Texas A&M University-Corpus Christi
CHEM4402 Biochemistry II Laboratory
Laboratory 5 - Electrophoresis & Quantification of PCR DNA
Please bring a portable memory device to lab

Once we have performed the amplification of our target DNA sequence using PCR, we must have a means of determining whether the reaction was successful. The most common method for determining the success of a PCR reaction is to analyze the contents on an agarose gel (electrophoresis). Agarose gel electrophoresis separates DNA fragments according to molecular weight. Agarose is a carbohydrate polymer. When hydrated, melted and allowed to cool, it produces a firm, gel-like substrate. If you were to view the gel at the microscopic level, you would see a very fine, complex meshwork of agarose polymers. DNA is added to wells in the gel, created by the insertion of "combs" in the melted agarose. Because it is porous, the gel can absorb and allow a solution to flow through it. If this solution contains electrolytes, such as those found in a number a buffers, application of a voltage will create a current in the gel. Negatively charged biomolecules, such as DNA, can then be induced to migrate to the positive electrode (figure 1). During the migration the agarose gel acts as a sieve, allowing smaller DNA fragments to travel faster than larger ones. Thus, any mixture of fragments of different sizes may be separated. If a DNA sample containing a series of fragments with known molecular weights (i.e. molecular weight standards) are run alongside a PCR product, the product size can be estimated by comparing its migration to that of the known standards. Today, we will be taking advantage of this fact to analyze the results of our PCR reaction. We will determine if the reaction was successful and estimate the molecular weight of the product. We will also clean up the remainder of our PCR product and estimate the concentration using a Nano-Drop spectrophotometer.

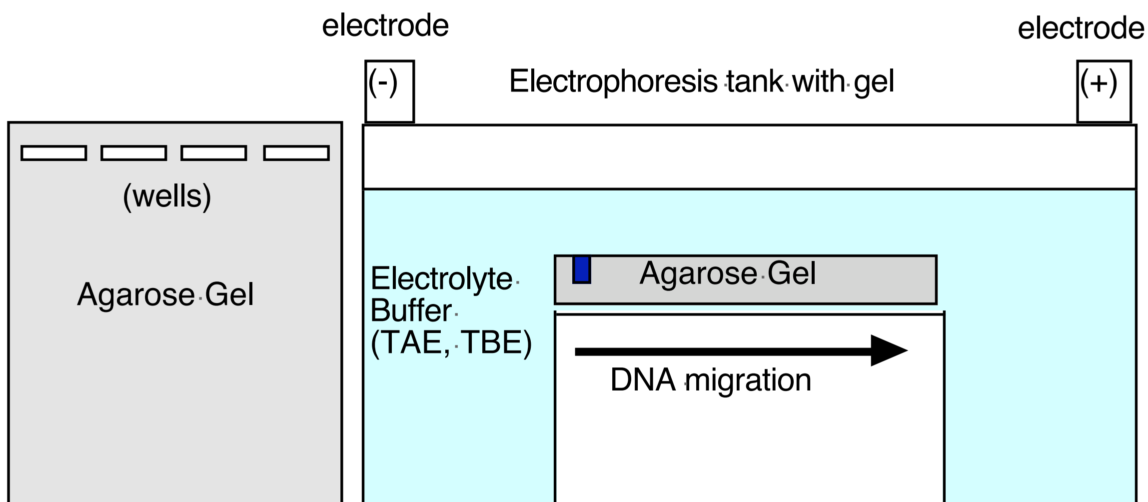

Figure 1. Top view of agarose gel (left), side view of electrophoresis tank containing buffer and gel (right)

Part 1: Agarose Gel Electrophoresis

Materials

Agarose	1X TAE Buffer (250 ml)	GFP PCR product
Gel box, tray and comb	125 ml erlenmeyer flask	Gel box Power supply
Hot plate	ethidium bromide (10 mg/ml soln)	37ºC water bath
microfuge tube	DNA molecular weight markers	

Procedure

1. Make **40 ml** of a 1% agarose solution in a 125 ml erlenmeyer flask by adding 0.4 g of agarose to 40 ml of 1X TAE (Tris – Acetic acid – EDTA) buffer.
2. Add a stir bar to the agarose solution. Mix on a hot plate until it boils. While waiting, set up your gel tray as indicated by your instructor.
3. Place melted agarose in a **37ºC water bath** to cool (~ 1 minute).
4. Obtain **4 microliters (ul)** of **ethidium bromide** from your instructor. Insert pipet tip into your melted agarose solution and dispense. (**Caution – ethidium bromide is a mutagen. Handle carefully and always wear gloves**). Swirl your gel solution gently to mix. Dispose of tips containing EtBr residue by **placing in marked waste container containing bleach solution**.
5. Pour melted agarose solution into a gel tray as indicated by your instructor. Be sure the tray has the “dams” on both ends up and screwed in and that it contains the 12-tooth "comb" which will produce the wells for loading your DNA samples (molecular weight standards and PCR product). Rinse agarose flask with bleach solution and wash with warm, soapy water.
6. Once your gel has set (it will have an opaque, grey-white appearance), gently remove the comb by pulling straight up and out of the gel. **Lower the “dams” on both ends of the gel tray** and place in the electrophoresis tank (**DNA wells closest to the black (-) electrode**). Fill the tank with 250 ml of 1X TAE buffer.
7. Load 10 ul of **DNA molecular weight markers** in the first well (left to right). Load 10 ul of your PCR product in a **middle** well (its OK if some spills out).
8. Insert the lid onto the electrophoresis tank. Connect the electrodes to the power supply (be sure to follow color-codes for connections).
9. Turn on your power supply, select *voltage* and set to 75 V. Press start (“running man” figure). Allow gel to run for **45 minutes**. While you are waiting, perform Part 2 “PCR Clean-up and Spectrophotometry Quantification”.
10. When your gel has finished running, shut off the current (press “running man” figure again), turn off the power supply and remove the electrodes connected to the gel box. Remove the top of the gel box and remove the gel tray (wear gloves). Go with your instructor to take a photo of your gel.
11. While you are waiting on the other lab groups, Rinse out your gel box, tray and comb with distilled water. There is no need for soap. Place on paper towel to dry. **DO NOT hand dry** as the platinum electrodes are quite fragile
12. Turn in a copy of your gel photo, properly formatted (see fig.3) with the size of your PCR product and the DNA molecular weight markers *neatly* labeled. This should be done

electronically in any number of software programs (Word, Powerpoint, Illustrator, etc.).See figure 2 below for molecular weight (in base pairs, bp) of the marker bands. (5 pt.)

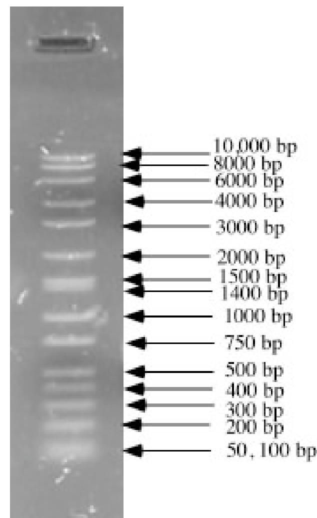

Figure 2. Molecular weight markers and size identity of bands

Figure 3. Example of formatted gel figure.

Title. Also include name, name of laboratory assignment, lab section and date

Identify size of Mol. Weight markers

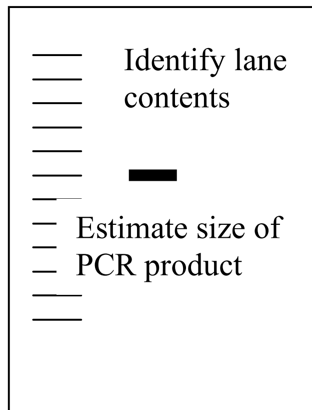

Figure legend. Explain figure. Include relevant information such as percentage of agarose gel, identify of running buffer, amount of sample loaded, and voltage/time for running gel, etc. See literature assignments for examples

Part 2: PCR product clean-up and Spectrophotometry quantification

The DNA template must be cleaned up to remove excess primers, dyes, salts, and other PCR components that can interfere with "downstream" protocols. After clean up, our PCR products will be quantitated using spectrophotometry (Nano-Drop instrument). An accurate knowledge of the amount of PCR product is also important for many downstream applications.

Materials

Buffer **PB**
Elution Buffer **EB**
2 ml collection tube (round botton)
Buffer **PE**
Spin Column
1.5 ml microcentrifuge tubes (conical bottom) (2)

PCR Product Clean-up Procedure

Before you start:
-Be sure appropriate buffers have had ethanol added (marked on bottles)
-All centrifugation steps should be performed at 13,000 rpm

1. Transfer PCR reaction to a microcentrifuge tube.
2. Add 5 volumes of **Buffer PB** to PCR sample and mix. (example: add 250 ul of Buffer to a 50 ul PCR reaction).
3. Place a spin column in a provided 2 ml collection tube.
4. To bind DNA, apply the sample to the spin column and centrifuge for 60 s.
5. Discard flow-through. Place the spin column back into the same tube.
6. To wash, add 0.75 ml **Buffer PE** to the spin column and centrifuge for 60 s.
7. Discard flow-through and place the spin column back in the same tube.
8. Centrifuge the column for an additional 1 min. (IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.)
9. Place spin column in a sterile (DNAse-free) 1.5 ml microcentrifuge tube.
10. To elute DNA, add 50 ul Buffer **EB** (10 mM Tris·Cl, pH 8.5) to the center of the spin column membrane. Let the column stand for 60 s and then centrifuge for 60 s. (IMPORTANT: Ensure that the elution buffer is dispensed directly onto the spin column membrane for complete elution of bound DNA.
11. Go with your instructor to measure the concentration of your PCR product DNA using the Nano-Drop spectrophotometer. Be sure to record your value in your laboratory notebook. When finished, your instructor will collect your samples for use next week (DNA ligation and Transformation).

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Laboratory 5 - Electrophoresis (17 pt)

READING ASSMT: Lehninger's Biochemistry Ch. 3.3 Working with Proteins, Ch. 9.1-9.2 (DNA Cloning: The Basics, From Genes to Genomes)

1. Include a formatted copy of your PCR gel, as outlined in the assignment. (5 pt)
2. Laboratory Performance (2 pt).
3. On what physiochemical basis are biomolecules such as proteins or nucleic acids separated using electrophoresis? (hint: it is more than just molecular weight?) (2 pt)
4. Is electrophoresis used as a large-scale purification technique? Why or why not? (2 pt)
5. Cloning of DNA fragments to bacterial vectors involves a process known as "transformation". What is this process and how does it work? (2 pt.)
6. How can we tell if a bacterial colony on an agar plate has been transformed? (2 pt)
7. What is a *DNA library*? In 2-3 sentences summarize how one is made. (2 pt)