Protein purification via agarose gel

1. Turn on water bath to 55C
2. Aliquot NID extraction buffer + 10% extra (150uL * number of preps * 1.1)
3. Add 100x Lysozyme (in corner of -20C Enzymes box) to extraction buffer
4. Mix by gently inverting 6 times
5. Pellet 0.5-3 ml of bacterial cultures at 6000 rpm for 1 min. Shake out supernatent over sink.
6. After drawing 150 ml extraction buffer into a pipette tip, the pellet was loosened off the tube wall with the tip without releasing the buffer. Then the extraction buffer was added and the pellet GENTLY resuspended by pippetting up and down slowly and just enough times to resuspend the pellet.
7. Incubate the bacterial suspension at 55C for 5 min.
8. Centrifuge at maximum rpm for 10 min or until a tight bacterial pellet is formed.
9. Decant supernatant careful not to disturb pellet and freeze @ -20C. This is your crude lysate miniprep
1. Mix 50mL of TAE and 0.25g of agarose to make a 0.5% gel
2. Put crude lysate miniprep in gel and run at 180V
3. Run for 30 minutes
4. Pause gel
5. Make a cut below the colored part for your protein
6. Place a piece of paper folded like an accordion and wetted in the cut so it will block the progress of the protein
7. Drop the voltage to 100V since at 180V the paper causes a hot spot that melts the gel and denatures proteins
8. Run the gel and keep checking every 5 minutes to see when all the color has accumulated in front of the paper
9. Stop the reaction and cut out the colored sections and put them in 650 mL eppondorf tubes
10. Freeze them at -80 for 20 minutes or more.
11. DO NOT THAW
12. Take tubes out, poke a hole in the bottom with a needle and then place the whole thing in a 1.5mL eppendorf tube (make as small of a hole as possible)
13. Centrifuge at max speed for 5 minutes.
14. The liquid should all collect in the bottom along with a little bit of the gel
15. Your protein will be entirely in the liquid and no more soap, DNA or RNA should be in it.