In Depth Protocol for Agarose gel electrophoresis with TBE

Reference: Sambrook, J. and D.W. Russell. Molecular Cloning: A Laboratory Manual 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Press, 2001.

*Before running a gel, be sure to read Chapter 5, Protocols 1 and 2 in Molecular Cloning Volume 1. Those protocols provide an excellent overview of agarose gel electrophoresis.

Before starting, ask the following questions:

**What size fragments am I resolving?*

Smaller fragments migrate faster through the gel matrix. Use pg. 5.6 in the Molecular Cloning Volume 1 to decide the appropriate agarose concentration.

**How many wells do I need?*

This is important to work out fully before starting. Will you need to run one sample more than once? Will you need a mass ladder? Do you need blank spaces between any samples (for example: to facilitate cutting fragments from the gel)?

**What voltage should I use?*

This varies greatly but generally is about 5 V/cm. To determine what constant voltage to apply, measure the distance the current travels through the gel between the cathodic (red, positive) and anodic (black, negative) electrodes. This distance is NOT a straight line from either end of the gel box but Using high voltage for long periods with TBE gels causes the gel and buffer solution to heat. This heat is uneven and may be sufficient to liquefy portions of the gel and denature the DNA contained within it.

Materials you will need:

1x TBE
agarose
Metal spatula
Weigh boat
250 mL Erlenmeyer flask
100 mL graduated cylender
Microwave
Heat resistant gloves
Distilled water
150 mL Erlenmeyer flask
1 mL transfer pipette

1) Assemble the gel casting materials:

a. For a 10-lane mini gel, firmly insert the two black rectangular dams on opposite sides of the raised center platform. Place the comb flush against the side towards the anodic electrode. The teeth of the comb should be 1 mm above the bottom of the tray. The teeth should not touch the bottom of the tray.

b. For a 20-lane Owl box, slightly moisten the rubber tubing on either side of the casting tray. Slide the tray evenly into the gel box so that the rubber tubing is parallel with the long sides of the gel box. The tray should fit snugly. If no rubber tubing is present, then the tray may also be taped with pressure sensitive tape. In either case, ensure that the molten gel will not leak from the casting tray.

2) Measure out the necessary amount of TBE and agarose
For a 2% (w/v) gel, use 2 g agarose per 100 mL 1x TBE.
Prepare 50 mL for the 10-lane mini gel and 100 mL for a 20-lane Owl gel.

3) Slowly add the agarose to the 250 mL flask. Try to get all of the agarose powder into the bottom of the flask and none on the walls. Gently pour the TBE into the flask. Mix the agarose and TBE by lightly swirling the flask. Tare the flask and record the value.

4) Pour approximately 30 mL of distilled water into the 150 mL flask. Place both flasks into the microwave.

5) Heat both flasks in the microwave for 45 seconds. Remove the 150 mL flask.

6) Heat the gel mixture for 90 seconds. The mixture will begin to boil; do not remove the gel mixture at this time. Wait until the mixture has boiled up, and then begins to simmer (usually 15 seconds after boiling starts). Remove the gel mixture and mass it. Add hot distilled water to bring the mass back to the pre-heat value. Gently swirl the flask to mix the water thoroughly with the molten gel. Heat the mixture for additional time if the water does not mix with the molten gel.

7) Run the base of the flask through a gentle stream of cold water. Swirl the flask gently through the stream, mixing the liquid gel as it cools. Do not mix the solution vigorously as that will introduce bubbles to the molten gel. When the gel is warm enough to touch (about 50 C), pour the solution into the casting tray.

8) Leave the gel to cool until completely set (approximately 30-45 min). The gel will change from transparent to translucent as it cools

*While the gel is cooling, add loading dye to all samples. Generally, loading dye is added at 1/10 of total sample volume. Loading dye is prepared as a stock and stored in the refrigerator. To prevent contamination of the stock, aliquot 1 mL of loading dye into a 1.5 mL microfuge tube for personal use.

When the gel is completely set, align the casting tray so the wells are located toward the anodic electrode. Pour 1x TBE over the top of the gel and into the reservoirs until the gel is covered by ~1 mm of buffer. Be sure that the gel is covered with buffer while resting on a level surface, but do not pour an excessive volume.

9) Remove the comb with one even, fluid motion. Allow the buffer and gel to equilibrate to the same temperature before loading samples. Pipette the desired amount of sample into each well. Typically, the same pipette tip may be used to load all samples. Between samples, the tip must be washed thoroughly by repeatedly taking up and releasing buffer from the anodic chamber. A fresh tip should be used if the fragments will be extracted or if the gel will subsequently be used for Southern hybridization.

10) Attach the lid to the gel box, covering the gel and buffer. The lid protects the user from receiving an electric shock as well as minimizing buffer volume decrease from evaporation.

11) Attach the electrodes. The anodic lead is attached closest to the wells. The cathodic lead is attached opposite the anodic lead. Use the mnemonic device ‘run towards red’ to remember correct placement of electrodes. Plug the leads into the power supply and apply voltage. If all leads are correctly attached, you will see bubbles generated at the cathode and anode due to electrolysis of water.

CAUTION, RISK OF ELECTRIC SHOCK: While the voltage is being applied to the gel, do not touch the gel or buffer solution. Do not touch the gel box or leads unless the power supply is off.

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12) While running the gel, the loading dye will separate into two blue dye fronts. The leading front, composed of bromophenol blue, migrates at about the same rate as a 300 bp fragment of linear double-stranded DNA in an agarose gel run in 0.5% TBE. The trailing front, composed of xylene cyanole FF, migrates at about the same rate as a 4 kb fragment of linear double-stranded DNA. Use these two fronts as indicators of when to stop the gel.

13) When the fragments have migrated the desired distance, turn off the power supply and disconnect the electrodes.

14) Prepare a staining solution by adding 10 uL ethidium bromide stock solution (10 mg/mL in water) per every 100 mL distilled water. Generally, 200 mL of dilute stain solution is sufficient to stain 10-lane or 20-lane gels.

WARNING: Ethidium is a carcinogen. Wear gloves when handling staining solutions or working in an area where gels are stained.

Pour the stain solution into a Pyrex dish and transfer gel to the stain. Do not contaminate gel boxes with ethidium bromide solution.

15) Stain the gel for 30-45 minutes at room temperature on a shaker. Long staining is usually only required when many bands need to be visualized (such as RAPD PCR products). Apply only a gentle rocking motion to the tray; the gel needs to remain covered by solution but not violently shaking.

16) Remove the stain from the tray by carefully pouring it into a clearly labeled lightproof jar. Stain solution can be repeatedly reused and supplemented with additional ethidium bromide stock before each use.

17) Rinse the gel and tray with cool tap water. The gel can be soaked in water for 20 minutes to minimize background fluorescence and sharpen fragments for photographing.

18) The gel may now be photographed using the Nucleotech Gel Expert apparatus (methods described elsewhere).