

Investigations in Biology and Chemistry II – Lab work

Name: \_\_\_\_\_

Date Due: \_\_\_\_\_

**Lab #30: The Use of DNA Fingerprints to Determine Wolf Breeding**

**Pre-Lab Activities**

1. Survey the headings and subheadings to familiarize your self with this lab.
2. Read the **Introduction**, completing the given tasks along the way. Now go back and re-read it, this time **highlighting** main ideas.
3. **Interactive Lecture:** What is a population’s gene pool?
4. Read the **Materials and Methods** completing the given tasks along the way. Now go back and re-read it, this time **highlighting** main ideas.
5. Read through the **Procedure** and circle any new or unfamiliar materials.
6. Set up your lab notebook for Lab #30. Be sure to transfer any important safety information and/or safety symbols into the “Safety Precautions” section.
7. Write the purpose of Lab #30 in your lab notebook.
8. **Interactive Lecture:** What are restriction enzymes and how are they used in biotechnology?
9. Complete *Recombinant DNA* Colorplate, pp. 104-105. Discuss the difference between the technology described in this colorplate, and the use of restriction enzymes in DNA fingerprinting.
10. **Interactive Lecture:** What is DNA fingerprinting and what materials and methods do we use during this type of protocol? This also will involve a discussion of lab techniques.
11. You are conducting another separation of a mixture in Lab #30. Complete the table below for this separation and one other lab experience of your choosing from the Lab #s 1 through 29.

**Separation of Mixtures**

| Question about Protocol  | Lab #30 | Lab # _____ |
|--|---------|-------------|
| What are the components of the mixture?  |         |             |
| What materials/equipment were used for the separation?                             |         |             |
| How does the mixture move through the separation medium?                           |         |             |
| What do the results look like?<br>How do you know you have separated your mixture? |         |             |

## Introduction

A genome is an organism's entire set of genetic information. In humans, the genome is made up of the information contained within the 46 chromosomes found in each *somatic* (body) cell. Remember, these 46 chromosomes are divided into 22 pairs of autosomes and 1 pair of sex chromosomes. To see maps of the human genome, reference the following url:

<http://www.ncbi.nlm.nih.gov/genemap99/map.cgi?CHR=X>. A genome map simply provides the location of the genes within a species' genome, known as a gene *locus*. It does not point out genetic variations among individual organisms within that species. In addition to the human genome, maps have been created for several bacteria; *Saccharomyces cerevisiae*, a yeast: <http://db.yeastgenome.org/cgi-bin/SGD/PGMAP/pgMap> ; *Drosophila melanogaster*, the fruit fly; and *Arabidopsis thalianam*, the mustard plant:

[http://www.ncbi.nih.gov/mapview/map\\_search.cgi?taxid=3702](http://www.ncbi.nih.gov/mapview/map_search.cgi?taxid=3702) . Work is currently underway to map many other species' genomes.

### *DNA Technology and Population Studies*

Most of the genome of each species is highly conserved, meaning that the genetic sequences remain constant from individual to individual. An interesting aspect of biological unity is the fact that genomes are highly conserved across species. For example, humans share 85 to 95% of DNA with mice! This molecular connection, and thousands of similar examples, is further evidence that living things are unified by common evolutionary ancestry.

In this lab we will focus on one fundamental biotechnology technique called *gel electrophoresis*. This technique permits us to study differences in a narrow section of a chromosome that we already know provides variation from individual to individual. It takes quite a bit of work to get to the point at which we know a) where traits are located within a given genome, b) whether the alleles for that trait are conserved or variable, and c) what those variations are within a given population or across populations.

### *Biotechnology and Forensics*

*Forensic science*, or *forensics*, is the application of scientific problem solving to the criminal justice system. Forensics includes many tasks that broadly fall into two categories: the investigation of the crime scene and the analysis of evidence. To perform these tasks, forensic scientists are trained in a variety of fields. The sole purpose of forensics is to provide admissible evidence to the court.

For many years, forensics scientists needed to rely on human and animal finger and footprints to determine the presence of a suspect at a crime scene. Savvy criminals used gloves and other equipment to ensure that they could escape the scene of the crime without a trace. With the introduction of PCR and gel electrophoresis techniques, it has become more difficult for criminals to be transparent. Any strand of hair, fleck of skin, or spot of blood or semen may be analyzed for its DNA content.

On television shows such as *CSI*, molecular evidence in the form of DNA fingerprints is highlighted. The admission of such evidence in courts of law became famous in the long and televised trial of O.J. Simpson. What was interesting about DNA evidence in that case was the question of precision. How close does the DNA match need to be before we can prove a suspect's

connection beyond reasonable doubt? What is reasonable doubt in a DNA fingerprint? We are really talking about the statistical likelihood that someone's DNA fingerprint is not actually unique, that another person may have the same genetic variation. This would be the inherent error in the DNA fingerprinting technique. In the O.J. case, the prosecution needed to prove that in the City of Los Angeles on the night of the crime (the defined place and time for the human population in consideration), if the DNA from the crime scene matched O.J.'s, we could rely on the precision of the methods to judge O.J. as guilty. The jury did not buy the prosecution's presentation of the DNA evidence, and O.J. was judged innocent in the final verdict. Even though we have biotechnology at our disposal, they do society no good unless people understand their functions and limitations.

### *The Case of Alaskan Wolves*

Wildlife experts study the relationships of populations other than humans to better understand the effects of processes such as inbreeding or crossbreeding. The organisms we are studying in this lab are wolves found in an isolated part of Alaska. These wolves belong to the species *canis lupus*. This population is rapidly decreasing in size. Explain some possible reasons for the dwindling population size in the space provided:

Forest rangers are hoping to increase the size and vigor of the population by breeding appropriate members of the population. They have currently trapped three wolves, X, Y and Z, and hope to breed either organisms Y or Z (both males) with organism X (a female) to produce the healthiest litter of wolf cubs possible. The scientists want to avoid *inbreeding*. Inbreeding is formally defined as the mating of members of the nuclear family (father/daughter, mother/son, brother/sister). Animal husbandry has utilized a more broad definition of inbreeding to include mating between aunts/nephews or cousins to propagate desired traits within a population. The remainder of this lab will utilize this second definition of inbreeding.

Inbreeding with the intent to obtain desired phenotypes is an old practice that continues today (for example, most flowers sold at florists have been inbred). However, inbreeding negates one of the benefits of sexual reproduction: the introduction of genetic diversity into a population. If two distantly related organisms of the same species mate, they produce offspring with what is most likely a genotype different from either parent. This new genotype could produce new variations that, if environmental conditions changed, could provide the organisms with a competitive edge. Conversely, crosses between closely related organisms can reduce the genetic diversity and therefore reduce a population's ability to withstand environmental change.

If the gene pool of a population contains only a few different alleles for a trait, then over time, all members of the population will start to exhibit similar phenotypes. For example, the number of cheetahs in the African savannah has declined sharply in the last fifty years due to many natural and human-caused factors. Cheetahs have been forced to mate with close genetic relatives. Over time, the number of variations within the population for any given trait has decreased. This lack of variation produces the possibility for extinction. Explain why the lack of genetic variation in the cheetah population could result in possible extinction.

Inbreeding can also increase the frequency of genetic illnesses that appear within a population. Human population studies often utilize isolates to help study this phenomenon. An *isolate* is a community whose members marry within the group for generations. Isolates are useful because 1) they often experience uniform living conditions, 2) they act as a “closed system,” which controls for many factors; and 3) they usually keep excellent genealogical records (generally due to their immense pride in their cultures).

One example of an isolate is the Old Order Amish peoples of Lancaster County, PA. This Amish sect originated in the seventeenth century in Switzerland. As a result of religious persecution, they immigrated to the United States in the early eighteenth century. The members of the sect rarely marry outside of the group and over time have become an isolate. A 1964 study of this isolate determined that several ordinarily rare genetic illnesses occurred with increased frequency within this population as compared to the United States population as a whole. One such recessive disease, Ellis-van Creveld syndrome, a type of dwarfism, was found to occur at a frequency of about 1/14. Among other groups, the frequency of this syndrome is 1/1,000. Propose a reason why this disease occurs at such a greater frequency in this isolate than in the general population as a whole:

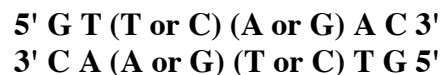
In the case of the wolf population of Alaska, decreasing range and increasing human occupation have resulted in the reduction of the wolf population. The wildlife experts are not concerned with breeding specific traits; they simply wish to produce wolf cubs that have the best chance of perpetuating a vigorous population. The data you gather will allow you to make a recommendation to the experts as to which two animals should be bred, X and Y or X and Z, in order to produce the healthiest offspring.

## Materials and Methods

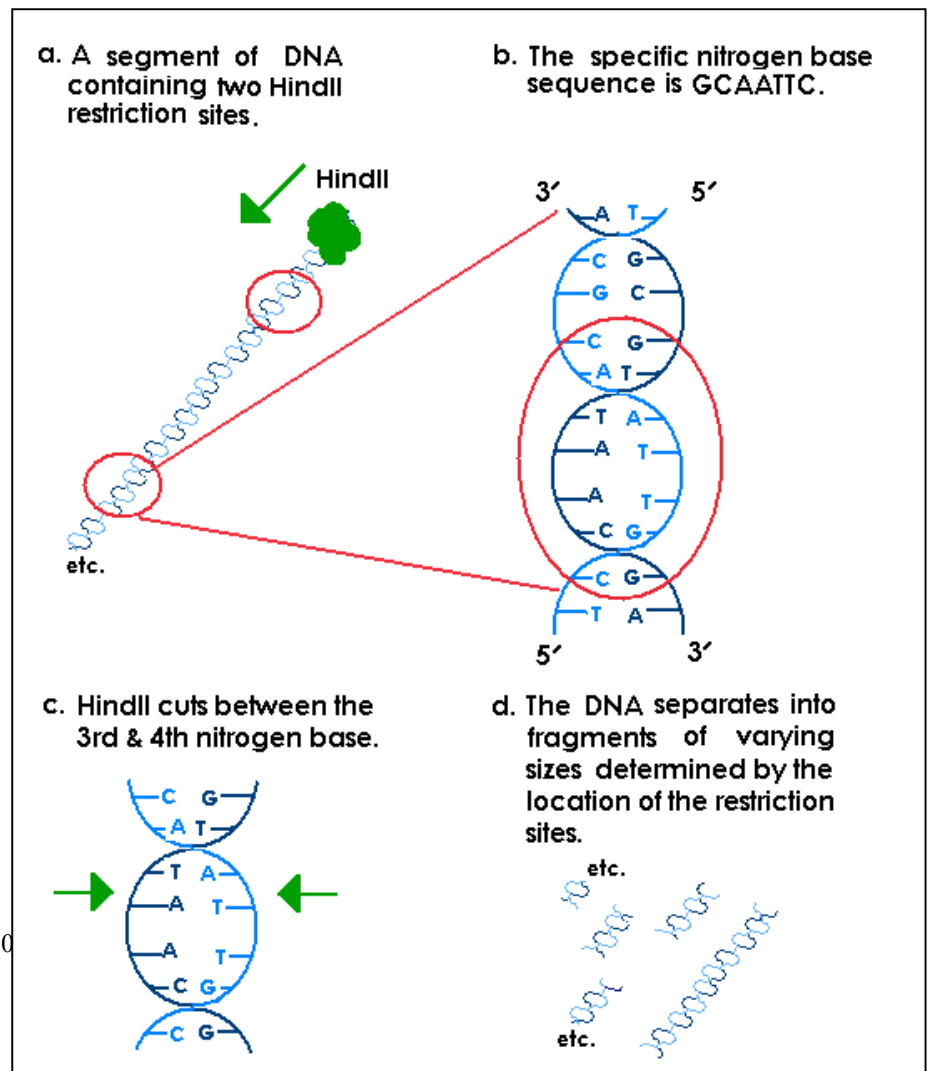
### *Narrowing the Field: Restriction Enzymes*

Three scientists at Johns Hopkins University (H.O. Smith, K.W. Wilcox, and T.J. Kelley) applied the specificity of enzymes to DNA. They found that the bacterium they were working with synthesized a class of enzymes that could recognize particular sequences of nitrogenous bases and would then cut the DNA in these regions and these regions only. This class of enzymes is called *restriction enzymes* because of their restricted behavior.

The particular restriction enzyme they isolated was found in the bacterium, *Haemophilus influenzae*. They named it Hind II, using notation that related its source (Hin- for *Haemophilus influenzae*) and its order of isolation (II for being the second such enzyme to be isolated from the bacterium). A restriction enzyme reads along a molecule of DNA much like DNA polymerase does. Just as DNA polymerase recognizes stop sequences, restriction enzymes recognize sequences of bases that can be 4 to 8 nitrogen base sequences long. This sequence is called a *recognition sequence*. Rather than triggering a “stop”, these sequences trigger the restriction enzyme to cut both helixes of the DNA molecule at a specific point in that sequence. This cut occurs between the phosphate of one nucleotide and the sugar of another along the same helix. The recognition sequence is specific for each restriction enzyme. Hind II recognizes the sequence:



When Hind II comes across the sequence, it cuts the DNA molecule between the 3<sup>rd</sup> and 4<sup>th</sup> nitrogen base within that sequence (See **Figure 1**). No matter what the source of DNA is (animal, plant, fungus, protist or moneran), Hind II will cut DNA wherever this sequence occurs. Where and how often this sequence occurs in a DNA sample determines how many DNA fragments will be created.



The research of H.O. Smith, K.W. Wilcox, and T.J. Kelley has aided our ability to work with discrete sections of DNA rather than entire strands. Since the isolation of Hind II and its use for fragmenting DNA, 900 or so restriction enzymes have been identified and applied to create biotechnologies. For example, if we take a sample of DNA, amplify it via PCR, and mix it with some specific restriction enzymes, the enzymes will cut the DNA creating smaller sequences. This process is often referred to as a *DNA digest*. Can you think of why this may be? Write your thoughts in the space provided.

### *Comparing Gene Segments: DNA Fingerprinting*

Meanwhile, during the 1960's, population geneticists developed a technique called *protein electrophoresis* to describe protein variation on the molecular level, and thus genetic variation, within wild and captive populations. We have already done a protein separation when we separated plant pigments using chromatography in Lab 20. This technique was soon applied to DNA molecules and developed into the DNA fingerprinting techniques we use today. It is based on the idea that restriction enzymes will cut DNA in the same place no matter whose DNA it is. So if we work with regions of DNA that are variable from individual to individual, all we have to do is let the enzymes do their thing, then separate out the resultant segments and compare them. This section explains how that separation works.

A porous, rectangular *agarose gel* is the separation medium. This gel has depressions in it at one end called *wells*. Agarose is derived from algal cells and forms a gelatin-like substance when a solution is cooled. Imagine an Olympic swimming race in which all members of the team are racing at the same time. Each DNA sample to be compared will be loaded into the wells lined up at one end of the "pool." At the starting gun, an electric current is applied to the gel containing DNA samples and the DNA "swimmers" will race toward the other end of the gel. In each *lane*, as it is actually named, the DNA segments begin to separate as the current pulls them along.

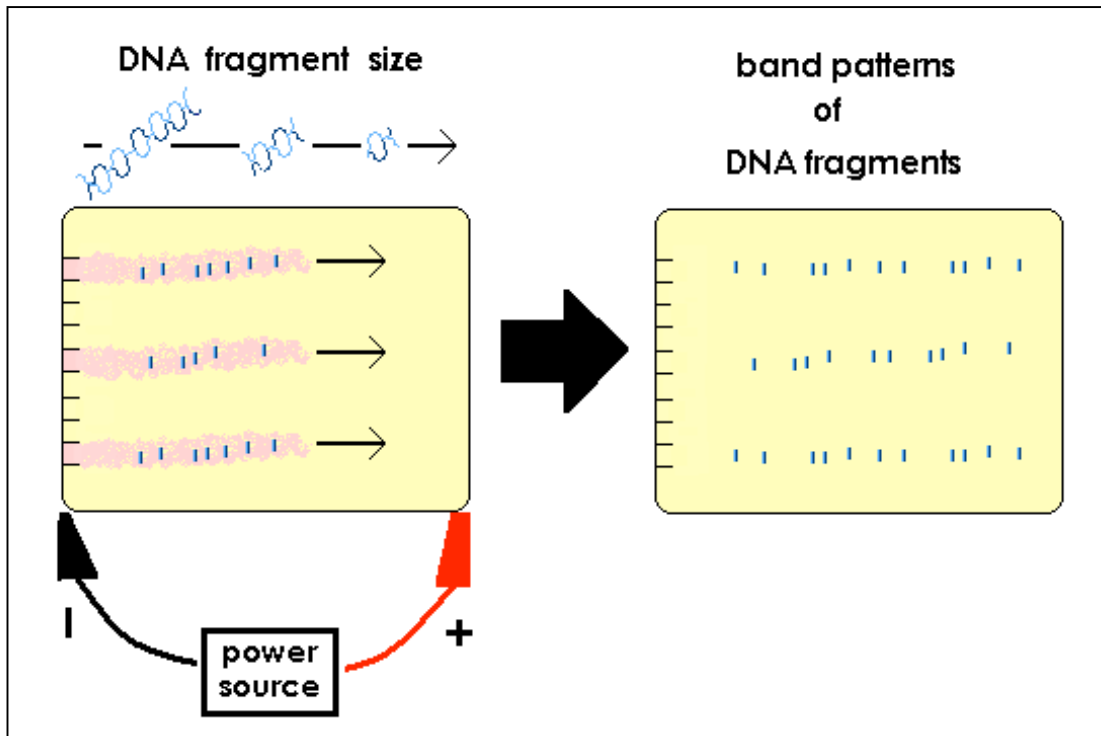
The gel matrix actually behaves like a *sieve*. The smaller DNA segments will fit through the holes in the "pool" better and move through the gel faster. The larger segments in each lane can only move so far. Once the smallest member of the DNA team reaches the end of the agarose pool, the race is over and we stop the current. The result is a pattern of bands in a given lane that demonstrates the sizes of the DNA segments (See **Figure 2** on the next page).

This technique works due to the effectiveness of the electrical current. The DNA has a slight negative charge; therefore, the DNA segments will be attracted to the positive electrode in the electrophoresis chamber.

Recall that each lane represents a different DNA sample, usually from different organisms that we wish to compare. Once samples are run through a gel, distinctive band patterns emerge based on

where the restriction enzyme cleaved the DNA strand. This banding pattern will be unique to each strand of DNA, since each individual nucleotide sequence is a little bit different. The banding patterns can then be compared to other samples. Several samples from the organisms in question must be digested with exactly the same enzymes and run in the same gel to reduce inherent experimental error.

**Figure 2: Electrophoresis**



In forensic science, a suspect's DNA will be compared to DNA found at a crime scene. If the patterns match, then there is a very strong likelihood that the suspect was at the scene of the crime. This banding pattern on the gel produced by the cleavage of the DNA is known as a *DNA fingerprint*. Why do you think it was named this? Write your thoughts in the space provided.

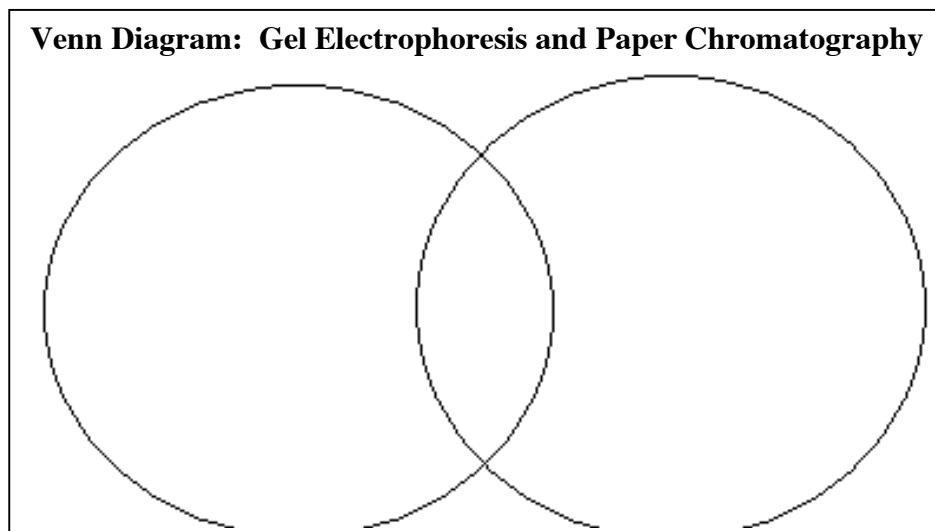
### *Sample Preparation and Use*

Lab #30 presents a problem to solve. We have three organisms from the same wolf population we are calling organisms X, Y and Z. We want to see which two organisms are the most closely related, so that we do not mate them. We take samples of their blood and divide each sample in half. Now we have 6 samples of blood, 2 for each organism. We will digest one sample from each organism with *Restriction Enzyme I* (REI) and the remaining samples from each organism with *Restriction Enzyme II* (REII).

Once the samples have been digested, we add a tracker dye to help us visualize the results and then we can load them into the wells of our gel using a *micropipette*. It is best if we load an organism's samples in consecutive wells, so wolf X's samples occupy the first two wells. We will load the remaining samples and then hook up the electrodes to run the current through the gel.

When the run is complete, we then need to make our results visible. This involves staining the gels. In previous labs and activities we have viewed prepared slides under the microscope. Most of these slides have been stained to help us see the structures we are looking for. For example, in Lab #7 you viewed cells from different Kingdoms. In order to see different organelles, the specimens were stained. If no stain had been used, we would not have been able to distinguish nuclear material from other materials. In the case of DNA gel electrophoresis, unstained DNA samples would basically appear as the color of the gel, making them invisible. *Methylene blue* is used to help us see the banding patterns. Once stained, the gel can be placed on a light source and the bands of DNA will be visible for comparison.

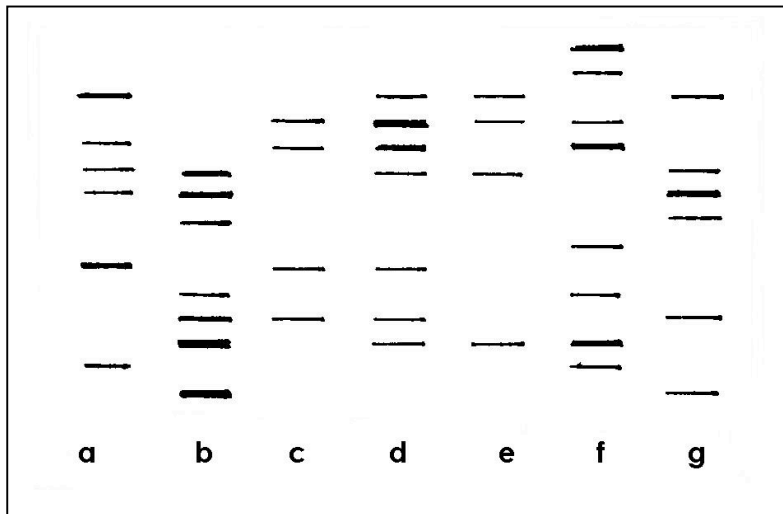
In many ways, DNA and protein electrophoresis are similar to the paper chromatography technique we employed in Lab #20. With paper chromatography, the paper strip was the medium through which pigment particles travel based on size and solubility. In DNA electrophoresis, the agarose gel is the medium through which charged particles travel based on size. Complete the Venn diagram comparing DNA gel electrophoresis and paper chromatography.



In the paper chromatography lab we completed, we only looked for the breakdown of pigments. However, it too could have been used to identify an unknown or to compare samples. **Figure 3** is a

sample DNA fingerprint. Take a moment and determine which lanes of DNA most closely resemble one another. Circle the two lanes that are the closest matches.

**Figure 3: Sample DNA Fingerprint**



Think about what information from your results will help you make your recommendations to the forest rangers as to which two wolves to breed. How will you use your results from this lab to help the rangers make a good decision?

**Materials (per group)**

- gel electrophoresis setup: gel boat, rubber dams, gel combs, electrophoresis chamber, chamber cover with electrodes, power source
- micropipette and tips
- 30mL of 55°C Agarose
- electrophoresis buffer solution
- waste beaker
- 30 to 40mL Methylene Blue Stain
- 200mL of distilled water
- 1 re-sealable plastic bag
- light source for visualization
- prepared DNA samples A-F
  - Sample A: Wolf X (female) DNA digested with restriction enzyme #1
  - Sample B: Wolf X (female) DNA digested with restriction enzyme #2
  - Sample C: Wolf Y (male) DNA digested with restriction enzyme #1
  - Sample D: Wolf Y (male) DNA digested with restriction enzyme #2
  - Sample E: Wolf Z (male) DNA digested with restriction enzyme #1
  - Sample F: Wolf Z (male)DNA digested with restriction enzyme #2

**Part A—Pouring Gels (completed by the instructor prior to the lab period)**

1. Obtain a gel bed, comb, and masking tape or rubber dams.
2. Close off the open ends by carefully placing a rubber dam on each end make sure it is seated securely. If you are using masking tape, take a  $\frac{3}{4}$  inch wide piece of tape. Place it smoothly across the end so the bottom of the tape seals the bottom edge of the bed. Fold the edges of the tape over the sides and bottom and press firmly so that the contact points are sealed.
3. Place the well-plate comb in the notches closest to the end of the bed. The comb should sit evenly but not quite touch the bottom of the gel bed.
4. Use agarose gel that is cooled to 55°C. Take a transfer pipette and carefully deposit a small amount of cooled agarose to the inside seam of both ends. Wait about 1 minute for the agarose to solidify.
5. Carefully pour cooled agarose into the gel bed until about  $\frac{2}{3}$  of the height of a comb tooth has been reached. Allow the gel to cool.

**Part B—Practicing Loading a Gel**

6. Obtain a practice gel and DNA sample; you will be sharing a micropipette with your lab group.
7. Carefully cover the gel with the practice buffer solution—the gel should be completely submerged.
8. Decide from which direction you wish to load the gel wells and make note of this. Generally wells are loaded from left to right.
9. Open the cap to the sample tube. With your eyes on the dial of the micropipette, rotate the adjuster slowly and smoothly to the desired volume. Make sure you know where the decimal point is on your instrument before starting. For this lab your desired volume is 30 $\mu$ L.
10. Holding the micropipette vertically (straight up), firmly and smoothly place a tip onto the end. Basically you are inserting the end of the micropipette into the top of a plastic tip in the box.
11. Once you have a tip, depress the plunger. This is like squeezing the bulb on a dropper before withdrawing liquid from a dropper bottle. Most micropipettes have “two stops” when depressing the plunger. What this means is that you will press down until you feel resistance, you will then be able to press more. The second stop generally discharges the tip so you do not wish to go that far when loading the sample or your will lose your tip.
12. Once you have depressed the plunger insert the tip into the sample tube. Remember, only the tip only touches the fluid and the fluid touches only the tip.

13. Release the plunger and the sample will be drawn up into the tip. It is important at this point that you keep the micropipette vertical so the sample does not run into the barrel of the instrument.
14. Carefully move the micropipette to the sample gel. Gently insert the tip of the micropipette through the buffer and into the first well at an angle. Be careful to not puncture the gel. You will most likely use two hands to do this.
15. Once the tip is in the well, start to press down on the plunger to let the sample into the well.
16. Once the plunger is completely depressed and the sample has been loaded KEEP THE PLUNGER PRESSED IN and remove the micropipette from the well. If you take your thumb off the plunger while the tip is still in the well, the micropipette will SUCK THE SAMPLE RIGHT BACK UP! NO GOOD!
17. Discharge the tip into the labeled waste crock by depressing the plunger to the second stop.
18. Repeat this procedure for each additional well.

***Part C—Loading gels with DNA samples***

19. Ensure that the gel is completely solidified and remove the rubber dams or masking tape.
20. Carefully remove the comb by slowly pulling straight up using two hands. You do not want to tear the wells.
21. Place the gel, still in its gel bed, in the electrophoresis chamber. It will need to be oriented with the wells at the anode as well as centered and level on the platform.
22. Carefully pour buffer solution into the chamber until the gel and bed are completely submerged with solution. Depending on the size of your chamber, this could take anywhere from 200 to 500mL of solution.
23. Obtain your six DNA samples from the warm water bath.
24. Load your DNA samples A-F using the techniques you developed in Procedure Part B. Sample volumes will again be 30  $\mu\text{L}$ . Be sure that samples are loaded consecutively from left to right. All group members should take a turn.

***Part D—Running the Gel***

25. After the samples are loaded, carefully snap down the cover, making sure that the cathodes and anodes are properly aligned.

26. Insert the electrode leads into the power source. Pair your source and leads carefully; generally black pairs with black/negative, and red pairs with red/positive. Remember: the DNA wells should be located closest to the negative lead.
27. Set the power source to “high.”
28. Turn the power source ON and check to see that the current is flowing properly: you should see bubbles forming at the electrodes.

The gel will run for the amount of time allotted by your instructor, which will be enough for the tracking dye to travel about 4 cm for adequate separation of DNA bands. *Your instructor will turn off the power source when the DNA samples are done running.*

**Part E—Staining with Methylene Blue (completed by the instructor following electrophoresis)**

29. Wearing gloves, pour Methylene Blue solution overall gel, until gel is completely submerged.
30. Allow the gel to sit for 45 minutes to an hour.
31. Heat 100 to 200mL of distilled water to 37°C.
32. After 15 minutes, transfer the gel to a large weigh boat and dispose of the sheet or stain solution.
33. Submerge the gel in 37°C water for ten minutes. Gently shake occasionally.
34. Pour off the water into the waste crock.
35. Repeat Steps 54 and 55 two more times. As you do so DNA bands should start to emerge. Place in sandwich bag.

**Part F — Visualizing**

36. Remove gel from sandwich bag and hold the gel up to the provided light source.
37. Sketch the visible bands of DNA in your lab notebook.
38. Dispose of the gel and stain rinse as instructed.

**Post-Lab Activities**

1. Construct a well-written paragraph that explains the genetic disadvantages of inbreeding.
2. Explain which two organisms are more closely related and how you know. Then, make a recommendation to the ranges as to which two organisms should be mated.
3. If you had an opportunity to further explore any of the concepts addressed in the lab, which ones would you choose and why?