AGAROSE GEL ELECTROPHORESIS OF DNA

Overview

DNA fragments can be separated by size using agarose gel electrophoresis, and the DNA is visualized by fluorescent staining using ethidium bromide. This procedure is used following restriction enzyme digests, cloning, DNA purification and numerous other procedures. It is probably the most commonly used molecular biology technique. It can be used to determine fragment sizes by comparison to a DNA size standard (ladder), or to estimate the purity or yield of DNA.
(Note: It is also possible to use polyacrylamide gels for separation of smaller DNA fragments, and other DNA stains are available. (See #.)

List of Materials

DNA samples
Loading buffer (6x)
Buffer (TAE or TBE) for preparing gel and tank
Gel electrophoresis unit and power supply
Ethidium bromide stain
Transilluminator
Camera with film

Preparation of the Gel

1. Set up the electrophoresis unit and determine the volume of the gel unit tank (where the buffer will be) and the gel tray. This can be done algebraically (h x v x w), or by testing the volume of water the tank and tray can hold. The running buffer in the tank should be high enough to cover the gel bed, submerging the gel by several mm. The agarose gel should be about 5-8 mm deep. Pour about half the running buffer into the gel unit.
2. To make a 1% agarose gel mix 1 g of agarose with 100 mL buffer in a 250 mL erlenmeyer (this leaves room for boiling). Cover with sponge cork, swirl to mix, microwave on low until the gel is melted. Swirl/mix again. Add ethidium bromide at this point if desired and mix again. Let cool just until the flask can be held in your hand (about 60 °C). Prepare the gel tray. Insert a comb in the tray being certain that 1-2 mm of clearance is between the comb and bed (slide a tip partially under the comb).
3. Carefully pour gel into the tray, tapping to remove bubbles. Let solidify about 30 minutes. (Premade gels can be stored in plastic bags at 4 °C)
4. Remove the comb by lifting from one side. Place the tray in the electrophoresis unit, and cover with the remaining buffer.

Click picture below to view a movie:

Preparation of the Sample

1. Determine the amount of DNA that will be loaded, recalling that 10ng of DNA per band can be observed using ethidium bromide fluorescent stain. (To determine DNA purity load more sample, as overloading the DNA will allow you to see small amounts of impurities such as RNA.) If necessary, dilute the sample with water or buffer.
2. Add 1 uL 6x loading dye per 5uL of sample, thus diluting the dye to a final concentration of 1x. Be sure the total volume will be less than the well volume. (For example, 10uL of 10ng/ul DNA will contain 100ng of DNA, and the final volume with dye will be 12 uL. If the well is 1mm x 5mm deep by 4 mm wide it can hold 20 uL sample.) Mix.
3. If necessary (ie for lambda ladders and digests) heat the DNA sample at 65 oC for a few minutes, chill on ice, and spin down.
4. Load the sample in the well. On a strip of tape record the sample order and other details such as the run time, voltage, type of gel and buffer. Place the tape on the unit. (Later the tape can be used to fix the photo in your notebook.)

Click picture below to view a movie:

Running the Gel

1. Hook up leads and turn the power supply on to about 80 volts. DNA is negatively charged so it will migrate toward the red, positive cathode. Run the gel until the dye has migrated about 2/3 of the total gel length. (Small DNA fragments migrate faster than the dye.)
2. Stop the run, and carefully remove the gel into a UV-transparent tray. Wear gloves!
3. Discard the buffer in the sink unless it contains ethidium bromide. Rinse the unit and gel trays.

Click picture below to view a movie:

Visualizing DNA

1. If the gel is not prestained with ethidium bromide, then place the gel in stain for a few minutes.
2. Place the gel on a transilluminator and visualize bands. Record with a camera, using Polaroid 667 film with UV filters. Wear a UV face shield to avoid serious burns and painful eye damage.

Click picture below to view a movie: