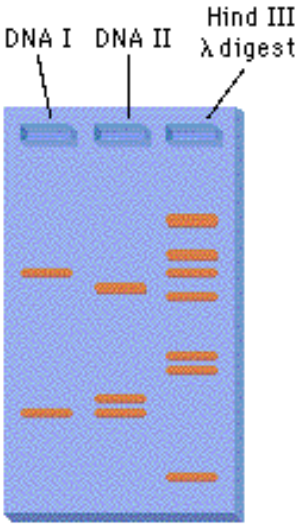
COLLEGE OF THE CANYONS INTRODUCTION TO BIOTECHNOLOGY: CUSTOM LAB



GEL ELECTROPHORESIS AND DNA ANALYSIS LAB

Version 7-5-12

- **One of the most basic and frequently used tools of the molecular biologist is electrophoresis.**
- **In this experiment, you will be using agarose gel electrophoresis to separate DNA fragments of different sizes.**
- **Electrophoresis means "to carry with an electrical current".**
- **It is possible to do gel electrophoresis with DNA because the DNA molecule has an overall negative charge.**
- **More specifically, the negative charge is directly proportional to the mass of DNA. This stable charge to mass ratio makes DNA an ideal candidate for gel electrophoresis.**
- **The different DNA fragments separate according to their size because the smaller fragments migrate farther than the larger ones which experience more friction from the agarose network of the gel.**

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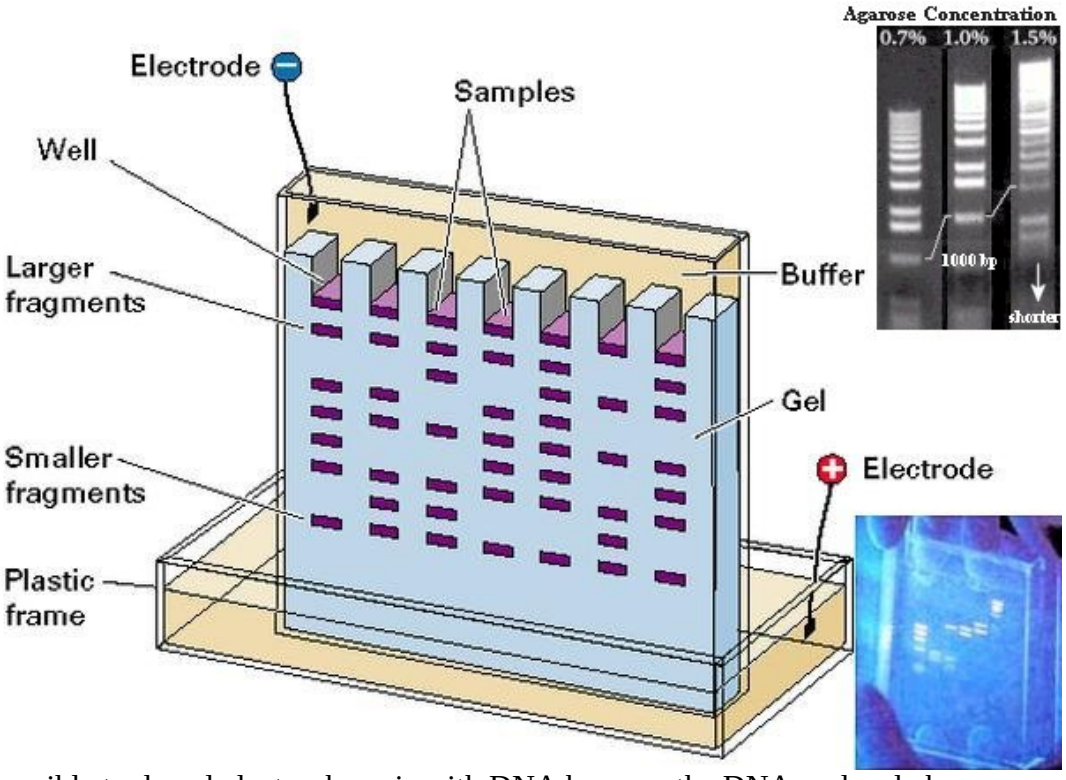
I. Objectives:

1. To have students review agarose gel electrophoresis as a tool for separating DNA fragments
2. To allow students to investigate some of the factors affecting electrophoresis of DNA including: voltage, % agarose, fragment size, staining resolution, and linear DNA vs. circular DNA fragments.
3. Review semi-log plotting, limits of stain resolution and range of agarose percentages in relation to fragment size separation.

II. Background:

One of the most basic and frequently used tools of the molecular biologist is electrophoresis. In this experiment, you will be using agarose gel electrophoresis to separate DNA fragments of different sizes. Electrophoresis means "to carry with an electrical current". What you will observe is that different sized pieces of DNA will be carried different distances by an electric current as the pieces migrate through agarose (a Jell-O like substance derived from seaweed) which you will apply in its melted state to a gel rig. The gel in the rig will be placed in an electrophoresis chamber filled with a buffer solution that contains the ions needed to conduct electricity. You will place your DNA samples into small wells cast at one end of the gel. Current supplied by electrodes at either end of the chamber creates an electric field across the gel. The DNA will migrate through the pores in the agarose toward the other end of gel. The diagrams below should prove helpful.



It is possible to do gel electrophoresis with DNA because the DNA molecule has an overall negative charge. The negative charge is due to the phosphate groups that alternate with the sugar, deoxyribose, to form the "rails" of the "twisted ladder" which makes up the double helix. When the current is turned on, the negatively charged DNA migrates along with other anions

away from the electron-rich negative electrode toward the electron- poor positive electrode. The different DNA fragments separate according to their size because the smaller fragments migrate farther than the larger ones that experience more friction from the agarose network of the gel. After separating the fragments, you will turn off the current and remove the gel. Agarose % ranges from 0.5% to 5%. The gels are very fragile and can separate larger pieces of DNA. 5% agarose is tougher and can separate much smaller fragments of DNA. 5% is about the upper limit of solubility for most agarose plates with 0.5% being the lower limit of solubility. The Table below correlates the relationship of % agarose solution to DNA fragment size.

Agarose gel concentration required for DNA separation		
% agarose concentration	Grams/100ml buffer	DNA fragment size (Kbp)
0.5	0.5	1-30
0.75	0.75	1-12
1.00	1.00	0.5-10
1.25	1.25	0.4-7
1.50	1.50	0.2-3
2 to 5	2 to 5	0.01-0.5

Note: Fragments cover a range from 100's to 10,000's of base pairs, so our %Agarose will go from the "low end" (0.8%) to a middle amount (2%).

While electrophoresis has many applications in state-of-the-art molecular biology laboratories, one of its most interesting and important uses is in the courtroom with a technique called DNA fingerprinting. DNA fingerprinting can reveal a DNA pattern which is unique to an individual and hence link a suspect to a particular piece of evidence with an extremely high degree of certainty. Since its first use in a l987 Oklahoma murder trial, it has been used in several thousand criminal cases and in thousands of disputed paternity cases. For example, a hair sample found at the scene of a crime is taken to a forensic laboratory. There the DNA in the cells in the root of the hair is extracted and then is cut into pieces by special bacterial enzymes called restriction enzymes. Restriction enzymes do not cut the DNA randomly but cut at specific base pair sequences called recognition sequences. There are several hundred restriction enzymes available, and each has a specific recognition sequence that is usually composed of four to twelve base pairs. For example, the double stranded recognition site for the enzyme EcoRI is The lengths of the fragments generated by a restriction enzyme digest depend upon the number

of cuts made in a given piece of DNA and the location of each recognition sequence. An individual's DNA is as distinctive as a fingerprint, since restriction enzymes cut each person's DNA into different sized pieces. The different sized pieces result from cutting non-coding regions of the chromosomes called simple sequence DNA that probably serves to maintain the structural integrity of the chromosomes. Each person's simple sequence DNA cuts uniquely with restriction enzymes. When the cut DNA is separated into its pieces by electrophoresis, the visible end result looks something like the bar code on a super market package. The DNA from the root of the hair can then be compared with the DNA obtained from one or more suspects. A sample of the victim's DNA is also run as a control.

<u>Use the practice problems (next page) to see an example of DNA fingerprinting.</u>
<u>The problems and accompanying information are FYI and should NOT appear in your lab notebook.</u>

The practice examples are vastly over-simplified. In actual criminal cases, several restriction enzymes may be used resulting in complex patterns consisting of dozens of DNA bands. The probability of any two individuals having identical band patterns is then computed. Usually the probability of two individual having the same bands is approximately one in 10^{19} which is many times the world's population. It is important to note that this method will also free an innocent suspect.

➢ FORENSIC SCIENCE - RAPE CASES

Assume that you are a molecular biologist involved in forensic medicine. Two women have been raped within a span of 2 weeks and you have been given the following evidence relating to the crime: Blood samples from both women, semen collected on each victim, and blood samples from three possible suspects. You purify the DNA from each sample, cut the DNA's with restriction enzyme and then perform agarose gel electrophoresis on the DNA as follows:

Well 1 -- Blood from victim A

Well 2 -- Blood from victim B

Well 3 -- Semen collected on victim A

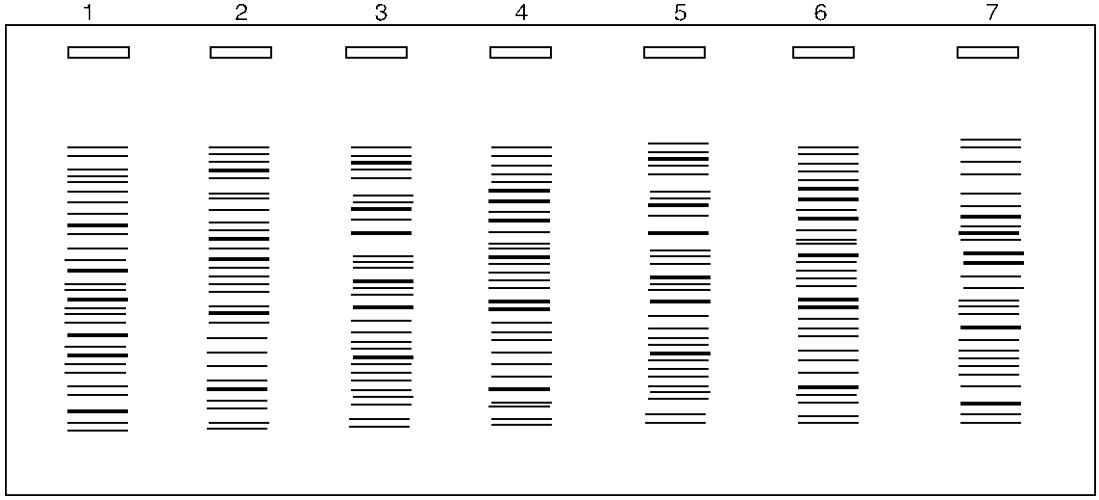
Well 4 -- Semen collected on victim B

Well 5 -- Blood from suspect X

Well 6 -- Blood from suspect Y

Well 7 -- Blood from suspect Z

Results from the electrophoresis of DNA after cutting with restriction enzyme: (next page).



Questions:

1. A. Did the same man assault both women? _____

   B. Explain your reasoning.

2. A. Which, if any, suspect or suspects, is/are involved?

B. Explain your reasoning.

- **OVERVIEW OF THE EXPERIMENT:**

The experiment will take two lab periods.

1. In the first lab you will prepare a range of samples. Linear and circular DNA will be assessed before and after digestion with restriction enzyme This process will take about 2 hours and during the "breaks" in lab students should take a few minutes to familiarize themselves with a gel imaging program, how to take photographs using the program, and how to save a gel on a jump drive.
2. The gel solution will be calculated by the student and a DNA visualizing agent (cyber) added by the instructor.
3. In the next lab you and your partner will pour two agarose gels with 10 wells each. The stain "Cyber green" will be added during the pouring phase.
4. After the gels have hardened you will place the gels in electrophoresis chambers that contain an electrophoresis buffer.
5. Loading dye will be added to your digested DNA samples and some predigested "standards" (AKA Ladders). All samples will then be loaded into the wells and the current will be switched on.
6. After a specified amount of time, you will turn off the current, remove your gels and stain the DNA samples with cyber green.
7. You will compare your DNA gels with other groups in lab and discern the effects of the various electrophoresis parameters (voltage, time and percent agarose) on gel integrity.

Day one procedures

1. <u>Cutting the DNA into fragments with restriction enzymes.</u>
    a. Get 16 1.5 ml tubes and label according to below matrix. Also include group initials and realize there are two sets of tubes. (See below).
    b. Use the matrix below as a checklist. Read down each column, adding the reagent to the appropriate tubes for 1-8 in each. Use a fresh tip for each new reagent. Create another set of tubes as noted below except change first number to 2 (e.g. 2C1, 2C2…).

Tube:	DI Water	Plasmid	2X Buffer	Standard	Enzyme
1C1		4 uL	7 uL 2x		2 uL HindIII
1C2		4 uL	7 uL 2x		2 uL HindIII
1B1	8 uL		1ul 10x	4 uL λ /BSTE	
1H1	8 uL		1ul 10x	4 uL λ /HindIII	
1LDR1	8uL		1ul 10x	4 uL KB ladder	
1LDR2	8uL		1ul 10x	4 uL KB ladder	
1P1	8 uL		1ul 10x	4 uL PBR 325	
1L1	8uL		1ul 10x	4 uL λ DNA	

c. Close the tubes and spin to collect in microfuge for 2 minutes. Place all 4 "C" tubes into a floating microfuge rack and place rack into 37 oC water bath for 1 hour. Use the incubation time wisely by familiarizing yourself with the gel imaging device, review your notebook to see if it is up to date. Revisit semi-___ plotting (either by hand or by excel).

d. Place all 16 tubes into rack and place in freezer (-20ºC) until next lab.

DAY 2 START:

2. Pouring the agarose gels. *Note: each group will make two gels. One with 2 % agarose and one with 0.8% agarose.The gel rigs hold about 40mls, the cyber green works at a concentration of 1.0 ul/10ml of agarose. So 4ul of cyber green will be added. Show the professor your math for both gels.*

   a. Set rig trays (2) on a smooth level section of lab bench with the combs (8 tooth) in place and gates up and locked (screws should be finger tight, do not over-tighten)!

   b. Obtain the previous prepared liquefied agarose solutions containers (one for the 2% agarose and one for the 0.8% agarose) and pour the gels by adding all 40 mls to the rig. The agarose should fill the rig to a height about 3 mm from the top of the rig. *Be careful not to create any air bubbles in the gels when pouring.*

   c. Allow the agarose solution to cool and solidify for at least 10 minutes or until cloudy.

3. Preparing for the electrophoresis combine agarose and 1x TAE buffer.Do not shake or swish the mixture as any agarose will not come off of the side of the flask. Microwave to dissolve. Place in the microwave for 30 seconds initially. Repeat for another 30 seconds or so until the solution begins to boil. Immediately remove the flask and swish the mixture. Inspect if all the agarose powder has dissolved. Repeat this step until the cloudy

mixture becomes as clear as water. Make sure to use heat proof mittens. Lastly let the agarose cool to exactly 85 degrees Celsius by placing in the bath. Add exactly 1.0ul/10ml of agarose of cyber green and mix. If you initially had 40ml of agarose you will be adding 4ul of cyber green.

a. Add 4μL of loading dye to each tube with micropipette using a new pipette tip for each tube. A total of 16 tubes should be set up. There are 4 digested plasmid samples, 2 undigested plasmids, 2 undigested λ and 8 ladders.

b. By now the gel should be solidified. It will appear cloudy. Press down on the gel rig and gently lift the comb.

c. Lift the tray with the gel and place onto the platform of the gel box with the wells toward the negative electrode. ***Make sure the gates are down!***

d. Add electrophoresis buffer to the chambers at the ends of the gel box until the buffer just covers the surface of the gel.

e. Set the micropipette to 17μL. Spin all 16 samples to collect all contents at bottom of the tubes. Add samples quickly and confidently from left to right into 0.8% gel. Leave first and last cones empty. Record lane positions on the provided gel diagrams (second to last page).

Lane 1: 1C1 - Dig. Plasmid	Lane 5: 1LD1 – KB ladder
Lane 2: 1L1 – undig. λ	Lane 6: 1C2 – dig. Plasmid
Lane 3: 1B1 – λ w/ BSTE	Lane 7: 1H1 – λ dig. w/ Hind
Lane 4: 1P1 – undig. plasmid	Lane 8: 1LD2 – KB ladder

4. Running the gel.

a. Close the lid of the gel box without disturbing the buffer. Be sure that there is no spilled liquid on the table under the gel box, wires, or power supply.

b. Turn on the power supply. Adjust the power supply to either 100 or 200 volts. (See instructor for voltage). Note the time. Look to see if bubbles are being produced at the electrodes in the gel box and that the colored dyes in the loading dye are migrating. Now load 2 % gel in similar manner (as noted in step E). Once samples are loaded, turn off power supply, plug in gel box and restart power supply. If you are running at 100 volts you will stop the gel in 40 min. If you are running at 200 volts you will stop the gel at 20 minutes.

c. Turn off the power supply after the correct amount of time. The bluish dyes in the loading dye will have migrated about 1/2 and 3/4 of the distance to the end of the gel. These dyes are not DNA, but they help you to tell at a glance if the electrophoresis has taken place.

d. Remove the lid of the gel box.

5. Photographing DNA in the gel.

   a. Transfer the gels to labeled trays. Note: ensure that you are wearing gloves! Label the bottom (tops are easily misplaced) of the gel tray with your group name, percent agarose, running time and voltage across a piece of tape.

   b. Photograph each gel four times (with help from instructor) if needed. Take your set of 8 gels (ensure you have 4 gel images/ per person) and completely label the lanes and gel treatment (see step 3-E above). Exchange a set of gel images with another team so that each person has the following gel images: (100V, 0.8 %, 40 min.), (100 V, 2%, 40 min.), (200 V, 0.8%, 20 min.), and (200 V, 2%, 20 min.). Put the labeled gel images (all 4) into the results section of your lab notebook.

   c. Discard or save the gels as instructed.

   d. Different dyes possible for staining: Methylene blue 10ug, ethidium bromide 1ug, cyber green 1ul/10ml.

III. Post-Lab Questions/Activities:

The following post lab questions are for your benefit. The questions will help you to address a range of topics relating to the lab activity. Along with the post lab handouts, these questions will help to ensure that you have both correct information regarding the lab data and crucial lab processes. Complete the post lab questions at the end of the lab and post lab handouts (keys for both of these are available from your instructor) before making any lab-notebook entries.

1. Why were your predigested samples slightly different in appearance to the samples you digested in lab?

2. Identify two gel lanes that are consistently good between all four gel samples. In a table format describe the effects of: time, voltage and percent agarose on the appearance of the gel image.

3. Using the provided semi-log paper and the data from the DNA ladder lanes, create a graph of distance migrated verses molecular weight. Use the linear axis for the distance migrated from the well and the molecular weight on the log axis. To do this, measure the distance from the gel well to the different DNA bands in mm. Using provided images, identify the weight of the DNA fragments. Create a list of distance migrated and accompanying weight of DNA (in units called base pairs or BP). On the linear access, put the distance migrated, and on the log access the MW in BP. See the instructor if you have questions about these steps. Draw dots at the intersection of these two axis, and draw a best fit line. You are encouraged to try this on Excel as well, but also do this by hand to ensure your understand the log axis. Now, using interpolation, predict how far a piece of DNA of 5,500 base pairs long would migrate. How big a piece of DNA would migrate 2.7 cm? How big were the pieces of DNA from the plasmid? BE SURE TO USE THE GRAPH TO SHOW YOUR ANSWERS TO THIS LAST QUESTION BY INTERPOLATION.

   Repeat above process using excel.

4. Why did the digested plasmid move at a different rate than the undigested plasmid?
NOTE: - The digested plasmid was cut just once making the fragment linear. Originally it was a loop.
- Why 3 different sizes? (loop, super coiled and dimers)
- Circular plasmids may form loops, super-coils or dimers.

IV. Notebook Entries:

Data from the lab should be the focus of this section and if there are any incorrect results, you should discuss this as well as expected results. Section V will contain both your results and discussion. Your data should drive the discussion. An informed discussion is dependent on understanding the post lab questions/activities.

Your intro should:

- Define gel electrophoresis.
- Explain why DNA is ideal molecule for this technique
- Factors being investigated.
- Technique of molecular mass determination.

Results should be:

- 4 gel images (completely labeled).
- Excel graph and interpolation of cut plasmid mass.

Discussion should consider the following:

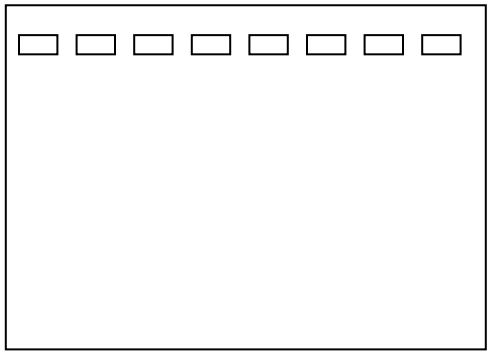
- Reference images very specifically and discuss each parameter specifically.
- Example: "voltage effects can be seen by comparing the 200 volt gel to the 100 volt gel. Smearing is noted in 100 volt lanes implying that low voltage decrease gel clarity".
- Please note: previous information is NOT CORRECT, it is just an observation.
- Discuss the molecular mass determination.

The previous lab protocol can be reproduced for educational purposes only. It has been developed by Jim Wolf, and/or those individuals or agencies mentioned in the references.

References:

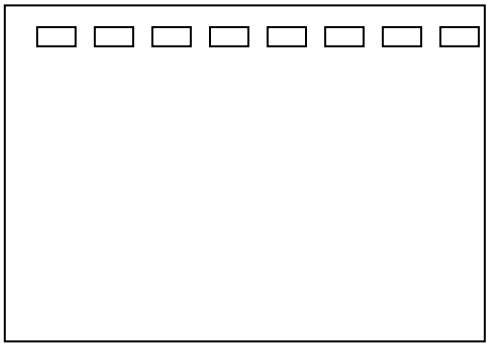
California Lutheran University Enriched Science Program: www.clunet.edu

100 volt, 40 min, 0.8%.



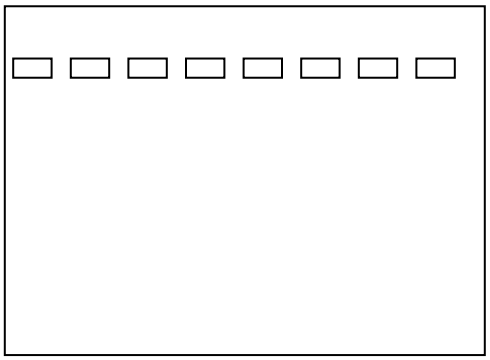
0.8%

100 volts, 2%, 40 min



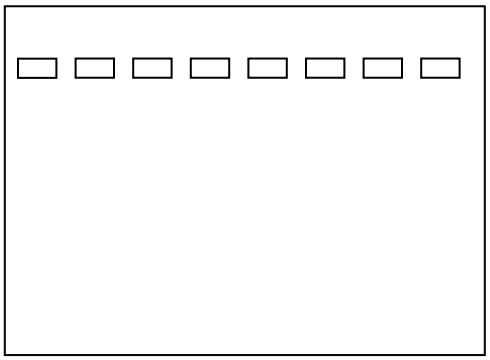
2%

200 volt, 20 min, 2 %



0.8%

200 V, 0.8%, 20 min



2%