

## **Polyacrylamide Gel Electrophoresis (PAGE)**

Used for DNA and other applications where very high resolution is required.

### **Apparatus Assembly**

- Note: The process is mostly FYI as pre-cast gels are commercially available and used in this lab.

### **Gel Components:**

Double distilled water

Tris Acetate Buffer (weak, good for short runs) or Tris Borate buffer (strong good for long runs)

Unpolymerized acrylamide solution (5 %) **Very toxic**, gloves and gas mask should be used.

TMED Tetra-methyl-ethylamine-diamine cross links the acrylamide

Ammonium persulfate (made fresh) is a catalyst for the reaction

Polymerization takes about 30 minutes and can be verified by looking at unused fraction left in flask.

### **Setting up the rig**

Install gel with shorter plate towards back (remember to remove comb and tape)

Center device and tighten slightly

Fill back compartment first and check for leaks. Ensure that the buffer covers about 1 cm above the back plate.

Fill front compartment.

Pre running gel and washing loading lanes are only required when working with freshly prepared gels (not for commercially available gels.)

### **Loading sample**

Tip is placed atop back plate and sample is allowed to dribbel" down into well.

DO NOT force tip between plates.

### **Running the gel:**

1-8 volts per cm of gel is average (so a 10 cm long gel can run from 10 to 80 volts.)

Lower voltage will preserve sample better and is less likely to damage the cells. Also lanes will run more uniformly (no smiles!)

### **Staining, visualization and molecular weight determination:**

Staining: Gently open plates holding the gel. Watch to see what plate the gel is sticking to and turn accordingly so that this plate is on bottem.

Gently transfer to staining tray and photograph using UV light (for DNA) and Polaroid film.

**NOTE** Interpolation of fragment mass. Standard curve is created using semi log paper. Distance migrated is on linear access, and molecular weight is on log access. Distance migrated is measures and this value is carried over to best fit line, and down to molecular weight ( this process is know as interpolation, know how to do this, as it is very useful.)