

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?

Laboratory Protocol

The exercise: You will be running your restriction digested PCR product (banana smell-generating enzyme) and vector (pSB4A5) from last week on a gel. You will observe the different length bands and cut out the appropriate bands. Then you will recover and purify the two DNA parts and quantify the amounts recovered.

Materials: 1% Agarose gel in 0.5X TBE buffer
TBE Running buffer
SybrSafe DNA Stain
Razor Blade
Qiagen Gel Extraction Spin Column and Buffers
Microcentrifuge
1.5 mL Microcentrifuge Tubes
Deionized water
UV Cuvettes

Equipment: Gel Box
Power Source
UV Table lamp
Mass Scale
Spectrophotometer

Protocol Part I – Gel Electrophoresis

1. The gel has already been poured, cooled, and placed in the gel box with the proper amount of 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).
2. On a piece of parafilm, make four 4 uL spots of 6X loading buffer. Make sure that they are far enough apart that they cannot mix!
3. Take 46 uL of your PCR product and add it to a loading buffer spot. Label below the spot with an appropriate title.
4. Take 46 uL of your PCR negative control and add it to a loading buffer spot. Label below the spot with an appropriate title.
5. Take 46 uL of the restriction digest from last week and add this to another spot. Label this spot pSB4A5 plasmid digest.
6. Take 46 uL of the undigested instructor pSB4A5 plasmid and add this to the final spot. Label this spot undigested pSB4A5 plasmid.

7. Mix the Samples by drawing the liquid in and out of the pipette several times.
8. Carefully pipette 10 uL of the instructor prepared 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 done gently, the sample will settle into the well.
9. **Very carefully** pipette the 50 uL of each sample with loading buffer into a different well of the gel. Again, be extremely careful as before! Make sure you record in your lab notebook which wells contain which samples!
10. 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.
11. Set the power supply voltage to 125 V and start the gel running. Observe which direction the blue spots begin to move.
12. 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.
13. Look at the gel on the UV-light table. Make sure you wear the appropriate face shield to protect you from the ultraviolet light.
14. Observe the ladder pattern. Identify the length of the various bands. Record in your lab notebook.
15. Your PCR product should have a band that is 1000 bp in length. The digested vector should be slightly longer (approx 3500 bp). If these bands are present, very carefully cut them out of the gel using a razor blade. Cut the smallest piece of gel possible which includes the bulk of the band. The slice should be very thin.
16. Place the gel slices into individual clean 1.5mL microfuge tubes and place them in the freezer until next week.

Part II – Gel Extraction

1. Weigh the piece of gel. (Hint: don't remove the gel from the tube, use an empty tube to tare the scale)

2. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 μ l). For example, add 300 μ l of Buffer QG to each 100 mg of gel. If your gel is larger than 400 mg, you will need to separate the gel into two or more pieces.
3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.
4. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color of the mixture is orange or violet, ask your instructor how to proceed.
5. Add 1 gel volume of isopropanol to the sample and mix. Do not centrifuge the sample at this stage.
6. Place a QIAquick spin column in a provided 2 ml collection tube.
7. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min at 13,000 rpm. The maximum volume of the column reservoir is 800 μ l. For sample volumes of more than 800 μ l, simply load and spin again.
8. Discard flow-through and place QIAquick column back in the same collection tube. Collection tubes are reused to reduce plastic waste.
9. Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min at 13,000 rpm. This step will remove all traces of agarose.
10. To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min at 13,000 rpm.
11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 13,000 rpm to remove all residual ethanol.
12. Place QIAquick column into a clean, labeled 1.5 ml microcentrifuge tube.
13. To elute DNA, add 50 μ l of water (pH 7.0–8.5) to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min at 13,000 rpm. **IMPORTANT:** Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA.
14. Elute a second time as in step 13 using 50 more μ l of water.

15. Pipette 70 uL of your gel purification products into separate, labeled UV microcuvettes. In another UV microcuvette, prepare a blank with 70 uL of deionized water.
16. Blank the Spectrophotometer using the water-filled cuvette
17. Record the absorbance of each of the samples at 230, 260, and 280 nm.
18. Pipette the DNA back from the cuvette in the microfuge tube. Store the microcentrifuge tube in a freezer at -20C.

Session 5: Post-Laboratory Exercises

Name: _____

Date: _____

- 1) Assume that you gel purified 200 μL of vector at 75 $\text{ng}/\mu\text{L}$, and you purified 50 μL of PCR product at 1 $\mu\text{g}/\mu\text{L}$. After purification, you eluted into 30 μL and obtained 10 $\text{ng}/\mu\text{L}$ for the vector and 50 $\text{ng}/\mu\text{L}$ for the PCR product. Calculate the % yield or recovery from you gel purification for the PCR product and vector. (Hint: % Yield = 100% x massout/massin). Show ALL work.

References & Additional Reading

Wikipedia:

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

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