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)
- 37°C 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 **37°C 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 210 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 reaction 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. **Bring a portable memory device (e.g. thumb drive, etc.)**
11. While you are waiting on the other lab groups to have their gel photo taken, 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.
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          Buffer PE
Elution Buffer EB         Spin Column
2 ml collection tube (round bottom) 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 entire remaining PCR reaction to a microcentrifuge tube.
2. Add 5 volumes of Buffer PB to your PCR sample and mix. (e.g. add 200 ul of Buffer to remaining 40 ul of 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. Centrifugate 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).
Assignment – Preparation of a formatted gel electrophoresis figure

Prepare a formatted figure of your gel electrophoresis results. This should be done electronically using any number of software programs (Word, Powerpoint, Photoshop, Illustrator, etc.). The figure should be publication grade quality, which includes a figure legend containing all the information usually requested by scientific journals. An example from one of Dr. Larkin’s publications is included below to serve as a model.

Figure 1. Temperature response in reverse transcriptase PCR results. Samples: 1) Lemont, 2) Panda, 3) Rexmont, 4) Toro-2, 5) Nato. Temperature (degrees Celsius) of endosperm development is indicated above individual lanes. Molecular weight markers indicated in lane 1 and between samples 4 and 5. Electrophoresis conditions: 2% agarose gel in 0.5X TAE running buffer.

Note the following:

1. The figure legend begins with a figure number so it can be easily referred to in the text of a publication, as most will include multiple figures. Of course in your case there is only one figure but still number it appropriately. (1 pt)

2. The number is followed by a one-sentence description of the figure. (1 pt)

3. All lanes containing a result or molecular weight markers are identified in the figure legend. In this example, particular samples occupy more than one lane (individual experiments run at different temperatures) so they are grouped together. In most cases, there will be only one sample result per lane. (1 pt)

4. The most relevant molecular weight markers are identified to enable the reader to easily estimate the size of the sample results. A good rule of thumb is to identify at least the nearest 4 size markers (2 larger and 2 smaller) for each sample result (i.e. you will need to identify even more if there is more than one band among your samples). The sizes (base pairs) of the molecular weight markers we used in today’s experiment are provided below. (1 pt)
5. Some information regarding electrophoresis conditions is presented in the figure legend. This can be especially helpful when the figure is included in a poster, where a detailed *Materials and Methods* section may not be included. (1 pt).

6. Of course, no figure is complete without both the figure legend and the figure it describes (the photograph in our case). (1 pt).
1. Laboratory Performance (knowledge & initiative, procedure, safety, etc.) (2 pt).

2. Formatted figure of your gel electrophoresis results (6 pt).

3. What was the estimated size (bp) and concentration (ng/ul) of your PCR product? How does the size compare with the size of sequence obtained in Laboratory 2, *Database searching*? (2 pt).

4. We used a 1X TAE buffer during our electrophoresis procedure. What does the TAE acronym stand for? What advantages and disadvantages does TAE have compared to TBE, the other widely-used electrophoresis buffer? (hint: see the Wikipedia page for TAE buffer). (2 pt)