Protein Gel Protocol (Lac Promoter Protein

1. In 5 mL LB + antibiotic, seed tube with frozen stock or from plate. Also make WT culture. Grow overnight.
2. Determine IPTG gradient you want to run (for example 0, .1, .5, 1, 2 mM). Add 50-100 uL overnight culture to new LB+antibiotic tubes. Grow to OD .7.
3. Defrost IPTG in the dark on ice.
4. Add IPTG in appropriate amounts. Grow to OD 1.5-5 (preferably 2+).
5. Make every culture sample the same OD by diluting with water (the lowest OD you have).
6. Take samples and spin down, dump supernatant. Wash 2x with 1 mL water.
7. Use ~5 OD. Add B-Per reagent (1862487 #78243). Mine was 1 OD so I used 200 uL reagent* (1 mL for 5 OD).
8. Re-suspend in reagent, incubate at room temp for 30 minutes.
9. Take 20 uL entire fluid to PCR tube (whole sample).
    a. Centrifuge remaining sample and take 20 uL supernatant (soluble protein).
    b. Wash 2x with water (original reagent volume*-sample extracted volume) –180 uL in this case.
    c. Re-suspend in small amount of liquid. Take 10 uL of sample (insoluble protein).
10. From protein supplies box (green, top shelf -20˚C freezer), take PLB 6x and add 5 uL to each sample.
11. Boil samples in Thermocycler 95˚C 10 min.
12. Take a gel from the bottom shelf of the 4˚C fridge. Wash gel in DI water from sink. Remove tape (bottom) and well comb (top).
13. Add gel with the flat side away from the front of the protein gel tank, do not clamp yet.
14. Add recycled running buffer to unclamped gel to the mark between the gels ~1.5” from the bottom. Buffer should be in front and behind gel.
15. Clamp gel in. Add buffer to fill line in front section of the gel.
16. Add 15 uL protein and dye to wells as well as ladder (in between the dotted lines marked).
17. Run 80-100V (current stays the same, leave that setting). Run for 110 minutes. If the gel samples are arcing in the middle, decrease the voltage to have a more uniform line. Run until the front end of samples reach the end of the gel (we added 20 minutes).
18. Fill tuperware half full with DI water, wet the sides with water to keep gel from getting caught and torn. Pry gel plastic open with scoopula flat edge. Run pipet tip down the outer edge of gel to free it. Drop gel into water and grain water.
19. Add staining solution overnight on rotor.
20. Dump staining solution back into recycled staining solution.
21. Add de-staining solution and a Kim wipe to absorb staining solution.
22. Take photo on laminated white sheet of paper of gel. Put in PowerPoint and adjust sharpness and exposure to optimal view. Use DI water to push gel back off paper and into container.