 hour add 5?l of Stop solution provided (which also contains the dye for gel electrophoresis).

8. Give your samples to your instructor who will store them at 4C until the following week.

Week 2 – Analysis by gel electrophoresis.

1. At the beginning of the lab, load your samples into the assigned wells in the gel that is provided.

2. The gel will be 'run' for at least 2 hours at roughly 100V and then stained. If the gel is not complete by the end of the lab period you can view it the following week.

3. While the gel is running generate the restriction map based on the data provided. Although there is no formal write-up the map is due by the end of the period.

Calculations

C1. From the gel, determine which of the three 'suspects' matches the crime scene DNA in the fragments generated by *both* enzymes.