GEL ELECTROPHORESIS OF DNA

Readings: Review pp. 352-354, 363-364, 369-370, & 380-381 in your text (POHS, 5th ed.).

Introduction

Gel electrophoresis is a technique that allows mixtures of molecules to be sorted into homogeneous populations separated by differences in length, shape or charge. In all types of electrophoresis, an electric current is passed through a gel which is in contact with a defined buffer solution within a chamber. The molecular mixture is loaded into a well formed in the gel. (While the gel is still liquid, it is poured into a mold; this is called *casting* the gel. A *comb* is inserted into the liquid cast gel; when the gel has solidified, the *comb* is removed, leaving a series of *wells*.) Once the mixture of molecules has been placed in the well, a current is applied to the system, and the molecular mixture enters (or *migrates*) into the gel. The smaller molecules migrate faster, and therefore further, into the gel. The type of solidifying agent (*gel*) used will also determine which subpopulation of molecular species will be separated.

Two gel systems are used extensively in basic research and in applied research (for example, medical diagnosis and forensic analysis): polyacrylamide and agarose. Polyacrylamide gels are typically rigid and are generally used to separate mixtures of proteins or small nucleic acids (tRNAs and DNA fragments smaller than 300 base pairs [bp]). Agarose gels are generally used to separate larger molecules because the pore size of the gel is larger; mRNAs and DNA fragments between 300 bp and 25,000 bp (or 25 kilobase pairs [kbp]) are typically separated on this kind of gel. The experiment in this exercise uses an agarose gel.

DNA fragments migrate through agarose gels based strictly on size because the charge density of each fragment is essentially the same. This net charge comes from the phosphate groups that alternate with sugar molecules to form the phosphate-sugar backbones of the double helix. Genomic DNA molecules, if extracted carefully from cells, are enormous macromolecules several hundred or thousand kbp long. Molecules of this size are too big to separate electrophoretically. In order to be able to use gel electrophoresis with DNA, the DNA must be cut, or *fragmented*, into smaller pieces. *Restriction endonucleases* are enzymes (...ase) able to cut specific (*restricted*) DNA (...nucle...) targets within a molecule (endo...), thereby producing specific fragments. Each restriction

endonuclease (at least 100 different ones have been discovered) cuts both strands of DNA whenever it finds its target sequence. The *target sequence* is a unique, linear array of adjacent base pairs 4, 6, or 8 bp long. The restriction endonucleases used in this exercise are *Eco*RI, *Hind*III, *Hae*III, and *Xho*I; their targets are:

*Eco*RI	*Hind*III	*Hae*III	*Xho*I
5'...GAATTC..3' 3'...CTTAAG..5'	5'..AAGCTT..3' 3'..TTCGAA..5'	5'..GGCC..3' 3'..CCGG..5'	5'..CTCGAG..3' 3'..GAGCTC..5'

Because DNA fragments migrate through agarose gels according to their lengths, a standard curve can be produced by graphing the migration distance vs. the fragment length in kbp of DNA fragments of known length. The DNA sequence of two bacterial viruses (*bacteriophages*) has been completely determined; this information allows us to predict where and how often any restriction endonuclease will cut the DNA. Digestion of these viral DNAs by various restriction endonucleases yields a population of molecules having known lengths. These DNA fragments are called *markers* and are loaded into one well per gel. The other wells can then be loaded with DNA fragments of unknown sizes. After electrophoresis is complete, a standard curve can be generated using the marker lane, and the unknown DNAs can be sized. A lane of markers is included in each gel to act as a reference for sizing other DNAs run in the same gel, because each gel varies slightly. See Appendix C for information about DNA size markers.

LAB GOALS

- Learn how to generate a standard curve and size unknown DNA fragments.
- Learn how to interpret data produced by agarose gel electrophoresis of DNA fragments.
- Examine the molecular model of the DNA double helix and become familiar with its structure.

EXPERIMENTAL PROTOCOLS and DATA ANALYSIS

*Note: Begin Protocol 2 first. While your gel is running, begin and complete Protocol 1.

Protocol 1. Work individually. You will be provided with a xerox copy of a photograph of DNA fragments on a typical agarose gel as visualized on an ultraviolet transilluminator. The DNA fragments in this gel have been stained with

ethidium bromide, a dye that intercalates between adjacent stacked bases in DNA. Ethidium bromide fluoresces in the ultraviolet range thereby producing the bright bands seen on your xerox. You will also be provided with a ruler and a sheet of 3 cycle semilog graph paper. Measure how far each of the Lambda/Hind III marker fragments migrated (measure from the well to the bottom of each fragment). Note these measurements in the following table.

Hind III fragment	Distance migrated from well
23.2 kb	
9.3 kb	
6.6 kb	
4.4 kb	
2.3 kb	
2.0 kb	
0.5 kb	

To generate a standard curve, plot the distance migrated on the horizontal axis vs. the DNA fragment lengths on the vertical axis. Expand the horizontal axis to make it as wide as possible. Set the "1" of the cycle closest to the horizontal axis equal to 100 bp (or 0.1 kbp). The "1" of the next cycle would then be equal to 1000 bp (or 1 kbp), and so on. Mark dots corresponding to the intersection of fragment size and distance migrated. Connect the dots by drawing a line from point to point – your line will be curved both at the top and at the bottom. Let your instructor check your curve before proceeding. Next, determine the sizes of the DNA fragments in the other lanes.

Data analysis for Protocol 1.

1. Compare the accuracy of sizing very small or very large DNA fragments to DNA fragments that run approximately in the middle of the marker size range.
2. Examine the target sequences of the restriction endonucleases used in this exercise; look at the sequences of both strands reading from 5' to 3'. What structural anomaly is shared by all of them?

Protocol 2. Work in groups.

1. You will be provided with tubes of DNA marked 1 through 10 and with a tube of DNA size markers. You will also be provided with a "practice gel" and a tube of loading dye which is a solution of glycerol and bromphenol blue.

2. Your instructor will demonstrate how to load your DNA samples into the wells in an agarose gel. Practice with a pipetteman and loading dye until you feel confident you can load your sample DNA into a well accurately.
3. Make sure each gel has at least one marker lane and one each of the unknown DNAs (1-10). Record which DNA is loaded into which well.The gels used in this exercise are 1.0% agarose in 0.5X Tris borate buffer, pH 8. Once all the DNAs are loaded, adjust the power source to 100 V.
4. Run the gel until the blue dye is almost at the bottom of the gel. *Turn off the power source* and then **carefully** remove the gel – it will be very slippery and will break if it is dropped.
5. Slip the gel into a glass dish of methylene blue staining solution; your instructor will help you determine when the gel is adequately stained. (Ethidium bromide is usually used as a DNA stain in agarose gels. We are not using EB in this exercise because it is a mutagen and requires elaborate disposal procedures.) Stain your gel for **exactly** 4 minutes.
6. Transfer your gel to a glass dish of destaining solution (deionized water) and gently agitate the gel. Change the water as soon as it starts to turn blue. Destain the gel in at least four changes of water. Monitor this process carefully. You want to remove the stain from the gel where there is no DNA without stripping it off the DNA as well. When you can resolve the marker fragments, remove your gel and place it on a square of Whatman 3MM filter paper.
7. Measure the migration of all the marker fragments, generate a standard curve as in Protocol 1, and then measure the migrations of all of the other fragments in the gel – make sure you know which DNA sample gave which size DNA fragment(s).
8. Place the gel on the Whatman filter paper on a stack of five paper towels on a glass sheet. Overlay the gel with a piece of Saran wrap, another glass sheet and a finger bowl full of water. The next day, this apparatus will be dismantled and the paper-thin gel sandwiched between the Saran and the filter paper will be placed outside the lab for you to view. If needed, you can re-measure the migrations of the DNA fragments at this point.

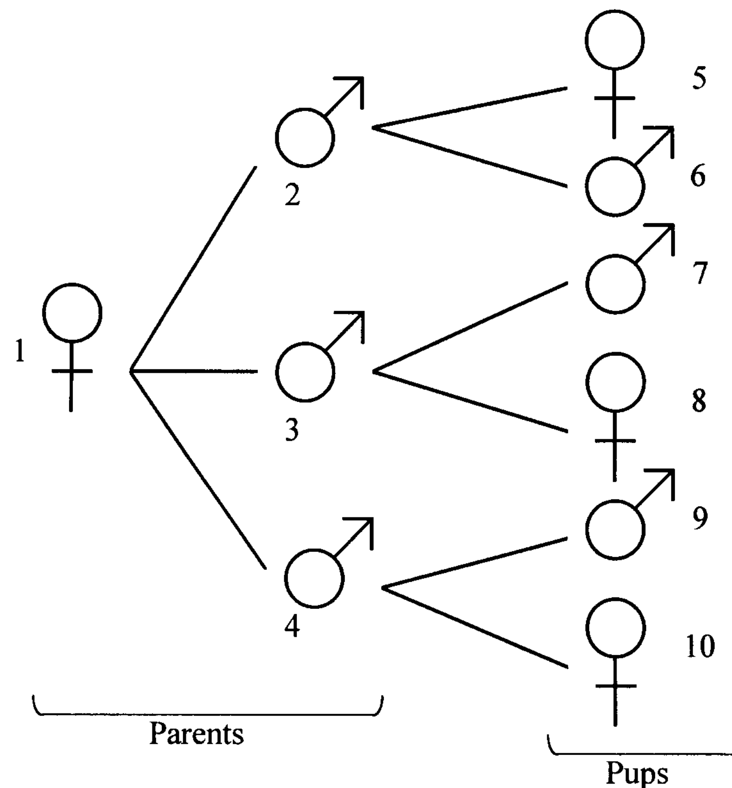
Data analysis for Protocol 2.
Using the data generated by your gel, read the following paragraphs, examine the pedigree below, and answer the questions at the end.

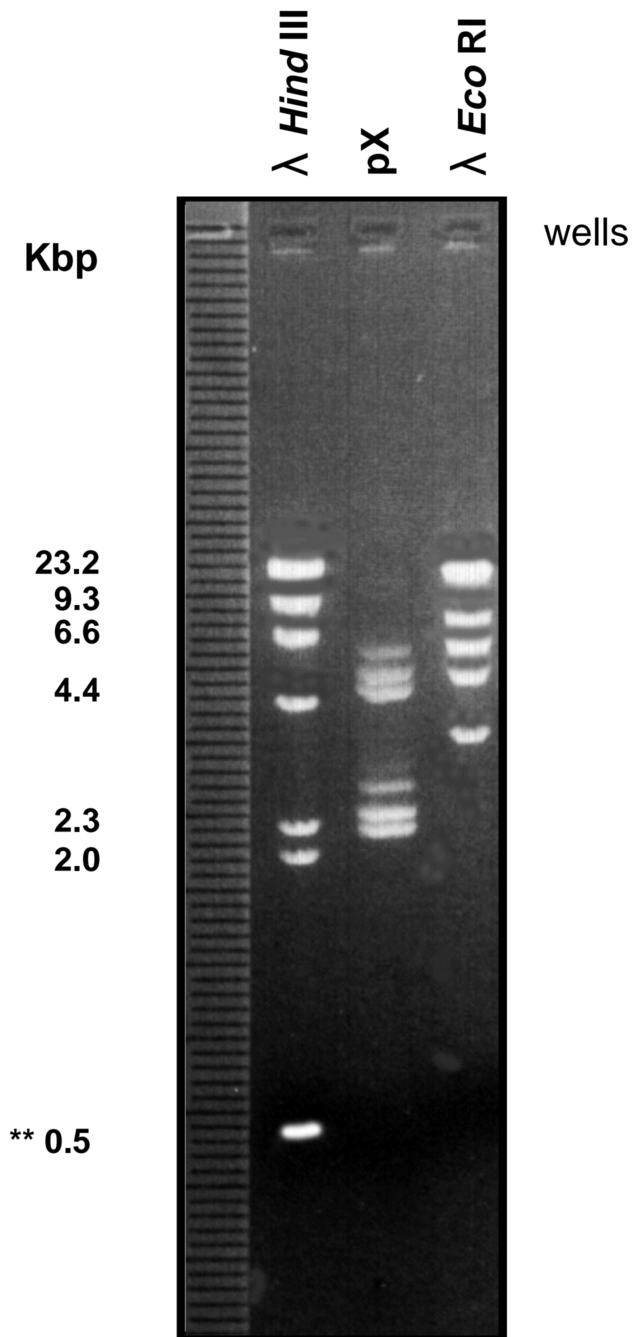
Diagnostic DNA Electrophoresis Puzzle:
A dominant mutation in a gene that leads to hip dysplasia in purebred Dalmations has been discovered by restriction fragment length polymorphism (RFLP)

analysis. This mutation consists of a single base pair change in the coding region of the gene that creates a new cleavage site for the restriction endonuclease *Xho*I. The normal (or wild type) gene yields a 4.6 kbp *Xho*I fragment. The mutated gene yields two *Xho*I fragments sized 1.3 and 3.3 kbp, respectively.
Hip dysplasia does not affect reproductive capacity but does substantially shorten the lifetime of the affected individuals. The onset of hip dysplasia is post-reproductive, so Dalmation breeders want to remove affected animals from the breeding pool.

A kennel owner has bred a champion bitch with three dogs. The kennel owner has just found out about RFLP analysis and wants to determine if (1) any of the dogs bred with the bitch are afflicted with the mutation, and (2) if any of the pups will be affected. Blood is drawn from all of the animals in question and DNA is prepared. The DNAs are then digested with *Xho*I and given to you for analysis by electrophoresis. You have run them in an agarose gel. The numbers next to the pedigree correspond to the DNA sample numbers.

Questions:
(1) Which parent has the mutation?
(2) Which pup(s) have the mutation?
(3) How many fragments would you expect to see in the affected pup's DNA?





0.8 % agarose gel

0.5X Tris Borate Buffer

** This fragment enhanced to allow detection