The Techniques of Molecular Biology: Forensic DNA Fingerprinting

**In addition to close-toed shoes, you are required to wear long pants or skirt (ankle length) and gloves (latex or nitrile) to participate in this lab. You will not be allowed in the lab without these minimum requirements.**

The techniques of molecular biology are used to manipulate the structure and function of molecules such as DNA and proteins in order to understand more about how they function. These molecular biology techniques are not limited to fields such as genetics and cell biology, but they are now commonplace in all areas of biological research including microbiology, evolutionary biology, and botany. For this reason it is important for you to begin to gain experience with the basic fundamentals of molecular biology in the laboratory. One of the most widely publicized uses of molecular biology techniques is for the purpose of identification of criminals through what is commonly called Forensic DNA fingerprinting.

**DNA Fingerprinting Overview**

DNA fingerprinting can be used to help identify individuals by revealing the differences in the DNA sequence among different people. Certain non-coding regions of DNA exhibit significant differences in base pair sequence among individuals. Such regions are called polymorphisms (meaning many forms). Thus, you can compare the base pair sequence of a polymorphic region of DNA found at a crime scene with the base pair sequence of a polymorphic region of DNA obtained from the suspects. If the sequence of one of the suspects matches the sequence found at the crime scene, then you can be certain that the suspect was present at the crime scene. In order to simulate this process we must carry-out the following four procedures.

1. Isolate genomic DNA from the crime scene and from the suspects.
2. Amplify (make many copies of) a polymorphic region of the DNA using a technique called polymerase chain reaction (PCR).
3. Treat the DNA obtained in step 2 with enzymes called restriction endonucleases which will cut the DNA into smaller fragments.
4. Subject the fragments of DNA produced from step 3 to agarose gel electrophoresis which will allow you to visualize the DNA fragments and determine their size.

Steps 1 and 2 will be conducted by the instructor before your lab begins. You will carry out step 3 the first week, and then step 4 will be conducted during a later lab period.

**Equipment Needed**

Almost all of the equipment needed for this lab will be provided to you; however, you will be required to wear gloves throughout all of the lab procedures. Latex and non-latex gloves are available in the GRU Bookstore and can also be purchased at local drug
or discount stores. You will also need to wear protective eyewear for some portions of the lab. We have goggles available for use in the lab, but if you would prefer to purchase your own, they are also available in the GRU Bookstore.

**DNA Fingerprinting Lab 1: Genomic DNA Isolation & PCR**

**Part 1: DNA Isolation**

DNA can be obtained from almost any tissue or biological fluid that is left at a crime scene. A hair, blood, and saliva are all possible sources of genomic DNA because all three will contain a few cells with nuclei. Isolating and purifying DNA from these cells is a very common technique in today’s world of biotechnology. However, the quantity of DNA obtained from samples such as these will be very small; therefore, the next step in the process is to increase the quantity of DNA using a technique called the polymerase chain reaction (PCR).

**Part 2: Polymerase Chain Reaction (PCR)**

The polymerase chain reaction is one of the most important research techniques ever developed, and it has revolutionized most fields of biological research. Its development started in 1983 by Kary Mullis and his colleagues at Cetus Corporation, and in 1993 Mullis won the Nobel prize for this work. In short, the technique uses the enzyme DNA polymerase to make virtually unlimited copies of a piece of DNA. This polymerase enzyme takes monomers (deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, dTTP)), and links them together to make the polymer DNA. Only tiny amounts of the original piece of DNA, known as the template, are required, and millions of copies can be produced in just a few hours. This template is placed in tube along with the DNA polymerase, the monomers, and very short DNA pieces called primers.

There are 3 basic steps to the PCR reaction, and these steps are repeated over and over to make millions of copies of the DNA product. Step 1 is to separate the double stranded template DNA into two single stranded DNA molecules. This step is called denaturating or melting the DNA. Because the double stranded DNA is held together by hydrogen bonds, the two strands are easily separated by heat (melted). Step 2 is to allow two short DNA primers to anneal (form base pairs) with the two single stranded templates. These primers must be present in order for the DNA polymerase to make a new DNA strand. The enzyme cannot start synthesizing a new DNA strand from scratch, but if bases are already present (the primers), it can add new nucleotides to it and thus synthesize a whole new strand. Step 3 is the actual synthesis of the new DNA strands which is called elongation. The DNA polymerase enzymatically joins new nucleotides first to the primers, and then to the growing DNA strand. The rate of nucleotide addition is very rapid; several hundred nucleotides will be polymerized within a minute.

This process can be repeated many times. The rate of synthesis of new DNA strands is exponential because with each repetition the number of DNA molecules doubles. By the end of the 30 cycles the region you are amplifying will have replicated
over a billion times. To see a great animation about PCR please visit the Dolan DNA Learning Center at: http://www.dnalc.org/ddnalc/resources/pcr.html. The polymerase chain reaction is also described in chapter 20 of your textbook.

### Part 3: Restriction Endonuclease Digestion

The goal of this exercise is to differentiate between different DNA samples based on differences in base pair sequence. While it is possible to determine the entire linear sequences of bases in DNA, it is a laborious and potentially expensive process. However, thanks to modern molecular biology techniques there is an easier way to determine small differences in base pair sequence between two or more DNA samples. One way is through the use of enzymes called restriction endonucleases. These enzymes, which were discovered in bacteria, recognize specific sequences of base pairs and cut the DNA only at those specific recognition sites. You will use two enzymes called *Eco*R1 and *Sal1*, and they cut DNA at the following recognition sites which are indicated in bold:

\[
\begin{align*}
\text{EcoR1 cuts here:} & \quad \text{GAATTC} \\
\text{CTTAAG} & \quad \text{CTCCAGTTGTGTAC}
\end{align*}
\]

Whenever the *Eco*R1 enzyme encounters this pattern of six base pairs, it will cut the DNA at that site.
How many pieces of DNA would result from this cut? ________

What differences are there in the pieces? ______________________________________

Sal1 cuts here:

Whenever the Sal1 enzyme encounters this pattern of six base pairs, it will cut the DNA at that site.

How many pieces of DNA would result from this cut? ________

What differences are there in the pieces? ______________________________________

Compare the following two DNA fragments. Identify the recognition sites for EcoR1 and Sal1 in the fragments – only single strands are shown for simplicity.

How many pieces of DNA would result from the cuts?

Sample 1:  # of fragments ______  Sample 2:  # of fragments ______

List the fragment sizes from sample 1 in order from largest to smallest.

List the fragment sizes form sample 2 in order from largest to smallest.
Because polymorphic regions of the genome may differ significantly in base pair sequence among individuals, restriction sites may also differ among individuals. Sites that are present in one person’s DNA may be absent in another person’s DNA. Thus, restriction digestion of a polymorphic region from two different individuals will result in different numbers and sizes of DNA fragments. Therefore, if the DNA fragments obtained from a suspect’s DNA exactly match the fragments obtained from DNA found at a crime scene, you can be certain that the suspect was at the crime scene.

*Restriction Endonuclease Digestion Protocol*

Each lab bench will be provided with a sample of DNA that was obtained from a crime scene and samples that were obtained from 7 different suspects. Each of the samples has already been amplified by PCR and is now ready to be digested with the restriction endonucleases. Each lab bench will also receive a tube containing the restriction enzymes EcoR1 and Sal1 in the appropriate buffer to allow the enzymes to function. You will pipet 5μl of the enzyme mixture into each of the tubes containing the suspects’ DNA samples and to the tube containing the crime scene sample. Be sure to use a clean pipet tip each time.
Part 4: DNA Fingerprinting Lab 2: Agarose Gel Electrophoresis

We will purify our PCR products using a common procedure called agarose gel electrophoresis. Agarose is a polysaccharide that is purified from seaweed. The powdered agarose is mixed with a buffer solution and then dissolved by heating the mixture in a microwave. The molten agarose is then poured into a small plastic tray, and as the agarose cools it solidifies to form a gel. A small plastic comb is inserted in the tray while the agarose is still in its molten state, and after the gel solidifies the comb is removed leaving behind small wells. The DNA sample will be mixed with a loading dye and pipeted into the wells, and an electric current will then be applied to the gel which will cause the negatively charged DNA molecules to migrate through the gel toward the positive terminal. Specifically, the DNA will weave its way through tiny pores in the gel as it is pushed/pulled along by the electric current. Because electrophoresis is just a modified form of chromatography, the rate of migration of the DNA molecules is determined by the size of the molecules. Thus, the smaller molecules will migrate faster than larger molecules. In the figure the band of DNA indicated as “B” is the smallest and the band of DNA indicated as “D” is the largest. In this example, the rank order of size from smallest to largest is B < C < A < D.

In order to see the DNA in the gel, a chemical called ethidium bromide (EtBr) was added to the agarose while it was still in its molten state. Ethidium bromide is a molecule that inserts or intercalates between the bases of double stranded DNA. Under UV light EtBr fluoresces, therefore DNA becomes visible under UV light in the presence of EtBr. EtBr is a potent mutagen (may cause genetic damage) and is moderately toxic. Always wear appropriate protective equipment, such as gloves and safety goggles, when handling EtBr or the agarose gels that contain EtBr. In addition, UV light is damaging to skin and eyes. Please use caution when near the UV light source and always wear safety glasses or a protective face mask.

Smaller molecules of DNA will migrate through the pores of the agarose toward the positive terminal faster than larger molecules of DNA. After a period of time, molecules of DNA of different sizes will be spread out within the gel. We will identify our bands of DNA under UV light when we compare our sample to the standard DNA markers we will also place in the gel.
**Electrophoresis Protocol**

You will have about 10μl of each suspect and crime scene sample to which you will add a loading dye (your instructor will tell you the exact volume). The loading dye contains several dyes which will migrate through the gel when the electric current is applied. The smallest of these dyes is designed to migrate through the gel at least as fast as any small fragments of DNA. Therefore, when the first dye is nearing the end of the gel, you will know that the DNA is not far behind. It also contains a complex carbohydrate called ficoll that helps the DNA settle to the bottom when the sample is pipetted into the well. This dye comes as a 6x stock, and you want its final concentration to be approximately 1x. Check with your instructor to determine the appropriate volume to add to your samples and follow the instructions they provide.

Your professor will pipet standard DNA markers, known as a ladder, into the first well on the left side of the gel. Next, you will pipet the suspects’ DNA (tubes 1-7) into wells 2-8. Finally, add the crime scene DNA into the last well. The gel will run at 100V for approximately 45 minutes and then the DNA bands will be identified by placing the gel on the UV light box.

**Interpreting the Gel**

Your professor will take a picture of the gel and either print it or post it online for you to download. Using the gel picture, estimate the sizes of all the bands by comparing them to sizes of the bands in the DNA ladder. Write that information into the following table.

<table>
<thead>
<tr>
<th>List DNA fragments by size: largest to smallest</th>
<th>crime scene</th>
<th>suspects</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
</table>

Do any of the suspects’ DNA bands exactly match those from the DNA found at the crime scene? __________

Which one(s)? __________