

ELECTROPHORETIC SEPARATION OF DNA FRAGMENTS

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http://biology.clc.uc.edu/fankhauser/Labs/Genetics/DNA_Electrophoresis/DNA_Electrophoresis.htm

NOTE: Latex gloves should be worn at all times to protect from ethidium bromide, and to prevent contamination of DNA samples with skin-borne endonucleases.

EQUIPMENT:

DNA quality clean glassware:
1 L beaker
1000 mL graduated cylinder
100 mL graduated cylinder
25 mL graduated cylinder
Two 250 mL beakers
horizontal electrophoresis unit
gel tray
power supply
well comb
micropipettors
UV transilluminator

SUPPLIES:

latex gloves
50x TAE*
DNA grade agarose
10 mg/mL ethidium bromide solution
(*CAUTION:* carcinogen)
masking tape
DNA samples:
undigested lambda DNA
assorted lambda DNA digests
(HindIII, EcoRI, BstII, etc)
6x loading dye
microcentrifuge tubes

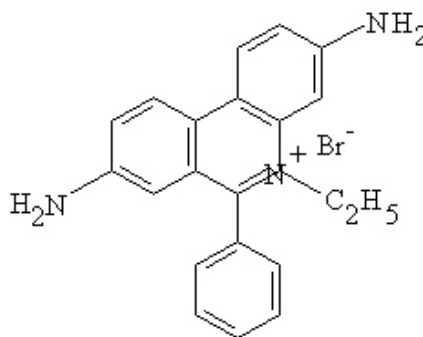
PREPARE THE RUNNING BUFFER AND GEL:

1. **Prepare running buffer:** Dilute 20 mL 50x TAE buffer to 1 liter in dH₂O, mix.
2. **Prepare the agar:** In 250 mL beaker, weight 0.64 g of DNA grade agarose. Add 80 mL 1x TAE buffer, microwave 1 min, swirl, microwave for 15 more seconds (95°C). Swirl to ensure complete solution. (Or heat over bunsen burner to 95°C.) Let cool to 50°C. *CAUTION, wear gloves:* Add 4 µL of 10 mg/L ethidium bromide solution to 80 mL agar to make it 0.5 µg/mL.
3. **Pour agar slab:** Tape the ends of the gel tray *securely* with masking tape, pour in cooled agar, place comb at one end, let sit on level surface until completely solidified.
4. **Carefully remove the comb** by wiggling and pulling straight up. Do not tear the agar. Remove the tape from the ends.
5. **Set up electrophoresis apparatus:** Lie the gel tray with prepared agar in the leveled electrophoresis chamber with the *wells to the right*, towards the black terminals (negative). Fill the apparatus with enough 1x TAE buffer to just cover the gel (filling the wells in the process).
6. **Prepare the DNA samples** so that they have 1 to 4 µg of DNA in up to 36 µL of solution containing 1x loading dye (i.e.: 5 µL sample plus 1 µL loading dye, or up to 30 µL sample plus 6 µL loading dye.) Mix either by flicking or by drawing up and down in the micropipet. Load 1 µg of undigested, 4 µg of digested DNA into designated wells.
7. **Load the samples** into the wells: Loading the pipet with the desired volume of sample, then with braced hands, insert the tip into the mouth of the well without touching the sides or bottom, *slowly and steadily* depress the plunger without shaking or causing bubbles. *Do not blow out.* When the sample is loaded, withdraw the pipet directly out of the well with a smooth movement. The sample should form a clean rectangle of blue with no irregularities surrounding it. Place standards (undigested DNA or lambda Hind III digest) in the outer wells.
8. **Run the gel:** After the wells are loaded, close the lid, attach the electrodes from the power supply, and turn on the DC power. Set the voltage for 150 volts, and plan to run it for at least an hour, preferably 2 to get the dye near the end of the gel.

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Ethidium bromide: A mutagen, intercalates between bases of DNA, fluoresces under ultraviolet light, allows DNA to be visualized even in small quantities.

Must be treated with great respect and disposed of as a hazardous material. NEVER get it on your skin



Ethidium Bromide