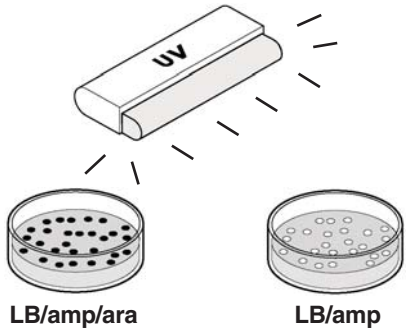
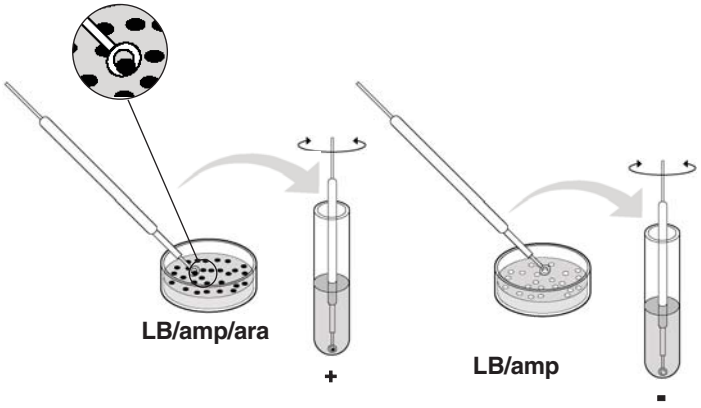
GFP Purification—Quick Guide

Lesson 2 Inoculation
Growing Cell Cultures

1. Remove the transformation plates from the incubator and examine using the UV light. Identify several green colonies that are not touching other colonies on the LB/amp/ara plate. Identify several white colonies on the LB/amp plate.



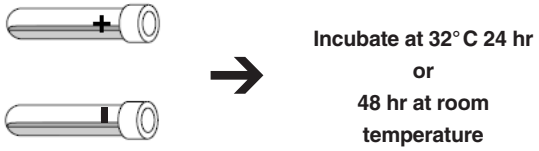
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick only a single colony). Spin the loop between your index finger and thumb to disperse the entire colony.



3. Cap the tubes and place them in the shaking incubator, shaking water bath, tube roller, or rocker and culture for 24 hr at 32°C or 2 days at room temperature.

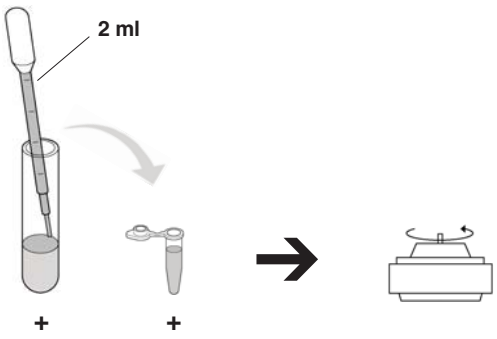
   or

   Cap the tubes and shake vigorously by hand. Place in the incubator horizontally at 32°C for 24–48 hr. Remove and shake by hand periodically when possible.



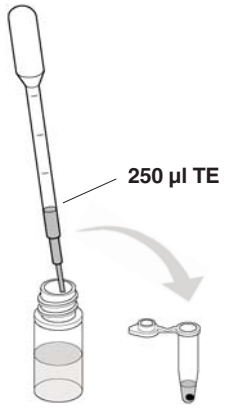
Lesson 3 Purification Phase 1
Bacterial Concentration

1. Label one microcentrifuge tube "+" with your name and class period. Remove your liquid cultures from the shaker and observe with the UV light. Note any color differences between the two cultures. Using a new pipet, transfer 2 ml of "+" liquid culture into the “+” microcentrifuge tube. Spin the microcentrifuge tube for 5 minutes in the centrifuge at maximum speed. The pipet used in this step can be repeatedly rinsed in a beaker of water and used for all following steps of this laboratory period.

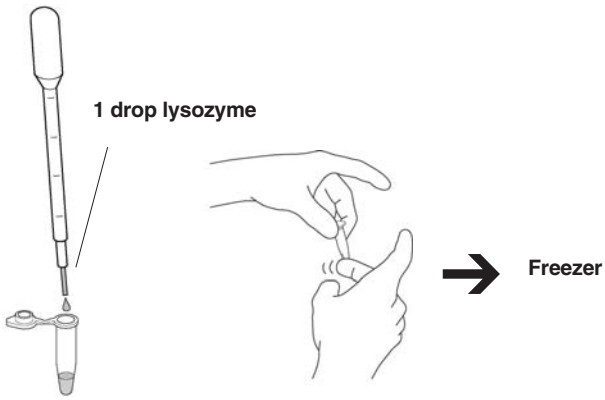


2. Pour out the supernatant and observe the pellet under UV light.

3. Using a rinsed pipet, add 250 µl of TE buffer to the tube. Resuspend the pellet thoroughly by rapidly pipetting up and down several times.



4. Using a rinsed pipet, add 1 drop of lysozyme to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.

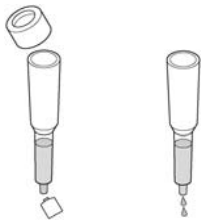


5. Place the microcentrifuge tube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

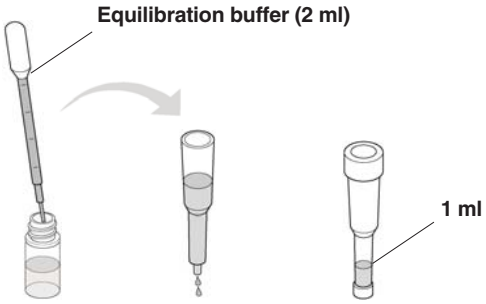
Lesson 4 Purification Phase 2
Bacterial Lysis

1. Remove the microcentrifuge tube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.

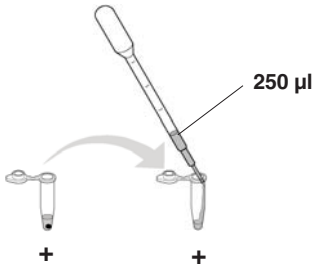
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



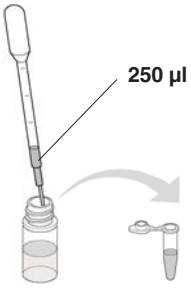
3. Prepare the column by adding 2 ml of Equilibration Buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipet. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



4. After the 10 minute spin, immediately remove your tube from the centrifuge. Examine the tube with the UV light. Using a new pipet, transfer 250 µl of the "+" supernatant into a new microcentrifuge tube labeled "+". Again, rinse the pipet well for the rest of the steps of this lab period.

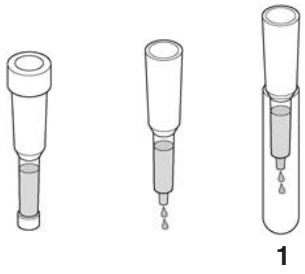


5. Using a well rinsed pipet, transfer 250 µl of binding buffer to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.

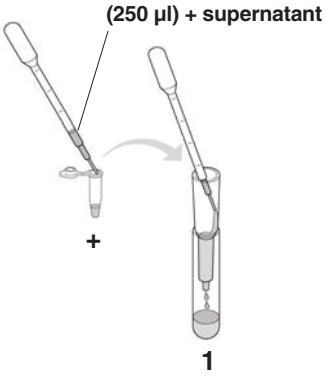


Lesson 5 Purification Phase 3
Protein Chromatography

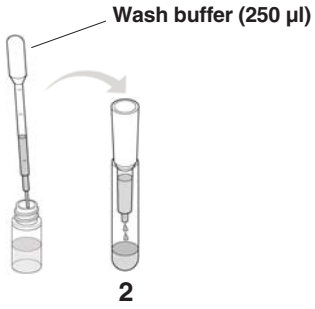
1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix proceed to the next step below.



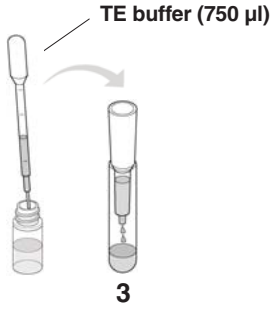
2. Using a new pipet, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipet tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light. Note your observations. After it stops dripping transfer the column to collection tube 2.



3. Using the rinsed pipet, add 250 µl of wash buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.



4. Using the rinsed pipet, add 750 µl of TE Buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations.



5. Examine all three collection tubes and note any differences in color between the tubes. Parafilm or plastic wrap the tubes and place in the refrigerator until the next laboratory period.