Green fluorescent protein (GFP) purification

How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?

This lesson is a continuation of the pGLO Transformation kit. Students remove a colony of transformed bacteria that results from that lab and treat it to remove and purify the green fluorescent protein (GFP) that it produces.

Protein such as insulin, can be created by bacteria in labs, purified, then used as medicine. This lab uses one technique, hydrophobic interaction column (HIC) chromatography to separate GFP from the bacteria that produced it. GFP can be used as a model protein to show that purification.

Students begin by using one colony of transformed bacteria from each condition in the pGLO transformation lab. The colonies are added to nutrients to grow, then incubated. After 24-48 hours, the bacteria is centrifuged, resuspended, then an enzyme is added to lyse the bacterial membranes. The samples are frozen to complete the breakdown of the bacteria, then centrifuged again to remove the bacterial debris. The remaining protein samples are then added to the chromatography columns which contain a "bed" of microbeads, these columns are treated with buffers of high salt concentrations that retain the hydrophobic (water-hating) protein (GFP). The final treatment rinses the protein from the column.

Reflection

1. Using the data table from Purification Phase 3, compare how your predictions matched up with your observations for each buffer.
   a. Binding buffer

   b. Wash buffer

   c. Elution buffer

2. Based on your results, explain the roles or functions of these buffers. Hint: how does the name of the buffer relate to its function.
   a. Equilibration buffer

   b. Binding buffer

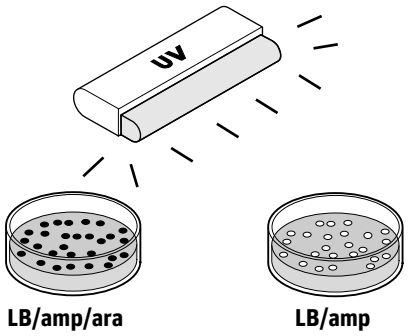
   c. Wash buffer

   d. TE (elution buffer)

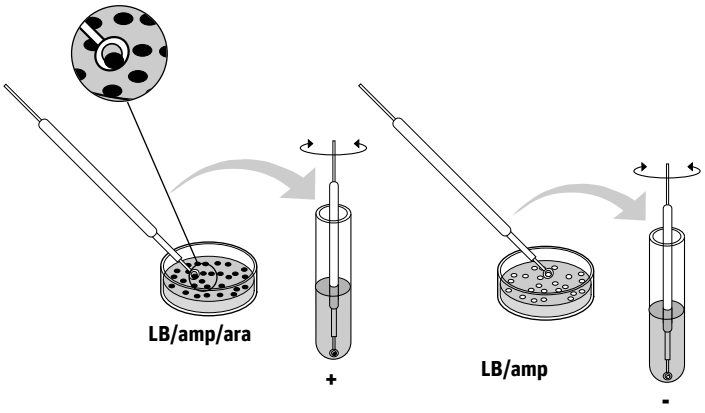
3. Were you successful in isolating and purifying GFP from the cloned bacterial cells? Identify the evidence you have to support your answer.

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.



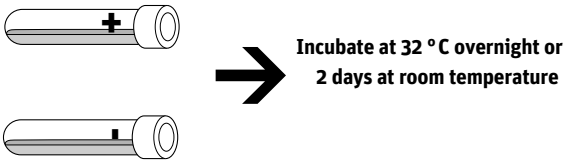
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick 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 or on the shaking platform and culture overnight at 32 °C or 2 days at room temperature.

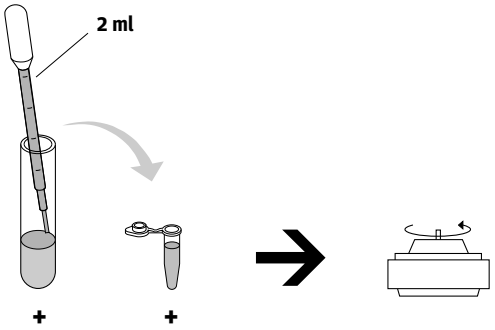
   or

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



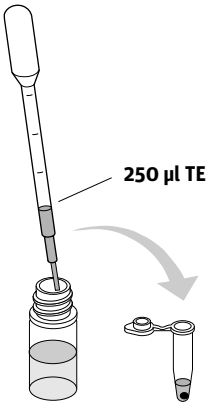
Purification phase 1: bacterial concentration

1. Label one microtube "+" 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 pipette, transfer 2 ml of "+" liquid culture into the "+" microtube. Spin the microtube for 5 minutes in the centrifuge at maximum speed. The pipette used in this step can be repeatedly rinsed in a beaker of water and used for all following steps of this laboratory period.

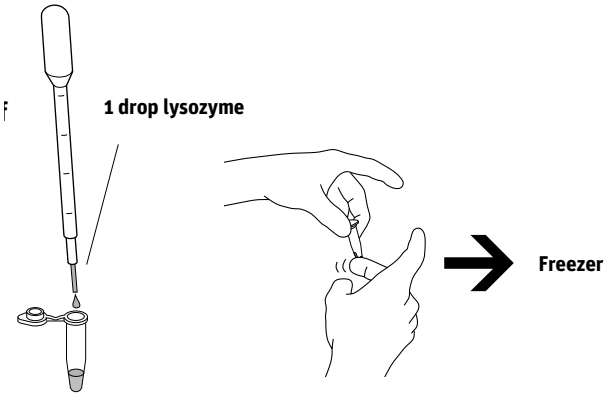


2. Pour out the supernatant and observe the pellet under UV light.

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



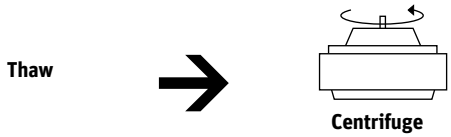
4. Using a rinsed pipette, add 1 drop of lysozyme to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.



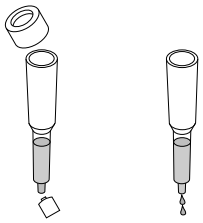
5. Place the microtube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

Purification phase 2: bacterial lysis

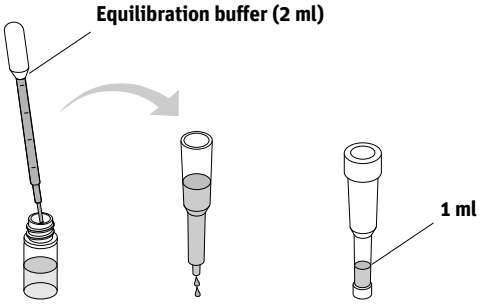
1. Remove the microtube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.



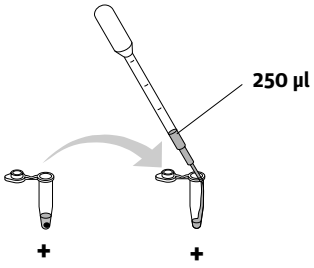
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



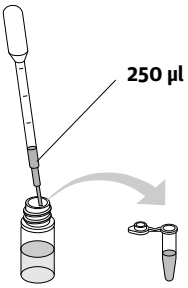
3. Prepare the column by adding 2 ml of Equilibration Buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipette. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



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



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



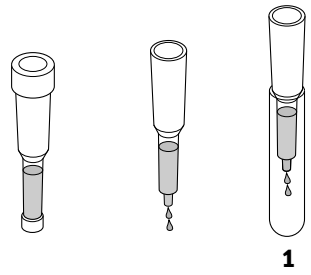
Equilibration buffer (used in preparation of the column): raises the salt concentration of the column to match the GFP lysate

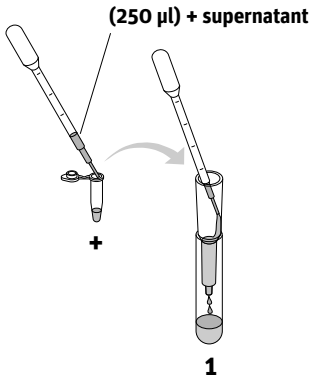
Binding buffer: raises the salt concentration of GFP to cause a conformational change in GFP (change in the tertiary structure of the protein), exposing the hydrophobic region of GFP

Wash buffer: washes away less hydrophobic, contaminating proteins from the column

TE (elution) buffer: removes GFP from the column

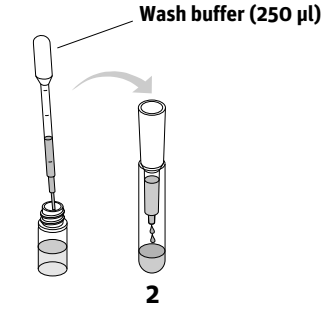
Collection tube number	Prediction	Observations under UV light (column and collection tube)
Tube 1 Sample in binding buffer		
Tube 2 Sample with wash buffer		
Tube 3 Sample with elution buffer		

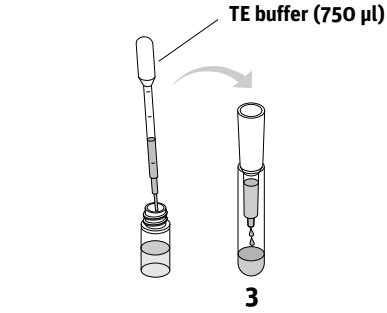


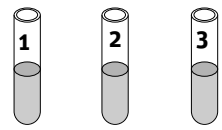


Purification phase 3: protein chromatography

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 pipette, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipette 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 pipette, 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 pipette, 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 Saran Wrap the tubes and place in the refrigerator until the next laboratory period.







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