BIOL 101: GENERAL BIOLOGY L - LABORATORY MANUAL

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1.11: Gel electrophoresis

In this exercise, gel electrophoresis (**Fig. 1**) of different electrophoretic dyes will be used to simulate the process of DNA fingerprinting (aka "DNA profiling"). DNA fingerprinting is a laboratory technique that forensic analysts use to compare a DNA sample collected at a crime scene with a DNA sample collected from a suspect. Even though 99.9% of the genome throughout the human population is the same, the remaining 0.1% of human DNA shows variation between individuals. These variable DNA sequences, called polymorphic markers, can be subjected to DNA gel electrophoresis to produce unique DNA banding patterns on an agarose gel. The DNA bands can then be used to differentiate or correlate individuals.

You will be tasked with analyzing the DNA of two individuals who are suspects in a crime scene from which human DNA samples (such as skin cells or hair) were recovered. Your goal is to match the DNA (in reality, this would be **DNA fragments** generated by **restriction enzymes**, explained below) from one of the two suspects to the DNA found at the crime scene.

If the DNA sample from a suspect matches the DNA at a crime scene, then that signifies that the suspect in question *was present at* the crime scene (although the suspect may not have committed the crime). If the DNA profiles from the crime scene do not match a suspect, then it can be concluded that the individual in question was not present at the crime scene.

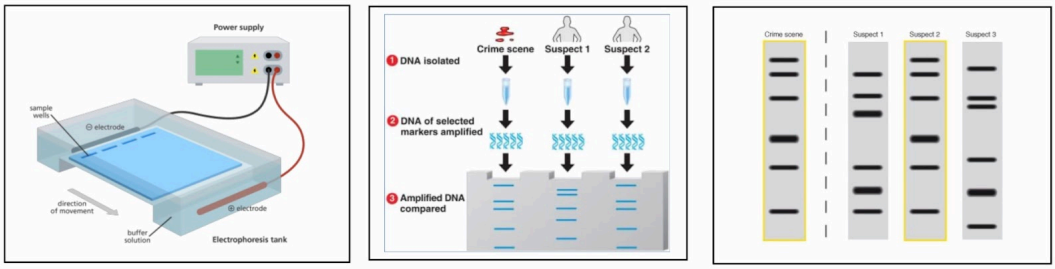


Fig. 1. Gel electrophoresis apparatus (left), (stylized) example of methodology used in this technique (center), and (stylized) example of gel from crime scene and three potential suspects (right). The point is to match characteristics of the DNA found at a crime scene with the DNA from potential suspects in the crime. In the right-most figure above, DNA from the crime scene matches DNA from Suspect 2.

DNA Fingerprinting:

DNA Fingerprinting (DNA profiling), similar to the exercise we are performing today, was first used in England in 1987, to help identify a murderer. This technique is now used routinely for identification purposes as diverse as the establishment or elimination of suspects in a crime, paternity suits, the verification of human remains after catastrophic events (e.g. plane crash), exoneration of the wrongly accused, or the establishment of family relations. Non-human DNA (such as that of endangered species, genetically modified plants, or disease-causing microorganisms such as *E. Coli* 0157:H7) can also be profiled.

Almost every cell in the human body contains DNA in the form of 23 chromosome pairs that collectively contain about 3 billion base pairs. On average, about 99.9% of the DNA in all humans is identical. However, the remaining 0.1%, which constitutes about 3 million base pairs, differs significantly enough among individuals (except identical twins) that it can be used to generate a unique genetic "fingerprint" for every person. Just like our physical fingerprints, "**DNA fingerprints**" are something we are born with and something unique to each person.

The unique 0.1% of our DNA contains **short, non-coding, sequences of repetitive DNA** that are 2-100 **base pairs (bp)** long. CTTG is an example of one such repeated unit (or simply **repeat**) that is 4 bp long. It might be repeated 3 to 100+ times as follows:

CTTGCTTGCTTGCTTGCTTGCTTGCTTG…..

Repeats are referred to by a variety of terms (sometimes confusing) depending on their size. For example, sequence repeats of 10 to 80 bp are called **minisatellites** or **variable number tandem repeats (VNTR)**. **Microsatellites**, also known as **short tandem repeats (STR)**, are smaller repeated units of 1 to 6 bp. Regardless of their size (number of base pairs) or names, DNA repeats show greater variation from one person to another than any other parts of our genome.

The number of times a given repeat (for example CTTG indicated above) occurs in any individual's DNA is a function of the DNA that a person received from his or her mother and father at conception. For example, three individuals (Mary, Jake, and Sue; **Fig. 2**)

could exhibit the following variation in the length of a particular repeat sequence on the chromosomes they received from their parents.

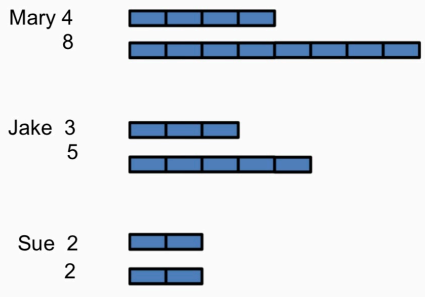


Fig. 2. Hypothetical variation in number of microsatellites in the DNA of 3 individuals. Blue rectangles represent microsatellite repeats on homologous chromosomes.

The process of DNA profiling uses molecular "scissors" called **restriction enzymes**, enzymes that cut DNA at specific nucleotide sequences. In this example, restriction enzymes would recognize particular nucleotide bases at the beginning and end of the repeating string of nucleotides (the **microsatellite region**). Consequently, one segment produced in this manner might be CTTGCTTG (2 repeats long) while another might be CTTGCTTGCTTGCTTGCTTGCTTG (6 repeats long). The DNA segments used in forensic investigations are, of course, much longer than this.

These DNA pieces of various lengths are separated using gel electrophoresis (see **Fig. 3** and text below).

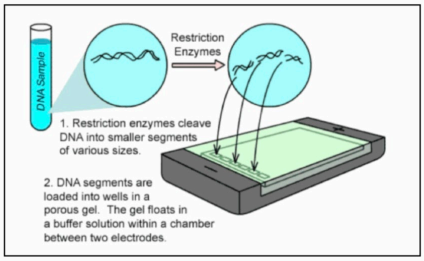


Fig. 3. How restriction enzymes are used in DNA fingerprinting.

Restriction Enzymes:

Restriction enzymes were first discovered in the 1970s. Restriction enzymes used in DNA profiling were developed from the 3,000 or more restriction enzymes (aka **restriction endonucleases**) that have been identified from bacteria and are a defense against the DNA of invading viruses. Specific bacterial restriction enzymes cut double-stranded viral DNA at specific locations (**base pair sequences**) into smaller non-infectious fragments (**Fig. 4**).

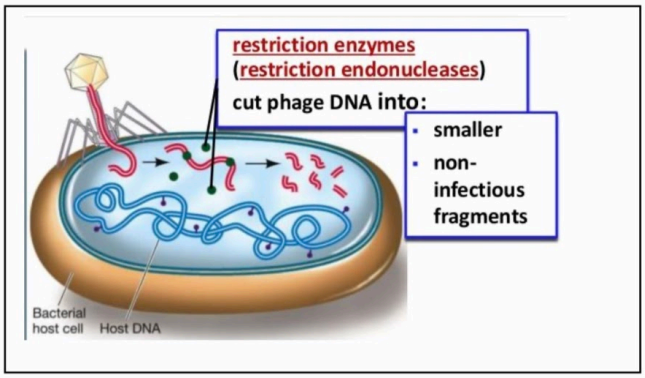


Fig. 4. How restriction enzymes function in bacteria.

When used in biotechnology, bacterial restriction enzymes act much as they do in bacteria. They locate and cut the DNA with which they are mixed (at specific restriction sites) to produce fragments.

Restriction enzymes are described by unique **acronyms** (abbreviations) that document the organism from which they were isolated. The first letter of the acronym is the first letter of the genus of the bacterium. The next two letters are the first two letters of the bacterium's species name. Additional letters and numerals indicate specific bacterial strains and their order of discovery. For example, *EcoR1* was the first restriction enzyme isolated from the RY13 strain of the bacterium *Escherichia coli.*

In the example below, the enzyme *EcoR1* has cleaved DNA between the G and neighboring A in the GAATTC recognition site (**Fig. 5, top**).

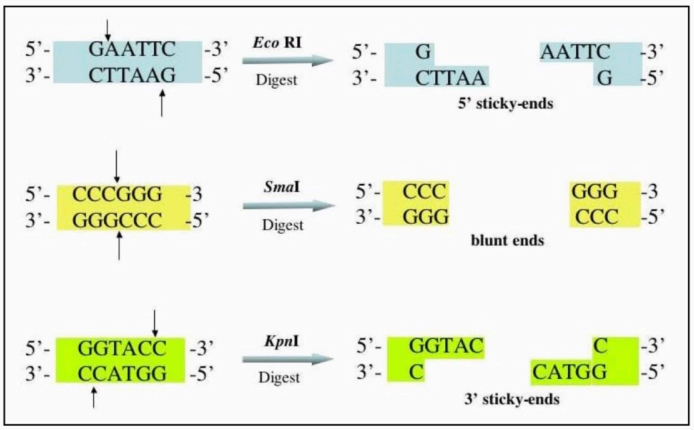


Fig. 5. Examples of how restriction enzymes can cut DNA.

It is important to note that the ends of the cleavage (cut) produced by *EcoR1* are staggered so that the resulting fragments project short overhangs of single-stranded DNA with complementary sequences. Such overhangs are referred to as "**sticky ends**" because the single strands produced can interact with (or stick to) other overhangs of single-stranded DNA with complementary sequences.

The discovery of restriction enzymes launched the **era of biotechnology** and has been a centerpiece for studies and advances in **molecular and gene cloning**, **DNA mapping**, **gene sequencing,** and various other endeavors including the DNA profiling discussed here.

Gel Electrophoresis:

Gel electrophoresis is a laboratory technique that allows macromolecules, such as DNA, or RNA fragments, or proteins, in a mixture to be separated according to their molecular size and/or charge. The molecules to be separated are placed in sample "wells" (depressions) in a thin porous gel slab (**Fig. 6**), which is then covered by a buffered solution and placed in a horizontal electrophoresis chamber (**Fig. 7**).

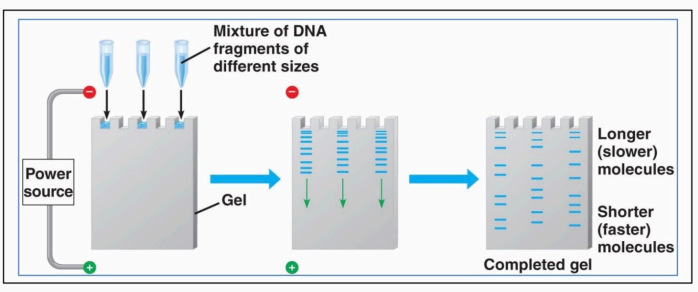


Fig. 6. The separation of DNA fragments in gel electrophoresis

The sugar-phosphate backbones of DNA are negatively charged. Consequently, if an electric current is passed through the chamber, **DNA fragments will migrate** through the pores in the gel, away from the **negative electrode** (where the wells are located) toward the **positive electrode**. Shorter DNA fragments move more quickly — and farther on the gel — than do larger fragments.

The different-sized DNA fragments that have migrated through the gel form distinct bands on the gel, which can be seen if they are stained with DNA-specific dye.

You will be given three samples that will simulate DNA from two suspects, as well as the investigator's DNA, that have been digested with a few restriction enzymes. (In reality, your samples contain electrophoretic dyes of different molecular sizes). Using agarose gel electrophoresis, these samples will form bands, which will then be compared to artificial DNA samples from a "crime scene" (that have also been digested with the same few restriction enzymes) and will run simultaneously in the same agarose gel.

The final step, following electrophoresis of the gel, is analyzing the suspect and investigator DNA sample profiles and comparing them for the presence or absence of particular bands in the crime scene sample profile. The more bands any given samples have in common, the more likely it is they came from the same person.

Components of the Electrophoresis Equipment:

Your instructor will explain and demonstrate how the gel electrophoresis chamber and its components function (**see Fig. 7 below**).

Power Supply: The high voltage power source (pictured below) connects to the electrophoresis chamber and sets up an electric field between the two electrodes — one positive and one negative. DNA-fragment samples (or in our case, electrophoretic dyes) loaded into the wells of an agarose gel are negatively charged and move through the gel toward the positive electrode as the agarose gel matrix separates the DNA molecules by size.

Electrophoresis power supplies typically have a variable output voltage allowing the user to set the output voltage for different size gel tanks and modify voltage for optimum results and convenience.

For our experiment, we will set the voltage on our power supply to **75 V**.

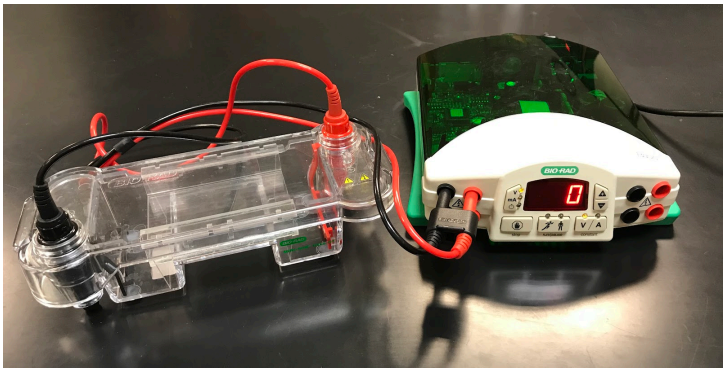


Fig. 7. Gel electrophoresis chamber and power supply (original photo)

Agarose, the main component of our gels, is a polysaccharide polymer extracted from seaweed. It is available as a powder, which is mixed with a buffered TBE solution (see below), heated until it dissolves, and then poured into molds where it solidifies (in about 20 minutes) into a gel slab (having the consistency of finger jello). A serrated "comb" is placed in the mold before the agarose solidifies to create **sample wells** that form in the finished gel.

TBE (Tris/Borate/EDTA) Buffer is diluted from a 20x concentrate to a final concentration of 1X. It is used to cover the gel in the electrophoresis chamber and contains ions that carry the current through the apparatus. It also maintains a constant pH for the experiment.

Micropipettes (**Fig. 8**) are used to dispense all the samples in preparation for electrophoresis. These devices are designed to transfer small amounts of liquid (<1ml). The scale on micropipettes is in microliters (1000 µl = 1 ml). Your instructor will demonstrate how to set the pipette for a particular volume of liquid and how to properly dispense the calibrated volume.

Fig. 8. Micropipette (BioRad) (original photo)

Procedures

Lab Safety:

- Gloves and goggles should be worn throughout the lab.
- Exercise caution when using electrical equipment and any device (such as a water bath) that produces heat.
- Wash hands thoroughly with soap and water at the end of the lab.

Materials:

- For pipetting practice:
  - Petri dish with 1% agarose gel with wells (optional)
  - Beakers with colored practice solution
  - Micropipettes and tips
  - Empty beakers (in which to dispense practice solution)
- Gel electrophoresis apparatus:
  - Gel tray (mold) with ends taped
  - 8-well comb
  - Electrophoresis chamber
  - Power supply
  - 1% agarose prepared in advance and kept at 65 degrees Celsius in water bath
  - TBE (Tris base; boric acid; ethylenediaminetetracetic acid, or EDTA;NaOH), 20x to be diluted to 1x (or 1x buffer already diluted)
  - Microcentrifuge (helpful to spin down samples)
  - Electrophoresis samples in labeled microfuge tubes
    - DNA ladder (standard) labeled “L”
    - Crime scene DNA labeled “C”
    - Suspect 1 DNA sample labeled “S1”
    - Suspect 2 DNA sample labeled “S2”
    - Investigator DNA sample labeled “I”

Exercise 1 - Preparing the Agarose Gel:

Shortly after the lab starts, you will be instructed to pour your agarose gel.

1. Obtain a gel tray (in which the ends have been taped to prevent leaking).
2. Retrieve an Erlenmeyer flask containing 35 ml of the heated pre-mixed 1% agarose gel solution. (The gel solution was previously made by weighing out 0.35 g of agarose, dissolving it in 35 ml of 1X TBE buffer, and heating it until boiling in a microwave.)
3. Pour the heated gel solution into your gel casting mold. Once you have poured the gel into the mold, carefully place the 8-well comb into the gel and position as instructed.
4. The gel will solidify in approximately 20 minutes. It is ready for loading when it is firm and appears semi-opaque (cloudy).
5. While the gel is solidifying, go on to Exercise 2 and practice pipetting with the micropipette.

Exercise 2 - Practice Pipetting:

Micropipettes are molecular biology tools that are designed to dispense very small amounts of liquid. Different micropipettes can be utilized for a range of volumes, for example 2 µl to 20 µl.

1. Attach a plastic disposable pipette tip to the tapered end of the pipette and fit securely in place.
2. Examine your micropipette. What are the numbers designated on the plunger of the pipette? The smaller number represents the smallest volume that should be measured with the pipette. The larger number represents the largest volume that should be measured with the pipette.
3. Locate the window on the side of the pipette. This window displays the volume currently set for the pipette. Set the micropipette to the largest volume the pipette can measure. For example, if the largest number is 20 µl, then rotate the dial until the correct volume appears in the display window.

4. Obtain the colored practice solution.
5. Before placing the tip into the liquid, depress the pipette plunger with your thumb to the ***FIRST*** stop to eject any air.
6. Place the tip into the practice solution and slowly release the plunger, gently "sucking" the liquid into the tip. Your tip now contains the measured volume of liquid displayed in the window. Remove the tip from the liquid.
7. Move your hand so that the tip of the micropipette is over the empty beaker. Insert the pipette tip into the empty beaker so that the tip is close to the bottom of the beaker. Touch the tip to the side of the beaker. Slowly press the plunger down to the first stop and then continue to press the plunger ***ALL*** the way down to the ***SECOND*** stop in order to release all of the liquid from the tip. ***Don't release the plunger yet!***
8. Pull the tip completely out of the beaker and away from the liquid, and then ***SLOWLY*** release the plunger back to the starting position.
9. Discard the tip, using the release button on the pipette. Use a new tip each time you use the micropipette.
10. Reset the volume in the display window to practice dispensing different volumes of practice solution.

Digested DNA Sample Simulation (Dyes)

Scenario:

DNA profiling may be used both to exonerate or convict criminal suspects. If a suspect's DNA is not found at the crime scene, the suspect can be excluded or - if they had been falsely accused - exonerated. Conversely, if a suspect's DNA is found at a crime scene that may or may not implicate them of the crime. In this case investigators must consider other factors, both biological (e.g. blood typing) and behavioral (e.g. motive and means). DNA alone is not sufficient evidence to convict, but it is sufficient evidence to exonerate.

In this activity you will play the role of investigator working a crime scene where you retrieved a sample of DNA. You suspect two different individuals of the crime and collected DNA samples from each of them. You send the samples to your analyst to conduct a DNA analysis. Per procedural protocol, you include a DNA sample of your own to rule out the possibility of DNA contamination at the crime scene. You assign a code to each sample to make sure the analyst conducts the analysis without bias. You code the samples as follows, with each code indicating the date of collection and a unique identifier. You ask the analyst to run a DNA profile for each of these samples hoping it will help you narrow your suspect pool.

Sample Code	Code KEY
1119_CS	Crime scene DNA
1119_BB	Suspect 1
1119_PG	Suspect 2
1119_MO	Investigator's DNA

The analyst receives your coded samples and proceeds with the analysis as follows.

Because of the difficulty involved in obtaining and storing stable DNA samples and the precision needed to perform a successful restriction digest, we will be simulating a DNA digestion using a mixture of dyes. Using dyes allows us to easily see the bands in the gel because of their different colors and because of how they separate on the gel. The table below shows information about the dyes we will be using.

Dye	color	Molecular weight (g/mol)	~DNA base pair equivalent movement	g/100ml*
xylene cyanol	blue	538	10,000	0.2
methyl orange	yellow/orange	327	4,500	0.2
Bromophenol blue	purple/blue	669	1,000	0.2
Ponceau G	pink	760	~100	0.2
*Each sample was made 0.2% by weighing out 0.2 g of dye and dissolving in 100 ml of 20% glycerol				

Exercise 3 - Loading, Running, and Analyzing the Gel:

Loading the Gel:

1. Retrieve your hardened gel.
2. Gently remove the tape from the edges. Gently remove the comb by lifting it slowly up out of the gel. Avoid tearing the gel. Leave the gel in the plastic mold.
3. Place the mold in the electrophoresis chamber. Place the gel so that the sample wells are toward the negative electrode (black).
4. Pour the 1X TBE Buffer into the chamber until the gel is completely covered.
5. Place the DNA samples into the microfuge and spin for 10 seconds. This will force all of the samples to the bottom of each tube.
6. Load 10 µl of each sample given to you by your instructor. Use the following table to run each sample in the appropriate lane. Make sure to use a clean tip for each sample!

Gel Lane (left to right)	Microfuge Tube	Contents (see key above)
1		
2	L	DNA Ladder (Standard)
3		
4	S1	1119_BB
5	S2	1119_PG
6	I	1119_MO
7	C	1119_CS
8		

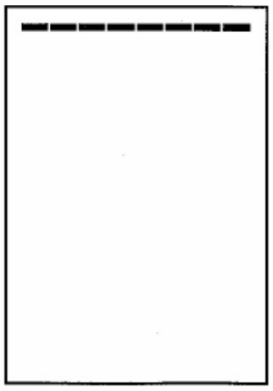
Running the Gel:

1. Place the lid on the electrophoresis chamber and connect the electrodes to the power supply, making sure you have “black to black” and “red to red”.
2. Set the power source to 75V and run the gel for approximately 60 minutes, or longer if possible.
3. Periodically check that the current is flowing correctly and the samples are migrating towards the positive electrode (red).

Analyzing the Gel:

You receive word that the DNA analysis is complete and rush to the lab to review the results. Working with the analyst you step through the results.

1. Lane 7 represents the Crime Scene DNA digested by restriction enzymes. It should yield distinct DNA banding patterns.
2. Lanes 4 and 5 represent the DNA samples from Suspect 1 and Suspect 2 respectively. Assume these DNA samples were digested with the same restriction enzymes used with the DNA in Lane 7.
3. Lane 6 represents your own DNA (called Investigator DNA). You ran your own DNA to ensure that you had not contaminated the DNA sample taken at the crime scene. Assume your DNA was digested with the same restriction enzymes used with the DNA in Lane 7.
4. To determine which suspect(s) was at the crime scene and which suspect(s) can be excluded, compare the banding patterns between each sample and Lane 7. A DNA sample that does not show any similarity to the pattern in Lane 7 can be excluded from your suspect pool. DNA samples showing even a partial similarity can not be excluded.
5. In the space below draw a representation of your gel. Use colored pencils to draw the results of the different colored fragments. Be sure to label each lane as well as the DNA standards (“Ladder”).



Investigator's Report: After examining the gel you prepare your report. You include answers to the following questions in your report.

1. Based on the DNA analysis, which suspect(s) can be excluded from your suspect pool? Explain how you came to this conclusion.
2. Based on the DNA analysis, which suspect(s) can not be excluded from your suspect pool? Explain how you came to this conclusion.
3. For suspect(s) remaining in your suspect pool, is this evidence alone able to convict them of the crime? Explain your reasoning.
4. Did your DNA (Lane 6) match DNA at the crime scene? What steps can investigators take to make sure they do not contaminate a DNA sample taken at a crime scene?

Questions for Review:

1. Which lane contained a sample with the smallest DNA fragment? Explain.
2. What is the relationship between the migration distance and the size of the DNA fragment? Explain.
3. Why were the sample wells placed toward the negative (black) electrode?
4. If you were pouring your gel to run molecules that had both negative and positive charges, how would you position your comb?

Practical Challenge Question

1. If you look at the molecular weights of the dyes we used, they are not separating on the gel by molecular weight (e.g. Ponceau G is the heaviest but moves the furthest). What might explain this?

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 9: Gel Electrophoresis, Restriction Enzymes, & DNA Fingerprinting," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY https://oer.galileo.usg.edu/biology-textbooks/18

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