Purification of Green Fluorescent Protein

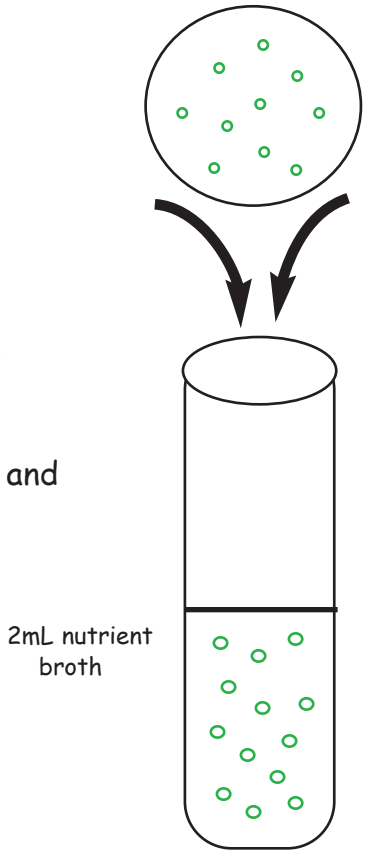
Using Hydrophobic Interaction Chromatography

Part I: Fluorescent Cell Culture

1. Use a small micropipettor with a sterile tip to pluck a single green fluorescent colony from the surface of an agar plate.

2. Place the tip inside a tube containing 2 mL of nutrient luria broth and eject tip.

3. Cap the tube and place in a 37 C shaking water bath or incubator overnight. Do not cap tightly. Cells need to aerate.



Part II: Collect and Lyse Cells

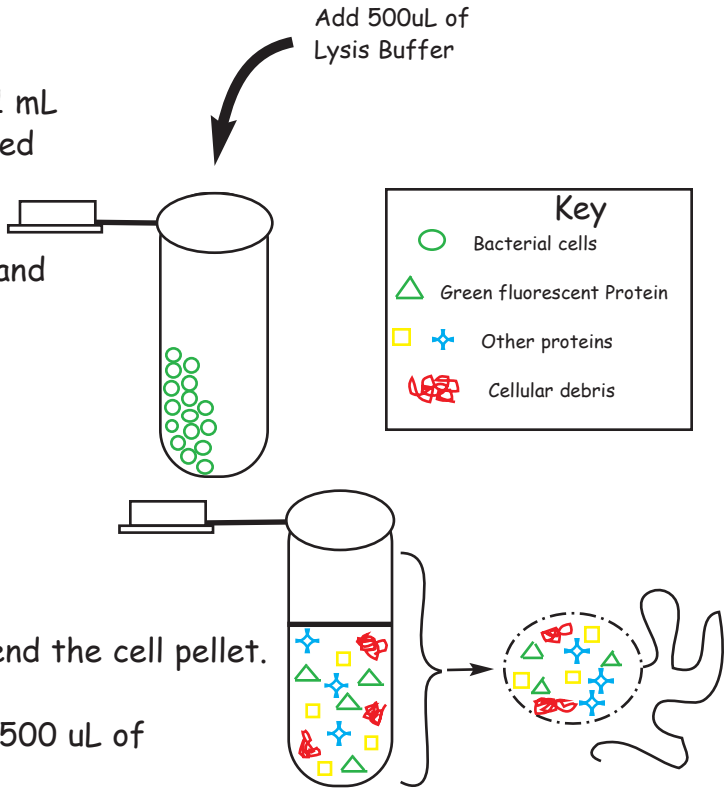
4. Using a marker, place your initials on a clean, 1.5 mL clear tube.

5. Using a large micropipettor, transfer 1 mL of the overnight culture into the labelled microcentrifuge tube.

6. Place the tube into a microcentrifuge and spin for 5 minutes. Be sure to balance the tubes.

7. After the cell culture is centrifuged, carefully pour off the supernatant so only a green cell pellet remains.

   Repeat steps #5-7.



8. Add 30 uL of GTE buffer and resuspend the cell pellet.

9. Use a large micropipettor to transfer 500 uL of Lysis buffer into the same tube. Mix the cells and buffer by pipetting up and down.

10. Incubate sample tube on ice for 10-15 minutes.

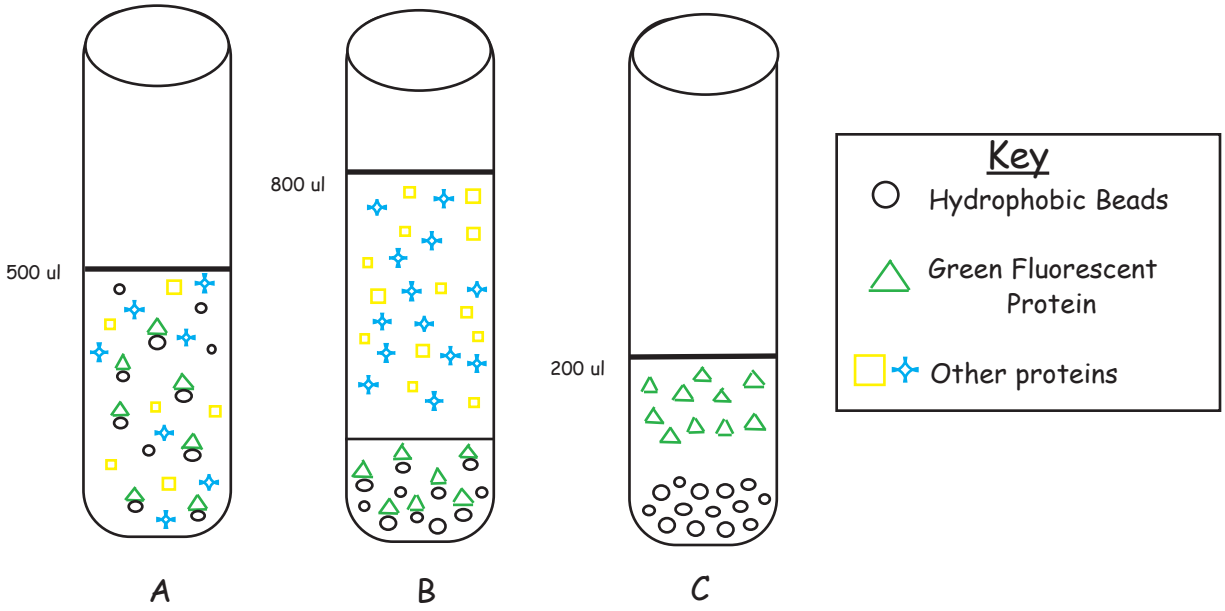
Part III: Purify the Protein

11. Place your tube in a microcentrifuge for 5 minutes to pellet bacterial debris.

12. The proteins are suspended in the supernatant. Collect 150 ul of the supernatant and transfer it to a clean 1.5 mL tube.

13. Add 150 uL of **binding buffer** to the 150 ul of supernatant. Mix tube by inverting several times.

14. Load 300 uL of the buffer/supernatant mixture to a tube containing 400 ul of hydrophobic **bead resin**. Shake tube and spin for 1 minute.(Figure A)



15. Remove the supernatant using a large micropipettor. Be careful not to disturb beads. Add 800 uL of **Wash buffer** to the beads. Shake and spin for 1 minute. (Figure B)

16. Remove the supernatant using a large micropipettor. Be careful not to disturb pellet. Add 200 uL of **Elution buffer** to the beads. Shake and spin for 1 minute. (Figure C)

17. This time the supernatant contains our protein! Collect this liquid and place it in a clean, clear 1.5 mL tube. Do you have purified Green Fluorescent protein in your tube? Place the tube under a UV light to find out!