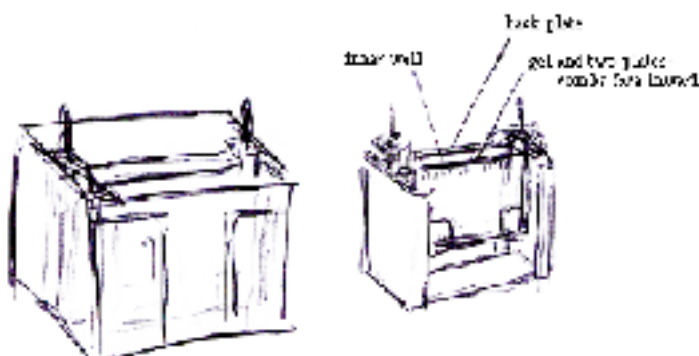


## Polyacrylamide Gel Electrophoresis

- (1) Resuspend and lyse the cell pellets from the induction.
  - Resuspend the pellet in 50  $\mu$ L TE
  - Add 50  $\mu$ L 2X Sample Buffer (SB)
- (2) Temperature shock.
  - Alternate 5 min. heating in the 100C block and freezing in the -80C freezer.
  - Heat, freeze, heat, freeze, heat. (Beware! Lid can pop open during heating.)
  - End on heat, because freezing can cause unrelated proteins to “globulate.”
  - Let cool to R.T. before loading.
- (3) Load the solution.
  - The sample with the lowest OD should be the 10 $\mu$ L (max. vol.) sample
  - Use vol. and OD in  $MV=MV$  to standardize the amount of protein being loaded.
  - Re-freeze unused solution at -80C.
- (4) Run at 100V until the dye hits the separating gel, then crank up to 160V.
- (5) Stain.
  - Remove, put in Gladware with ventilated lid.
  - Cover in Fairbanks stain (contains Coomassie Blue).
  - Microwave for 40-50s.
  - Put Gladware on shifty thing in hood for 10 min.
- (6) Destain.
  - Pour stain in waste bin.
  - Add Fairbanks destain.
  - Microwave for 50s.
  - Add two small Kimwipes.
  - Put Gladware on shifty thing in hood.
  - Check after 30 min-1 hr to preview the gel.
  - Remove Kimwipes, repeat first five destain steps, let destain overnight.

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Setting up the gel rig:



1. Lock gels into place. Make tops flush with bottom ridge on green padding.
2. Mix buffer soln: 450 mL H<sub>2</sub>O + 50 mL 10X Running Buffer.
3. Fill inner well to top first. Push off bubbles. Wait to see if it leaks.
4. Pour remainder of soln into outer well.