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