Bio101- LAB 8

Name: _____

CRIME SCENE INVESTIGATOR: DNA PROFILING

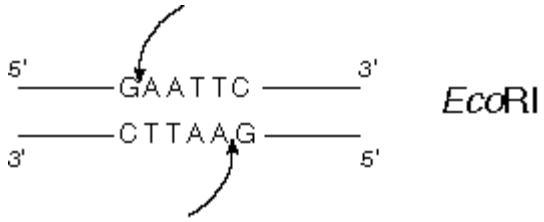
OBJECTIVES:

- To review the structure and function of DNA
- Understand and perform DNA digests
- To gain experience using the micropipettes and gel electrophoresis
- To explore the mechanisms for DNA profiling

Background:

Our ability to manipulate DNA for a variety of applications, including forensics, gene cloning, and making genetically modified organisms (GMO's), depends heavily on our ability to cut and paste DNA in a predictable and precise manner. For this reason, **restriction enzymes** are valuable tools in the laboratory. Restriction enzymes are naturally occurring enzymes that cut DNA at specific sites. (Bacteria use them as defense mechanisms to destroy any invading DNA from bacterial viruses.)

There are thousands of different restriction enzymes, each named after the bacteria it was originally isolated from. For instance, the restriction enzyme we'll use in this lab is known as *Eco*RI; it was the first restriction enzyme purified from *Esherichia coli* bacteria. Each restriction enzyme recognizes a different specific DNA sequence, and cuts a molecule only at that specific sequence (its **restriction site**). The restriction enzyme *Eco*RI cuts DNA at the sequence GAATTC as illustrated at right. Notice that the enzyme always cuts between the G and the A on each strand. Thus the fragments that are generated after such a cut have **sticky ends**, or short single-stranded regions. Note, too, that these are ***randomly occurring*** sequences; the enzymes do not distinguish where on the DNA molecule the restriction site is found. They simply cut the DNA molecule whenever this particular sequence is found!



The arrows indicate where the enzyme cuts the DNA

DNA profiling is a general term encompassing a variety of techniques used to distinguish one human being from another. This powerful tool is now routinely used to investigate crime scenes, missing persons, mass disasters, human rights violations, and paternity testing. For example, crime scenes often contain biological evidence, such as blood, semen, hairs, or saliva, from which DNA can be extracted and amplified (copied). If the DNA profile from the evidence matches the DNA profile of a suspect, the individual is included as a suspect; if the DNA profiles do not match, the individual is excluded from the suspect pool.

Our human genome, or total set of DNA, consists of ~3 billion bases. And more than 99.5% of this genome does not vary between human beings. Thus the challenge of a DNA profile is to focus on that small percentage of the human DNA sequence (<0.5%) that does vary; these regions are known as ***polymorphic*** ("many shapes") sequences. By convention, the polymorphic DNA sequences used for DNA profiling are neutral and do not control any known traits or have known functions.

In today's experiment, we will focus on a particular region of this variable, or polymorphic, DNA that does often vary between individuals in the human population. In particular, we'll look at a position (or ***locus***) on one of our chromosomes that often contains variability between individuals.

In our lab today we will simulate the process of DNA profiling using DNA digests and gel electrophoresis to distinguish multiple DNA samples, including one from a "crime scene", and samples from four potential suspects. These techniques should allow us to determine if any of our "suspects" are a close match to the DNA left at the "crime scene"!

To cut DNA in today's exercise, we'll mix different DNA samples with a restriction enzyme. This enzyme will literally slide along the DNA molecule until it recognizes (binds to) a specific sequence of base pairs, the enzyme's restriction site. The enzyme will then cut the DNA molecule at this site. Because these sites are randomly occurring, we expect that several restriction sites could occur on any given DNA molecule. For instance, if a given piece of DNA contains three restriction sites for *Eco*RI, the enzyme will cut the molecule at each of these locations, producing four fragments of varying lengths.

Part 1: DNA Digests

Step 1: Obtain Supplies

Each lab team will need microtubes (Eppendorf Tubes) containing: *Eco*RI enzyme solution, restriction buffer, distilled H2O, Crime Scene DNA, and four tubes of different 'Suspect DNAs'. Keep these on ice if possible! The tube color and labels are as following:

Tube color	Label	Ingredients
Clear	**R1**	*Eco*RI enzyme
Clear	**Buf**	Restriction buffer
Yellow	**CS**	Crime Scene DNA
Violet	**S1**	Suspect 1 DNA
Green	**S2**	Suspect 2 DNA
Orange	**S3**	Suspect 3 DNA
Clear	**S4**	Suspect 4 DNA

NOTE: YOU MUST LABEL EACH TUBE WITH YOUR GROUP NAME!

Step 2: Set-up Restriction Digests

Use the micropipettes to accurately measure out the following volumes of liquids into each of your tubes from Step 1. (Your instructor will provide additional instructions on the use of the micropipettes.) Be sure to use a clean pipette tip each time you pipette, and that the solutions are added directly to one another and not allowed to splash on the side of the tube. While adding your final ingredient (*Eco*RI) you can use the pipette tip to gently stir mixture.

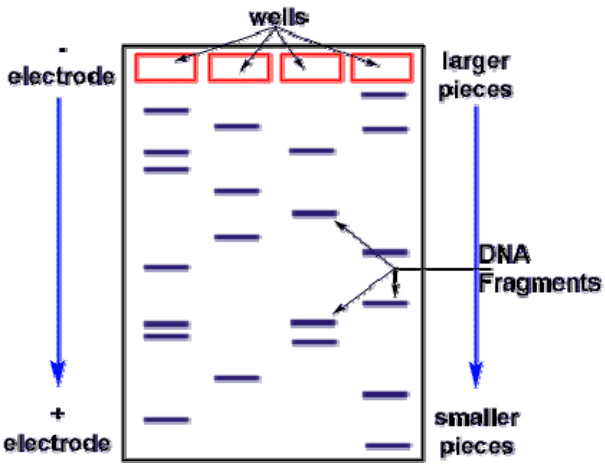
To Tube:	Add Buffer	Add EcoRI
CS	5 ul	1 ul
S1	5 ul	1 ul
S2	5 ul	1 ul
S3	5 ul	1 ul
S4	5 ul	1 ul

Step 3: DNA Digestion/ Pouring the Agarose Gel

After adding both ingredients, cap your tubes and mix your digests carefully. You may do this by flicking the tubes with your finger. Make sure to bring all of the volume back down to the bottom of the tube (instructor will give you tips on this). Now, place all tubes rack in the 37^{o}C water bath approximately 60 minutes.

Day 2- Part 2: DNA Electrophoresis

How can I tell if my digests worked? Unfortunately, you cannot see individual DNA molecules with either your naked eye or our light microscopes. So we will need another technique to allow us to determine whether or not our digests were successful and to determine if the lengths of the DNA fragments vary in length. The technique we will use is known as ***DNA electrophoresis***. (See diagram at right.)



Agarose gel electrophoresis separates DNA fragments according to their size (molecular weight, in kilobases, kB). In DNA electrophoresis, a collection of DNA molecules is placed in wells at one end of a dense matrix called an agarose gel. An electrical current is then conducted across the gel. As DNA is a negatively charged molecule, it will be drawn towards the positive pole. (Opposites attract!) Thus our DNA will move through the gel, and smaller fragments will travel faster, and therefore farther, than larger fragments. This allows us to sort the DNA fragments from each digest based on their size. (The rate of travel is inversely proportional to molecular weight.)

Part A: Preparing an Agarose Gel

1. Each group will receivea kit containing a DNA electrophoresis box and tray. Locate the tray in your kit, and use the tape provided to carefully seal off both ends of the tray. ***Tape carefully!! You will be pouring a hot liquid into this tray and leaks are common!*** Place a comb in one of the sets of notches located at either end of the tray.
2. Obtain a flask of melted agarose from the water bath. Carefully pour the agarose solution into your prepared tray until the agarose reaches the line on the tray, and covers the lower 3 mm or so of your comb.
3. Leave the gel sitting on a level surface to cool. In 10-15 minutes your gel should appear slightly opaque, and be firm to the touch.
4. When your gel is cooled, carefully remove the tape from each end of the tray, and gently pull out the comb.
5. Place your gel in an electrophoresis box. Recall that DNA is negatively charged, thus it will migrate towards the positive pole (red). This means that **the wells in your gel should be at the negative (black) end of the box**!
6. Carefully pour 1X buffer (at front of lab) over the surface of your gel until you have filled the chambers at either end of your gel.

Part B: Preparing your Samples.

1. Retrieve your digest samples. (Remember that these tubes have thin walls and should be handled gently.) Dark blue loading dye will already have been added to your samples. Loading dye serves 3 functions: the dye increases the density of the sample so that it sinks into the well of the gel, it helps to visualize an otherwise colorless sample, and finally, the loading dye contains indicators that migrate with the DNA giving us an estimate of how far the DNA samples have moved through the gel.

Part C: Loading your Gel. When your group has reached this point, your instructor will demonstrate how to load a gel by loading a **DNA ladder** on your gel. This ladder contains pre-cut DNA fragments of different, known sizes (see below) and can be used to identify the size of the DNA fragments generated by your digest. You will be given a tube labeled **'L'** contaning DNA ladder. **After** your instructor has demonstrated the process, you may proceed.

1. Using a fresh tip each time, carefully remove 10 ul of the digest/Loading dye mix. (Be sure to use the correct pipette!) Place the pipette tip **at the top** of the well, and slowly dispense the solution, allowing it to sink into the bottom of the well. Note that one of the most common errors is to push the

tip too far down and poke a hole in the bottom of the gel! It is also equally common to 'miss' the well entirely and shoot the sample into the buffer.

2. Record which well you placed the sample in, and continue as above to load the remaining samples.

Part D: Running the Gel.

1. When your gel is loaded, carefully place the cover on your electrophoresis chamber, taking care to align red with red and black with black.
2. Plug the ends of the wires into a power supply. We will run our gels at 100V for approximately 45 minutes. At the end of 45 minutes, turn of the power supply.

Part E: Staining your gel.

1. Carefully remove your gel from the electrophoresis box by lifting it out on its tray. Note that these gels are warm, slippery, and fragile at this point!
2. Slide your gel of the tray and into a large weigh boat or staining dish. **Clearly label** this weigh boat with your group's name.
3. Pour enough 100X Fast Blast solution into the weigh boat to completely cover the surface of your gel. Allow this solution to sit for 5 minutes.
4. After 5 minutes, carefully pour the stain into the collection beaker provided (not down the sink!) Wash your gel in warm water for 5-10 minutes, shaking occasionally to facilitate diffusion of excess dye out of your gel.
5. At the end of this wash, you should begin to see bands appear and are ready to analyze your results!

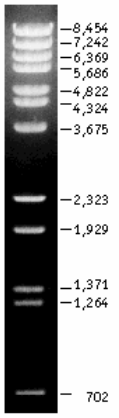
Clean-up: Before you leave the lab today, make sure your electrophoresis chambers and gel trays have been rinsed with tap water, dried, and returned to their storage boxes. Place these boxes on the carts in the classroom. Micropipettes and unused pipette tips should also be returned to their boxes. Take care to wipe down your workspace if any solutions have been spilled. The empty plastic tubes may be thrown in the trash.

What will I see as my gel runs? We mixed our DNA digests with a colored loading dye. This loading dye will make it easier to see and load our samples. In addition, the dye itself is also negatively charged; thus is will also migrate through our gel when the current is applied. You will see this dye moving. The DNA fragments generated in the DNA digests, however, are still not visible and have no natural color. To see these DNA molecules, we will have to apply a DNA stain, FastBlast. This stain will bind to the DNA molecules and allow us to see them. Depending on your lab schedule, your instructor may ask you to stain your gels overnight.

Analyzing the data

Your gels have now been stained in the Bio-Safe FastBlast stain, and destained in distilled water. The blue dye has bound to DNA in the gel, and the excess dye has been washed out of the rest of the gel (destaining) leaving a pattern of blue bands on your gel. We'll use a digital camera to capture an image of this gel.

With your picture in hand, you're now ready to determine the approximate sizes of each of the restriction fragments generated in the digest. You can do this by simply comparing the restriction fragments to DNA fragments of known size (our DNA ladder, shown below). Using the ladder, "read" the approximate size of the bands on your gel. Compile this information in the table below.



Questions to Answer:

1. In the space below, sketch the results you see AFTER your gel has been stained. Completely LABEL your gel. You should label your sketch clearly enough so that someone who did not participate in the experiment could clearly interpret your results, and the different samples involved.

2. Based on this sketch, fill in the right-hand column, indicating the gel results of each sample.

Lane #	Sample	Number of bands	Approximate Size of DNA Band(s) in kB
1	DNA Ladder		
2	Crime Scene Digest		
3	Suspect A Digest		
4	Suspect B Digest		
5	Suspect C Digest		
6	Suspect D Digest		

3. Based on your results, is it possible to connect any of the suspects to the crime scene? Explain. Can you exclude any suspects?

4. Suppose the criminal in this investigation has an identical twin. If the twin provided a DNA sample, and the DNA sample was digested with *Eco*RI, would you expect the generated DNA fragments to be similar, identical, or different when compared to the criminal DNA fragments? Explain your answer.

5. Could an identical twin be wrongly accused of a crime they did not commit, based on DNA evidence?

4. In your own words, define a restriction enzyme.

5. Consider a linear DNA molecule with four randomly occurring *Eco*RI restriction sites. If this molecule were completely digested, how many fragments would you expect to see on your gel?

6. Can agarose gel electrophoresis be used to distinguish the following two DNA molecules? Explain.

DNA #1: ATGGGCGTATGTGACGTAGCTAGCTAGCTTGCCGTACG

DNA #2: ATGTTTAGTATCGTATGGTCTGGATAGCCCCGTAGTAC

7. What was the role of tube "L" in your experiment? Do you expect to see multiple bands in this sample? Explain.