

Session 5

Gel Electrophoresis & DNA Purification from a Gel

Learning Objective:

In this lab exercise you will become familiar with the procedure for separating DNA fragments by size using gel electrophoresis, and the procedure for recovering specific DNA from a gel for further use.

Introduction

The ability to separate mixed DNA fragments by their length is critical to molecular biology. This technique allows us to quickly identify unknown DNA products, enables high efficiency cloning through careful selection of proper products, and plays a key role in the Sanger method of DNA sequencing. Gel electrophoresis is a simple method for separating DNA pieces by length.

Background:

Gel Electrophoresis Physically Separates DNA by Length

To physically separate different-length DNA fragments, gel electrophoresis takes advantage of the physical properties of nucleic acids. DNA is an *electrically charged* molecule; each nucleotide carries a negative charge on its phosphate group. Recall that similar charges repel, and opposite charges attract. As a result, in the presence of an electric field, DNA will migrate toward the positive charge at the anode (and away from the negative charge at the cathode).

Because each nucleotide carries a charge, the whole DNA strand has a total charge that is proportional to the number of nucleotides, or to the length of the strand. The longer the DNA, the greater the total charge. The electrical field exerts a force on the DNA in proportion to this total charge. Thus, the electrical force on longer pieces of DNA will be larger:

$$\begin{array}{ll} F = q * E & (q = \text{charge}; E = \text{electric field strength}) \\ q = C * \text{length} & (C = \text{charge per nucleotide}) \end{array}$$

But the DNA also has a *mass* that is proportional to its length. Therefore, the *charge-to-mass ratio* of DNA should be the same for any DNA fragment, no matter its length. Since acceleration depends on both mass and charge, we may expect all pieces of DNA to be accelerated uniformly:

$$\begin{array}{ll} m = K * \text{length} & F = m * a \rightarrow a = F / m \\ (K = \text{mass per nucleotide}) & a = (q * E) / m \\ & a = (C * \text{length} * E) / (K * \text{length}) \rightarrow \text{length cancels out} \end{array}$$

But if all pieces of DNA are uniformly accelerated by the electric field, how will they be separated by size? The answer lies in one factor we have not yet accounted for: the **drag** on the DNA caused by the gel medium.

Agarose is a polymer that forms a gel when dissolved at the proper concentration. The gel is a 3-D mesh or net-like structure, which impedes the motion of molecules through it. The gel has relatively large pores, and most of its volume is taken up by liquid, so molecules can still move through, just more slowly than if they were moving freely in water.

The DNA moving through the gel gets entangled in the gel fibers, and its motion is held up. Longer pieces of DNA get entangled more often, and get held up for longer periods of time, than shorter pieces of DNA. The overall effect is that DNA fragments of a certain length experience an average drag, or resistance to flow, which depends on that length. The driving force from the electric field and the drag force from the gel will balance out at a certain velocity; longer DNA moves slower. Thus, when you run a mixed sample of DNA through a gel for a long time, the small fragments will get ahead and the large fragments will be left behind. The fragments will separate into “bands” at different locations on the gel.

To see the location of the DNA in the gel, we use stains that selectively bind to DNA, typically between the bases. Most stains used in gel electrophoresis are fluorescent. The stained gel is illuminated with a certain wavelength of light (which depends on the particular stain used). The stain molecules respond to the illumination by emitting light of a second wavelength, which can be seen and photographed. The stain from a single molecule of DNA cannot be seen, but when enough DNA molecules are concentrated together, the band can be seen. ***Warning: DNA stain molecules, naturally, bind to DNA. Chemicals that bind to DNA can cause mutations. Thus, most DNA stains used on gels are potentially carcinogenic.***

To figure out the length of the DNA in a given band, we need something to compare it to. We use a pre-made mixture of DNA fragments of known lengths, commonly called a ***ladder***. A typical ladder mixture might have 10-20 fragments ranging from 100 to 10,000 base pairs long. Comparing the distance traveled by bands in your sample and in your ladder will give you an estimate of the length of DNA in each band. For example, a DNA band that traveled farther than the 2500bp ladder band, but not quite as far as the 2000bp ladder band, might be about 2300bp long. Every gel must have its own ladder, because the distance that DNA fragments travel depends closely on factors such as the exact composition of the gel, the exact voltage, and the exact length of time the gel is run, which will inevitably vary between gels.

DNA Purification

We know that we can get the pieces of DNA separated based on their size using a gel, and we also know that we can identify the correct length DNA with the use of a ladder. However, to actually use the DNA for our cloning application, we must also be able to get the DNA back out of the gel. To do this, the first step is to physically cut out the piece of gel containing the desired DNA band. Then, we can dissolve the gel from this small piece and recover the DNA from the solution. This is done with a silica membrane that selectively binds DNA, similar to the plasmid mini-prep.

The overall process of gel electrophoresis and DNA purification will improve the efficiency of the next steps in the cloning process, because we will be able to combine only the desired vector and insert sequences of DNA, discarding the “other” parts of the original plasmids. However, this benefit comes with a drawback: as a result of inefficiencies in the purification process, some of the desired DNA is also lost.

Laboratory Protocol

The exercise: You will be running your restriction digested insert and vector from last week on a gel. You will observe the different length bands and cut out the appropriate bands for later purification by the instructors.

Materials: 1% Agarose gel in 0.5X TBE buffer
TBE Running buffer
10X blue loading buffer
SybrSafe DNA Stain
Razor Blade
Microcentrifuge
1.5mL Microcentrifuge Tubes
0.5mL Microcentrifuge Tubes
Deionized water
UV Cuvettes

Equipment: Gel Box
Power Source
UV Table lamp
Mass Scale

Protocol

1. Add 40 μ L of your restriction-digested vector DNA to a 0.5mL tube. Label the tube “vector digest”.
2. Add 40 μ L of undigested vector DNA from the instructors to a 0.5mL tube. Label the tube “vector control”.
3. Add 40 μ L of your restriction-digested insert DNA to a 0.5mL tube. Label the tube “insert digest”.
4. Add 40 μ L of undigested insert DNA from the instructors to a 0.5mL tube. Label the tube “insert control”.
5. Add 10 μ L of 10X blue loading buffer to each of your tubes. Mix by gently pipetting the liquid up and down several times.
(note: we are using the blue loading buffer at a final concentration of 2X, rather than 1X.)
6. Come to the instructor bench, where gels have already been poured, cooled, and placed in the gel box with the proper amount of running buffer. Observe the orientation of the gel in the gel box (note the red and black electrical connections and where the wells of the gel are situated). Remember “RUN to RED”.

7. Carefully pipette 10 μ L of DNA ladder into each of the wells on the sides of the gel. When pipetting into wells you must be very careful not to pierce the bottom of the gel and you must pipette slowly so that the liquid does not come out of the wells. NO BUBBLES. The loading buffer is more dense than the TBE running buffer so, if you pipet gently, the sample will settle into the well.
8. **Very carefully** pipette the 50 μ L of each DNA sample into a different well of the gel. Again, you must pipette extremely carefully! **Record in your lab notebook which wells contain which samples.**
9. When all of the wells are filled, place the cover on the gel box. Connect the cables (red and black) to the appropriate terminals on the power supply.
10. Set the power supply voltage to 125 V and start the gel running. Observe which direction the blue spots begin to move.

STOP HERE – FINISH PROTOCOL AFTER GEL RUNS

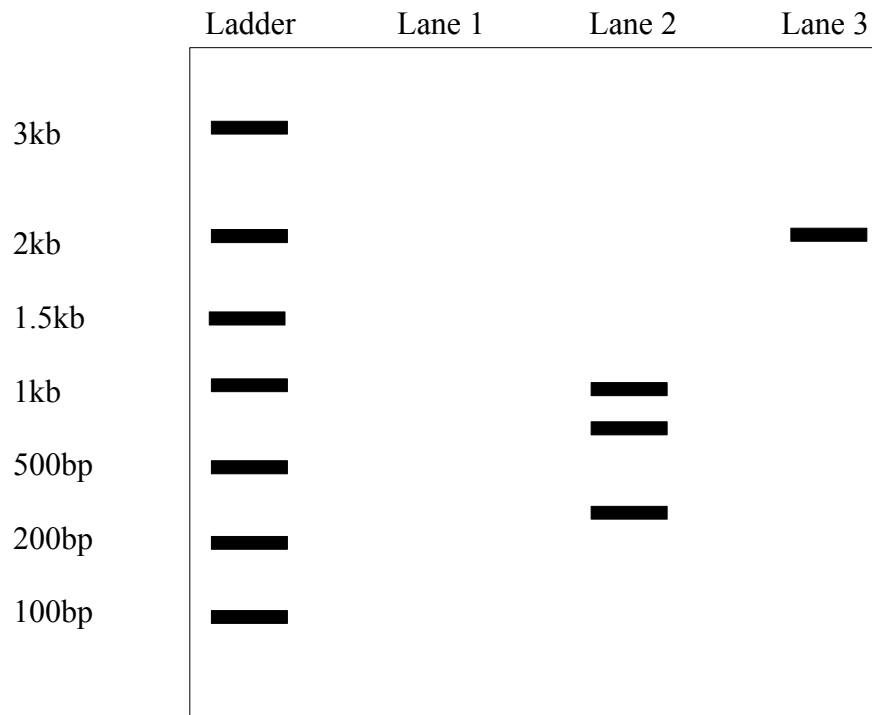
11. After the gel has run for 1 hour, you will see the blue color of the loading buffer has moved most of the way down the gel. Stop the gel running by turning off the power supply.
12. Look at the gel on the UV-light table. Make sure you wear the appropriate face shield to protect you from the ultraviolet light.
13. Observe the ladder pattern. Identify the length of the various bands. Record in your lab notebook.
14. The desired band in your restriction-digested insert is about 2kb long. The desired band in your restriction-digested backbone is either 3kb or 3.2kb long. If these bands are present, label two clean 1.5mL tubes with “vector” or “insert” and your group color.
15. Very carefully cut each desired band out of the gel using a razor blade. Cut the smallest slice possible that still contains the whole band; don't leave a lot of extra gel on your slice.
16. Place the gel slices into the labeled 1.5mL tubes and turn them in to the instructors.

Session 5: Post-Laboratory Exercises

Name: _____

Date: _____

- 1) Consider a plasmid of total length 2kb (kilo- basepairs). You do not know the whole sequence of the plasmid, but you do know that there is an EcoRI cut site at position 0 and a PstI cut site at position 700, and you think there are no other restriction enzyme sites in the plasmid. Suppose you cut this plasmid with both EcoRI and PstI, and ran the resulting DNA on a gel to verify that your restriction digest procedure worked correctly.
- a) On the gel below, draw the bands you would expect to see in Lane 1. Make sure to compare their positions to the positions of the appropriate ladder bands.



- b) Suppose that instead of your expected result, you got the result shown in Lane 2. What might have gone wrong to cause this result?
- c) Suppose that you saw the result in Lane 3. What might have gone wrong to cause this result?

References & Additional Reading

Wikipedia:

Gel Electrophoresis: http://en.wikipedia.org/wiki/Gel_electrophoresis

Qiagen

