Polyacrylamide Gel Electrophoresis

(1) Resuspend and lyse the cell pellets from the induction.
- Resuspend the pellet in 50 mL TE
- Add 50 mL 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 10mL (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 Coomasie 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.

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 H2O + 50 mL 10X

	3. Fill inner well to top first. Push off bubbles Wait to see if it leaks. 4. Pour remainder of soln into outer well.