

DNA ELECTROPHORESIS

As with all experiments in this course, come to the lab prepared to work efficiently in order to get everything done in three-hour blocks of time. Read through the background material in this document and protocols associated with these experiments ahead of time, and come to the lab each session with an outline and timeline of tasks to complete that afternoon. Be prepared to answer questions on the science behind what you are doing, as well as the specifics of the tasks you are undertaking in each lab session. Take the time, before the lab, to grasp the physics behind the studies you are performing, so that the experiments can serve to enlighten these concepts.

ABSTRACT

DNA is a charged *polymer*. As such, in the presence of an electric field, it feels a force $\mathbf{F} = q\mathbf{E}$, where q is the net charge on DNA and \mathbf{E} is the electric field. Because the charge on DNA scales with length, different lengths of DNA feel different forces in an applied electric field. By forcing the DNA to migrate through a porous gel, it is possible to use an applied electric field to separate DNA of different lengths. The ability to do this opens up a wide range of applications, including DNA sequencing, preparation of a *monodisperse* polymeric sample for physical studies, and recombinant gene expression.

I. OBJECTIVES

- Determine how DNA responds to an electric field and moves through a gel
- Learn about different topological forms of DNA
- Learn how to purify plasmid DNA from *E. coli* and digest it at a specific site
- Determine a quantitative relationship between the length of DNA and its mobility in a gel

II. BACKGROUND

DNA is a *polymer*—a long chain of covalently bonded *monomers* (chemical groups known as nucleotides). Each nucleotide consists of a charged phosphate group bound to a sugar and a base. The phosphate and sugar groups are the same for each nucleotide; what is chemically variable is the base. There are four different bases: adenine (A), thymine (T), cytosine (C) and guanosine (G). The arrangement of these four bases along the DNA is called its *sequence*, and different sequences of DNA encode different genes. Sequencing of DNA involves, in part, separating it by length. The technique used to do this is electrophoresis, the focus of these experiments.

In vivo, DNA is generally found in a double-stranded form with a characteristic double-helix geometry (see Fig. 1). The sugar-phosphate backbone of the DNA runs along the exterior of the helix, while the strands are held together by interactions between the bases (complementary hydrogen bonding between A+T and C+G).

Because of the presence of the phosphate groups along the backbone, DNA is negatively charged. In solution at neutral pH, each phosphate has one negative charge; thus, for double-stranded DNA, each basepair in the length of the polymer contributes $2e^-$ of charge. In solution, these charges are partially screened by counterions in solution, e.g., Na^+ , Mg^{2+} , etc. Nonetheless, a net negative charge is associated with the DNA.

In the presence of an applied field \mathbf{E} in a buffer solution, DNA is subjected to a force and will migrate with a velocity $\mathbf{v} = \mu\mathbf{E}$. Here, μ is the *mobility* of the DNA (measured in units of $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$). A larger mobility implies a larger length travelled in a given field for a given time period. The movement of particles in an applied electric field is termed electrophoresis (*electro* = electric; *phoresis* = movement of small particle by an applied force). Note that it is a vector relation: the DNA moves along the field direction (in an isotropic medium such as a liquid solution or uniform gel).

In a solution that has charged ions, a charged object such as DNA will attract counterions of the opposite sign. The electric field from the charged object will then decay exponentially away from the object, with the decay length (the *Debye length*) a strongly decreasing function of ion concentration (salt concentration) in the liquid. For the high-salt conditions typically used in experiments, the Debye length is only a few nm or less, comparable or smaller than the radius of a DNA molecule. In such conditions, the force exerted by the field on the DNA is proportional to the contour length of the polymer. But the fluid drag also grows with the length of the polymer, L . Thus, both pulling force and resistance are proportional to L and the net mobility is independent of the length.¹

We thus cannot separate DNA by length if we put it in solution and subject it to an electric field. But in a gel, the situation is different. Several things happen: first, the DNA is forced to extend (in solution, entropy forces it to coil up into a fluctuating sphere of average radius R_g (the “radius of gyration”).² Second, the DNA must “snake” its way through the mesh of the gel (think of a polymer fishnet filled with water). The friction due to the gel matrix is a complicated, nonlinear function of the polymer length. Essentially, long polymers have many ways to get snagged temporarily by the gel matrix fibers. Thus, short molecules progress faster through the gel than long ones, and this relation can be used to separate different lengths of DNA: Put them in a gel, subject them all to a uniform field, and watch the different lengths spread out at different speeds.

In these experiments, you will put these ideas into practice, to determine how the mobility of linear DNA scales with its length, L . You will do this by applying an electric field across an agarose gel (using DC voltages across positive and negative electrodes to create the field) and measuring how far different lengths of DNA migrate through this gel in response to the applied field. Note that to properly determine mobility μ ($\text{cm}^2\text{V}^{-1}\text{s}^{-1}$), one would need

¹ This description neglects the three-dimensional conformation of DNA—its shape in 3d—as well as screening by counterions, but the basic argument holds.

² The radius of gyration reflects the radius of the compact random coil, if it is approximated as a spherical blob. The radius of gyration of linear DNA in free solution can be estimated from $R_g = \sqrt{\frac{1}{3}pL}$, where p is the persistence length of DNA ($p \approx 50$ nm in standard buffer conditions and reflects the length over which DNA can be considered to be straight) and L is its contour length (0.34 nm/basepair \times # of basepairs).

to perform this experiment at a variety of field strengths \mathbf{E} , as μ depends not only on length but also on the strength of the applied field [1]. (The mobility is also temperature dependent—usually, electrophoresis is performed at room temperature.) Here, we seek to investigate only the dependence of mobility in a gel on the length (and topology) of DNA, and so perform the experiments at a single field strength by fixing the voltage across the electrodes to a constant value.

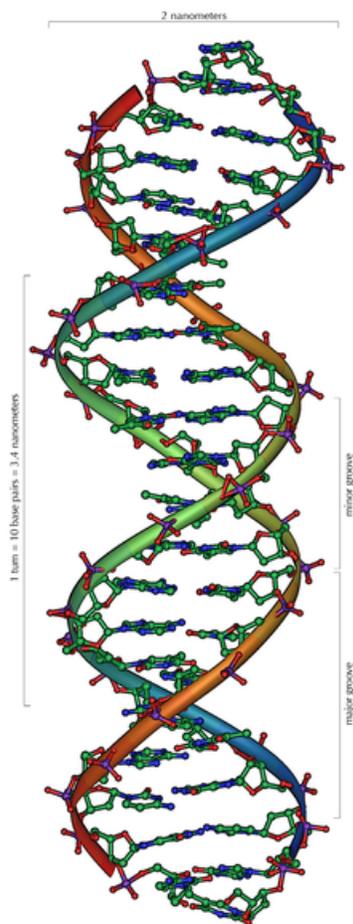


FIG. 1. The helical structure of dsDNA. Note its dimensions. (From wikipedia.)

The movie *Observation of Individual DNA Molecules Undergoing Gel Electrophoresis* demonstrates beautifully the dynamics of phage lambda DNA and yeast chromosomal DNA as they migrate in solution and through a gel in the presence of an applied field. This movie was recorded back in 1989 by Steven Smith, then at the University of Washington, and was the first demonstration of imaging single molecules during gel electrophoresis. You will see this movie after the completion of these electrophoresis experiments.

While the common picture of DNA is that of a linear double helix, it can adopt other forms. For example, in bacterial genomes, the DNA is commonly circular—i.e., it forms a closed loop. The *transfection* of cells for *recombinant gene expression* also commonly uses

circular DNA, known as *plasmid* DNA. The use of circular, rather than linear, DNA is important because of the stability of the DNA in the host. Because it can be degraded at its ends, the ends are unstable during replication and may not be completely copied before a cell divides. Thus, biologically, the use of circular DNA by cells can be extremely important. Circular DNA is also interesting from a physical and mathematical perspective, as a closed chiral loop leads to different topological structures: circles, circles with extra turns (supercoiled structures), concatenated rings, etc. In *E. coli*, DNA is usually negatively supercoiled, with an average supercoil density of $\sigma = -0.06$. Being in a native supercoiled state makes the DNA pack more tightly and fit more easily within the cell. Cells have developed specialized proteins such as *topoisomerases* to deal with the various topological structures of DNA, and investigating these machines is a very active research field.

Pre-lab question 1: For the *E. coli* genome size of approximately 4 Mb, and under the (incorrect) assumption that it is linear,^a determine R_g . How does this compare to the typical micron-sized dimensions of *E. coli*? Will *E. coli*'s genome fit in the cell without further compaction?

^a Although eukaryotes have linear chromosomes, those of prokaryotes including *E. coli* are usually circular. It is possible, however, for *E. coli* to survive with a linear version of their genome. See T. Cui et al., *EMBO Reports* **8**, 181–187 (2007).

Pre-lab Question 2: Sketch, to scale, a DNA molecule of this length in extended (straight) form and randomly coiled in solution. Be sure to indicate a scale bar on your sketch.

In this series of experiments, you will investigate the electrophoretic mobility of DNA through a polymeric, agarose gel. You will prepare and investigate DNA of different topologies, and will also determine the mobility of linear DNA in an electric field as a function of its length. Phys 833 students will, in addition, investigate how the mobility depends on electric field strength.

III. INVESTIGATIONS

1. Measure, and then mathematically determine, how DNA's electrophoretic mobility depends on its length (Phys 433 and Phys 833) and on applied electric field (Phys 833).
2. Determine how different topologies of DNA migrate in a gel.

IV. MATERIALS AND EQUIPMENT

To assist in your investigations, the following sources of DNA are recommended:

1. DNA ladders are size standards used to identify lengths of DNA in gels. A variety of these are available, each containing different lengths. Use the DNA ladders labelled “1 kb ladder” and “Lambda/Hind III ladder” found in the $-20\text{ }^{\circ}\text{C}$ freezer.
2. B. When a population of plasmid DNA is harvested from *E. coli* cells, it generally is found to be in a mixture of topological states, including uncoiled and supercoiled. Grow *E. coli* cells containing the plasmid pBluescript-KS(+) (see plasmid map on Fig. 2, next page), and harvest the plasmid DNA from these cells. To determine how the different topologies migrate, subject some of this plasmid to a nicking enzyme, which will make cuts in the sugar/phosphate backbone on only one of the two DNA strands. Take a second portion of the plasmid DNA and cut it with a restriction endonuclease, an enzyme that cuts the sugar/phosphate backbone of both DNA strands to make a complete break. You can then study the migration of these three populations of DNA: uncut, nicked, and linearized, and compare how DNA of the same length but different topology migrates in a gel.

A. DETAILED LIST OF EQUIPMENT AND SUPPLIES

1. Lab coat
2. Disposable nitrile gloves
3. Micropipettes and tips
4. Microcentrifuge
5. 1.5 mL microcentrifuge tubes
6. Waste bottle
7. Sterile, disposable inoculating loops OR metal wire inoculation loop
8. Microtorch
9. Autoclaved culture tubes + caps (3)
10. Rack (appropriate size) to fit culture tubes
11. Sterile, disposable plastic 5 ml pipettes
12. 10 ml pipette filler
13. Permanent pen (e.g., Sharpie) to label tubes

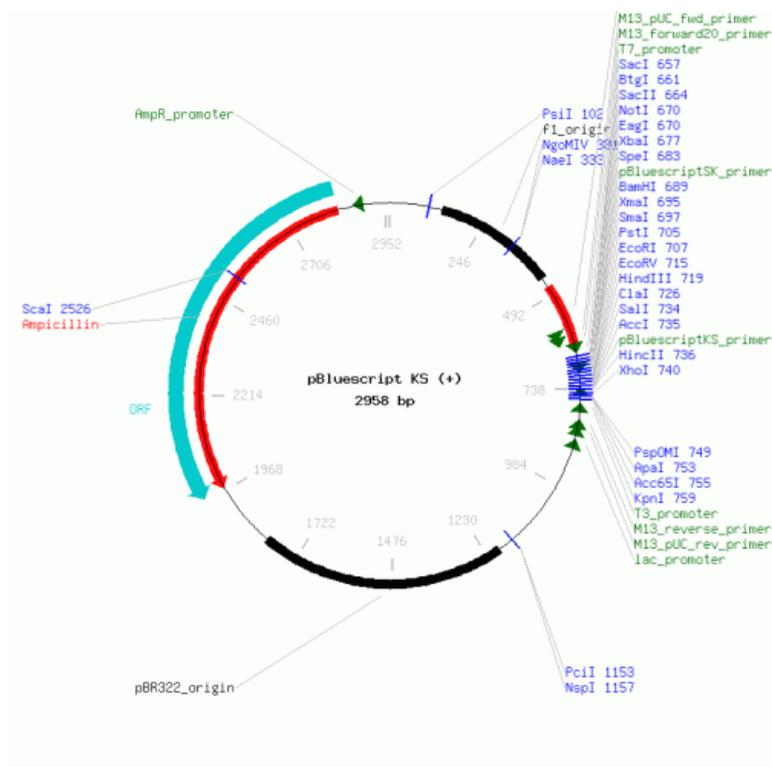


FIG. 2. Plasmid map of pBluescript-KS(+) showing ampicillin-resistance gene (APr—big red arrow on left), and various unique restriction enzyme cleavage sites (note location of EcoRI, used in these experiments). Figure from addgene.org; plasmid sequence also available at this site.

14. 37 °C room and shaker (or 37 °C shaking incubator)
15. Spectrophotometer and cuvettes
16. Heat block at 37 °C
17. Lab timer
18. Microwave oven
19. Hot gloves (oven mitts)
20. Electrophoresis apparatus (boat, tray, feet, comb) + power supply
21. Blue light and gel documentation camera

B. Culture growth supplies

1. 10% (v/v) squirt bleach bottle + paper towels to clean up potential bacterial contamination/spill

2. Streak plate with single isolated DH5? / pBluescript-KS(+) colonies
3. LB media, autoclaved in glass screw cap bottle (allow enough volume for 2×5 ml per overnight culture + 5 ml for negative control)
4. 50 mg/ml filter sterilized ampicillin stock, frozen (allow enough volume for $15 \mu\text{l}$ per 5 ml overnight culture + $15 \mu\text{l}$ for neg. control)

C. Plasmid purification, digestion, electrophoresis supplies

1. QIAgen miniprep kit with waste bottle
2. 1 kb DNA ladder
3. Lambda/Hind III DNA ladder
4. EcoRI
5. 10X Buffer H
6. Nb.BtsI
7. 10X NEBuffer 4
8. 100X BSA
9. dH₂O
10. TAE buffer
11. Agarose
12. SybrSafe dye
13. DNA loading buffer

V. SUGGESTED TIMELINE

Day 1: Start overnight culture of cells containing plasmid DNA. (*Protocol: Plasmid preparation*) Come having answered pre-lab question 1 from this Electrophoresis document and pre-lab questions 1-3 from Plasmid preparation document.

Day 2: Harvest plasmid DNA from these cells and quantify the amount of DNA recovered (*Protocol: Plasmid preparation*). Complete Table 1 in *Protocol: Enzymatic Digestion of DNA*, and have it checked by the instructor. If you have not completed this table by the end of Day 2, you **must** complete it and have it checked by the instructor before coming to the lab on Day 3.

Day 3: A busy day! Digest a small portion of the harvested DNA with a restriction enzyme (EcoRI). Incubate another small portion of the harvested DNA with a nicking enzyme (Nb.BtsI). (*Protocol: Enzymatic Digestion of DNA*) Cast, run and image gel containing samples of DNA ladders, uncut DNA, nicked DNA and digested DNA. (*Protocol: Agarose Gel Electrophoresis and Protocol: Electrophoresis Gel Imager*) Come having answered all pre-lab questions in these protocol documents.

VI. ITEMS TO INCLUDE IN LAB WRITE-UP

Spreadsheets (e.g., in Igor or Matlab) showing raw data of positions of DNA bands as a function of time can be submitted electronically. All other information, including sample calculations, should be included in the hard-copy report.

1. Answers to all pre-lab questions
2. Plasmid map of pBluescript-KS(+), clearly indicating the site(s) of digestion by EcoRI and site(s) of nicking by Nb.BtsI, including the strand(s) cut by each enzyme. What is the length of linearized plasmid, in nm? What is its radius of gyration, R_g , in free solution?
3. Absorbance spectrum of plasmid DNA. Ignore wavelength regions where there is no meaningful signal.
4. Results of spectrophotometric quantification of plasmid DNA concentration and its purity
5. Final image of gel, with bands clearly labelled
6. Raw data in table format: DNA length L and distance migrated z (in mm) as a function of time (table for each E-field used for Phys 833)
7. Plot of z versus time for at least three DNA lengths (the shortest, longest and an intermediate length) to determine the velocity of DNA electrophoresis at a given value of electric field
8. Determine the electrophoretic mobility, $\mu(L)$, for these three lengths of DNA
9. From the final image in which DNA has migrated farthest, determine and plot z vs L including all linear DNA bands, showing experimental points and fit to a model
10. Discuss the relationship you have found between length and distance travelled for linear DNA
11. Estimates and discussion of errors in your measurements
12. Phys 833: A plot for each E-field studied of z versus t for three DNA lengths. Determination of $\mu(L; E)$ for each length and E-field.

13. Phys 833: Determine and discuss a relationship between E-field and mobility for each of the three DNA lengths.
14. Discuss how, for a given length of DNA, its topology affects its mobility. Use the results from your plasmid DNA, nicked plasmid and linearized plasmid how did you assign the different bands in these lanes?
15. Discuss the more significant experimental difficulties encountered, including the appearance/shape of the DNA absorbance spectrum. What limits various estimates?
16. Discuss at least two (Phys 433) or all (Phys 833) of the “questions to ponder,” below.

VII. QUESTIONS TO PONDER

- How would your results change if you used a higher percentage of agarose when casting your gel?
- How would your results change if you used a higher voltage across the electrodes?
- How would your results change if you used a buffer with a higher ionic strength?
- How would your results change if you used a buffer with a higher pH?

VIII. FURTHER INFORMATION AND ADDITIONAL READING

Agarose is a polysaccharide (linear polymer of sugar molecules) consisting of D-galactose and 3,6-anhydro L-galactose (see Fig. 3). Commercially, agarose is extracted from seaweed and purified for use in electrophoresis.

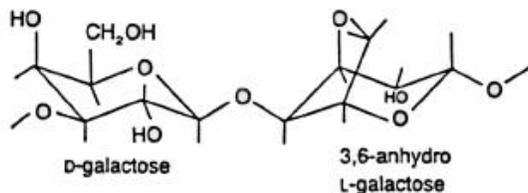


FIG. 3. Structure of agarose.

The pore size in an agarose gel can be determined from measurements of DNA’s mobility as a function of field strength, taking the limit of $\mu_{E \rightarrow 0}$ [2]. The pore size ξ can be calculated using the expression $\xi = (6Lp \mu_{E \rightarrow 0} / \mu_0)^{1/2}$, where L is the contour length of DNA, $p = 50$ nm is the persistence length of the DNA and $\mu_0 = 4.3 \times 10^{-4} \text{ cm}^2 \text{V}^{-1} \text{s}^{-1}$ is the intrinsic mobility of DNA in TBE buffer solution. This model is valid only for DNA molecules of chain length much larger than the pore size. This restriction along with the requirement that $E \rightarrow 0$

results in very long durations of electrophoresis (typically several hours) [2]. For more on agarose gel pore sizes, see [3]. Pore sizes in 0.7% agarose gels are typically a few hundred nanometers.

Topoisomerases are a class of enzymes (protein machines) responsible for removing excess positive or negative supercoiling from DNA. A recent review article describes single-molecule biophysical assays to understand their mechanisms of activity [4].

Two-dimensional electrophoresis can be used to separate different topoisomers of DNA. In this technique, one dimension is the applied field; the second involves adding a small-molecule drug to reduce the amount of supercoils [5]. The cited reference also includes details of the electrophoresis in its supplementary information [5].

Proteins can also be separated by size using electrophoresis. There, the gel is typically made from *polyacrylamide*, and the proteins are first coated with ionic detergent molecules (SDS) in order to impart to them an effective charge that scales with length. SDS-PAGE (SDS-polyacrylamide gel electrophoresis) is a standard technique for separating proteins, determining their relative abundance, measuring the molecular weight, etc. Acrylamide gels are also used to separate shorter DNA molecules by size, particularly single-stranded DNA molecules (or RNA molecules) of lengths < 100 bp.

REFERENCES

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