

Session 5

Gel Electrophoresis Purification

Learning Objective:

In this lab exercise you will become familiar with the procedures for running gel electrophoresis and recovering DNA of a target length after separation.

Introduction

The ability to purify DNA selectively by length is critical to molecular biology. This technology allows for the quick identification of DNA products, enables high efficiency cloning through careful selection of proper products, and facilitates the Sanger method of DNA sequencing. Gel electrophoresis is a simple way by which DNA is separated by length.

Background:

Gel Electrophoresis Separates DNA by Length

Gel electrophoresis is a way in which nucleic acids of different lengths can be separated. This process takes advantage of the physical properties of nucleic acids. DNA is a charged molecule; each nucleotide carries a negative charge. As a result, in the presence of an electric field, DNA will migrate toward the positive charge at the anode (away from the negative charge at the cathode). Because each nucleic acid carries a charge, the whole strand has a charge that is proportional to the length of the DNA; the longer the DNA, the greater the charge. Thus, the force of the electric field on the DNA will be larger:

$$F = q * E$$

$$q = \text{constant} * \text{length}$$

But the DNA also has a mass that is proportional to length and therefore the charge to mass ratio of the DNA is essentially constant.

$$F = m * a$$

$$m = \text{constant} * \text{length}$$

Because of this, we may expect all pieces of DNA to be accelerated uniformly in proportion to the electric field. However, we have not accounted for the drag on the DNA caused by the gel medium.

Agarose is a linear polymer that forms a gel when dissolved at the proper concentration. The gel is a 3-D mesh or net-like structure which hinders the motion of particles. The volume in the gel is still mostly liquid and the size of the pores caused by the gel are

large, so particles can still move through, just more slowly. The DNA moving through the agarose gel gets slowed up in the tangles of the gel. The longer pieces of DNA are larger and get held up for longer periods of time than the shorter pieces. The overall effect is an average resistance to flow or drag on the DNA which depends on the size of the DNA. For DNA running in a gel, the forces from the electric field and the drag from the gel will balance (yielding no additional acceleration) at a given velocity which is dependent on many factors including: the concentration of the gel, the strength of the magnetic field (i.e. the voltage across the electrodes), and most importantly the length of the DNA. Since, for a given gel, the electric field and concentration of the gel are constant, the velocity of DNA depends primarily on its length; longer DNA moves slower. Therefore, in the same amount of time, shorter pieces move farther than longer pieces. In this way, you are able to separate the DNA by different lengths. It is also important to note that the configuration of the DNA (linear, circular, or super-coiled) also plays an important role on the speed in the gel; two pieces of DNA with identical length but in different forms will run at different speeds in a gel. The same principle for gel separation can be used with gravity as the driving force for flow.¹

To see the location of the DNA in the gel, we use stains that selectively bind to DNA, typically between the strands. Typical stains used in gel electrophoresis are fluorescent. The stained gel is illuminated with a specific wavelength of light which depends on the type of stain used, and then viewed for the emission of light. The stain from a single molecule of DNA cannot be seen, but when enough DNA molecules are concentrated together, the band can be seen.

Now that we can separate DNA based on length and visualize the DNA in the gel, we need to know the lengths of the different bands. This is accomplished by running DNA of known lengths (called a ladder) in a different lane on the gel. Then, the distance travelled by the sample is compared to the bands on the ladder to determine the length(s) of the DNA sample.

DNA Purification

We know that we can get the pieces of DNA separated based on their size within a gel and that we can identify the correct length DNA with the use of a ladder. However, to be useful for our cloning application we must also be able to get the DNA back out of gel. To do this, we can simply cut the piece with the desired piece out of the gel. Then, we can dissolve the gel from this small piece and recover the DNA from the solution. This is done in the same fashion as with the plasmid mini-prep. By eliminating DNA of incorrect length, we can greatly increase our efficiency in cloning. However, this benefit comes with a drawback, decreased DNA product; as a result of inefficiencies in the purification process, some of the desired DNA is also lost.

¹ Please note: the mechanism of DNA transport through a gel is extremely complicated. The explanation given here is a simplification using the concept of drag and should not be interpreted as a precise explanation of the underlying principles.

Pre-Laboratory Exercises

Name: _____

Date: _____

- 1) Does DNA have an electrical charge? If yes, is the charge positive or negative?

- 2) Toward which electrode does DNA migrate?

- 3) What is responsible for separating the DNA strands of different lengths?

- 4) How is the DNA found again after it is in the gel?

- 5) How do we determine the length of the DNA in a sample?

- 6) How is the correct DNA recovered from the gel?