

## PRACTICE LOADING AND RUNNING ELECTROPHORESIS page 28

David B. Fankhauser, PhD, Professor of Biology and Chemistry

22 Feb 02, 2 Jan 03, 24Jan06, 18Dec08, 20Jan09, 29Dec09, 25Jan11

[http://biology.clc.uc.edu/fankhauser/Labs/Genetics/DNA\\_Electrophoresis/DNA\\_Electrophoresis.htm](http://biology.clc.uc.edu/fankhauser/Labs/Genetics/DNA_Electrophoresis/DNA_Electrophoresis.htm)

**PRACTICE:** The class will divide into three teams. Each will prepare a gel using bactoagar instead of DNA-quality agar (which is much more expensive). Each team will prepare 400 uL of 1x loading dye. Each student will practice, and loading 30 uL into one large well (32 uL) and 10 uL into each of two small wells. Do not tear sides nor puncture bottom of the wells.

### EQUIPMENT PER TEAM:

1000 mL beaker  
1000 mL graduated cylinder  
100 mL graduated cylinder  
25 mL graduated cylinder  
250 mL beaker  
horizontal electrophoresis units  
gel tray, and pouring frame (or masking tape)  
power supply (to be shared)  
well combs large and small toothed

### SUPPLIES:

10 uL, 200 uL, 1000 uL pipettors and tips  
latex gloves  
50x TAE\*  
Bacto-agarose  
masking tape and/or slab prep form  
10x loading dye  
microcentrifuge tubes (need not be sterile)  
Ringstand, wire mesh and bunsen burner  
thermometer

### Wear gloves for the duration of the exercise:

#### Prepare sample, each team:

- 1 400 uL water into microfuge tube.
- 2 Add 1/9th volume of 10x loading dye (44 uL), mix by flicking the tube.

#### Prepare a gel with two combs per team:

- 1 Prepare 1000 mL TAE buffer: 20 mL 50x TAE\* , *q.s.* to 1000 mL with dH<sub>2</sub>O. Mix.
- 2 Weigh out 0.64 g agar (Bacto agar for the practice, DNA-quality agar for "real.")
- 3 Add 80 mL TAE buffer in 250 mL beaker.
- 4 Stirring with thermometer, bring to boil, remove from fire before it boils over.
- 5 Let cool to 60°C.
- 6 Prepare tray to receive melted agar (tape if necessary)
- 7 [If for "real," add 4 uL 10 mg/mL ethidium bromide to 60 C agar, but not for this practice.]
- 8 Pour 60°C agar into prepared tray on level surface (with ethidium bromide if for "real").
- 9 Insert comb(s) into tray, allow to cool to solid.
- 10 When solid, carefully remove combs, place tray in electrophoresis tank, wells to R (black)!
- 11 Fill tank with TAE buffer until wells are just filled.
- 12 Add sample to wells (30 uL to large wells, 10 uL to small wells).
- 13 Cover, attach cables, turn on power supply, adjust to 150 volts.
- 14 Check after 2-3 minutes to confirm that dye is running correctly.
- 15 Run for 1-1.5 hours. Determine velocity of bromthymol blue band movement.

#### \* For 100 mL 50x TAE buffer:

24.2 g TRIS (base)  
5.71 mL glacial acetic acid  
10.0 mL 0.5 M EDTA (14.6 g/100 ml)