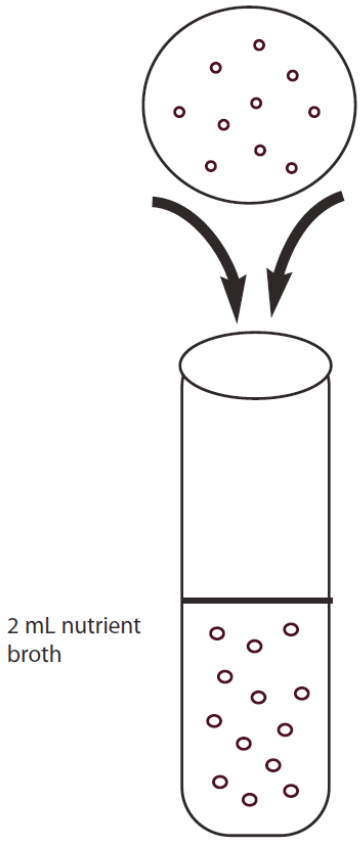
# Purification of Green Fluorescent Protein

## Using Hydrophobic Interaction Chromatography

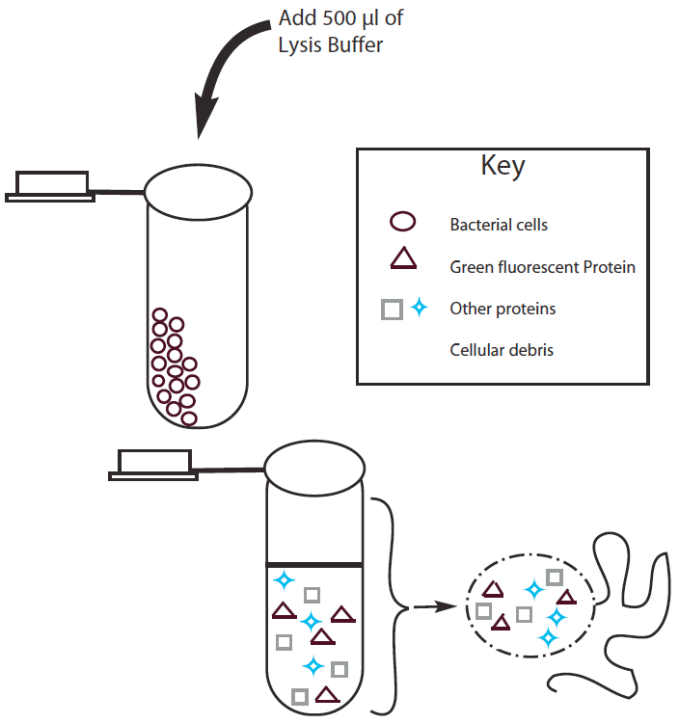
## Part I: Fluorescent Cell Culture

1. Use a small micropipette 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 Luria broth and eject tip.
3. Cap the tube loosely and place in a 37°C shaking water bath or incubator overnight. DO NOT CAP TIGHTLY! CELLS NEED TO AERATE.



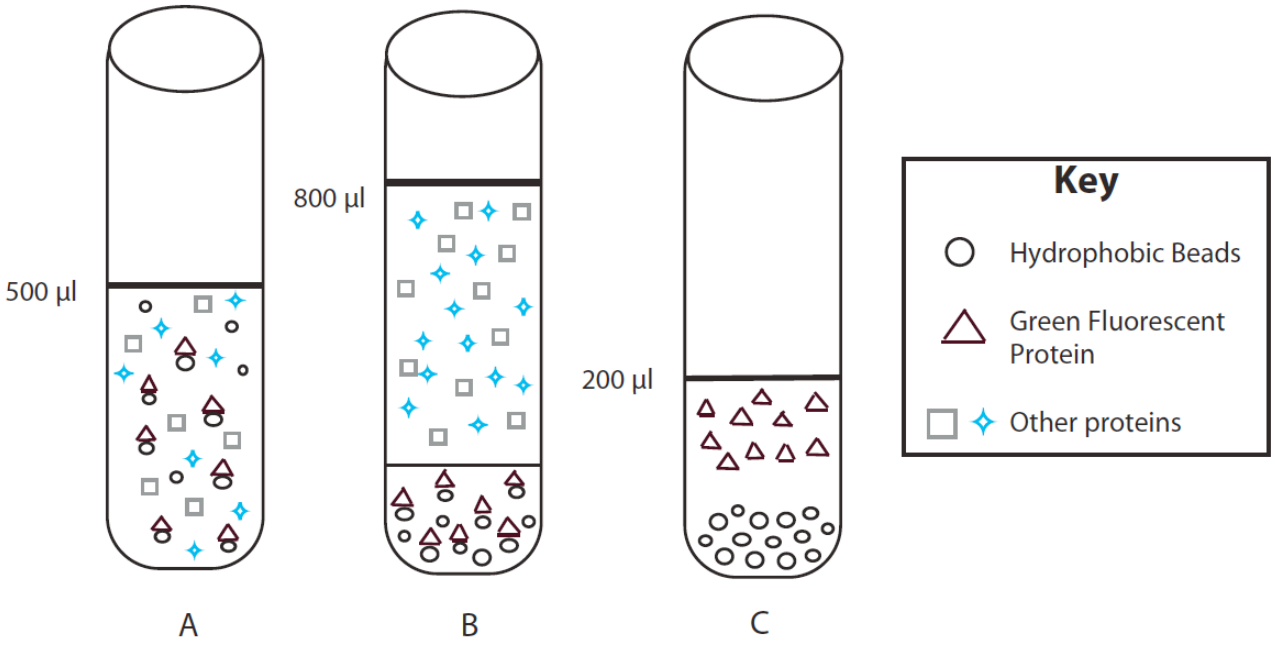

## Part II: Collect and Lyse Cells

4. Using a marker, place your initials on a clean 1.5 ml tube.
5. Using a large micropipette, transfer 1 ml of the overnight culture into the labeled micro-centrifuge tube.
6. Centrifuge for 5 minutes.
7. Carefully pour off the supernatant until only a green cell pellet remains.
   **Repeat steps #5-7.**
8. Add 30 µl of GTE buffer and resuspend the cell pellet.
9. Use a large micropipette to transfer 500 µl of lysis buffer into the same tube. Mix the cells and buffer by pipetting up and down.
10. Incubate tube on ice for 10-15 minutes.



## Part III: Purify the Protein

11. Centrifuge sample for 5 minutes to pellet bacterial debris.
12. Collect 150 µl of the supernatant (the proteins are suspended in the supernatant) and transfer it to a clean 1.5-mL tube.
13. Add 150 µl of binding buffer to the 150 µl of supernatant. Mix tube by inverting several times.
14. Transfer 300 µl of the buffer/supernatant mixture to a tube containing 400 µl of hydrophobic bead resin. Shake tube and centrifuge for 1 minute. (Figure part A)




15. Remove the supernatant using a large micropipette. Be careful not to disturb beads. Add 800 µl of wash buffer to the beads. Shake and centrifuge for 1 minute. (Figure part B)
16. Remove the supernatant using a large micropipette. Be careful not to disturb beads. Add 200 µl of elution buffer to the beads. Shake and centrifuge for 1 minute. (Figure part C)
17. This time the supernatant should contain only the purified Green Fluorescent Protein! Collect this liquid and place it in a clean, clear 1.5-ml tube. Return bead resin to instructor!
18. Place the tube under a UV light to see if your experiment was a success.