Protein purification for GFP (option 2)

Focus questions	How can proteins be purified? What is the mechanism used in biotechnology to extract proteins of interest?
Vocabulary	Hydrophobic interaction column chromatography, lyse, supernatant, hydrophobic, hydrophilic

This lesson uses Bio-Rad pGLO™ Bacterial Transformation Kit, Catalog #166-0005EDU available from **explorer.bio-rad.com**. *This lesson is a continuation of the BioRad pGLO™ Transformation kit.*

Proteins such as insulin, can be created by bacteria in labs, purified, then used as medicine.

> Genes can be cut out of human, animal, or plant DNA and placed inside bacteria. For example, a healthy human gene for the hormone insulin can be put into bacteria. Under the right conditions, these bacteria can make authentic human insulin. When allowed to multiply in gigantic vats (fermenters) these bacteria can be used to mass produce the human insulin protein. This genetically engineered insulin is purified using protein chromatography and used to treat patients with the genetic disease, diabetes, whose insulin genes do not function normally.
>
> A common problem in purifying genetically engineered "designer" proteins from transformed bacteria is contamination by endogenous bacterial proteins. Chromatography is a powerful method used in the biotechnology industry for separating and purifying proteins of interest from bacterial proteins. Proteins purified in this manner can then be used, for example, as medicines to treat human disease, or, for household agents such as natural enzymes to make better laundry detergents.
>
> The cloning and expression of the GFP gene (pGLO™ Bacterial Transformation kit), followed by the purification of its protein in this kit, is completely analogous to the processes used in the biotechnology industry to produce and purify proteins with commercial value.

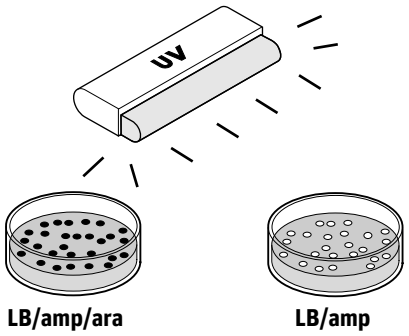
(**bio-rad.com/webroot/web/pdf/lse/literature/4006099.pdf** page 1)

This lab will use one technique, **hydrophobic interaction column (HIC)** chromatography to separate GFP from bacteria that produced it. GFP is being used as a model protein to show a process of purification.

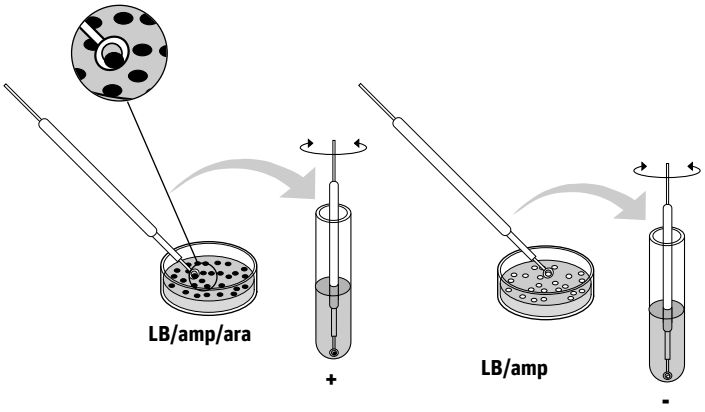
Procedure

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.



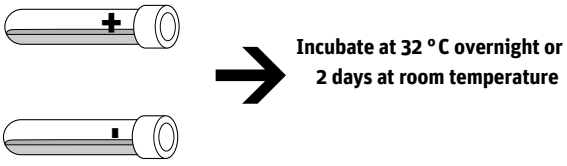
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick 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.

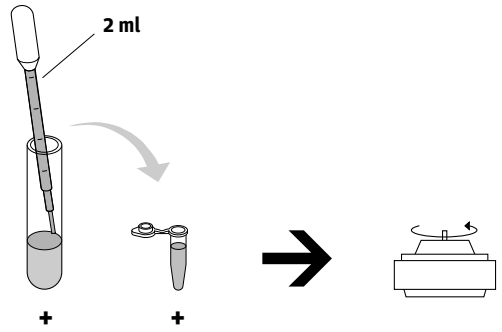
   or

   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.



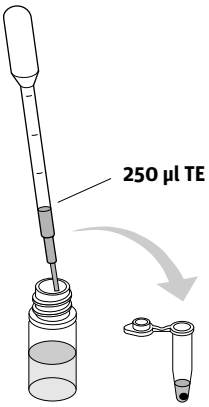
Purification phase 1: bacterial concentration

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.

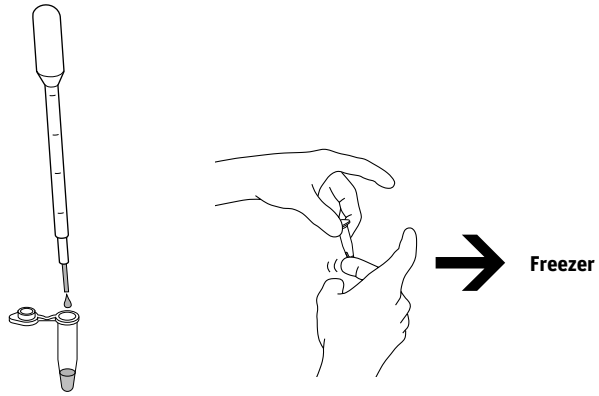


2. Pour out the supernatant and observe the pellet under UV light.

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.



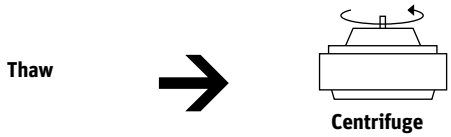
4. Using a rinsed pipette, add 1 drop of _____ to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.



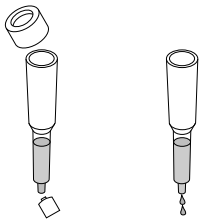
5. Place the microtube in the freezer until the next laboratory period. The freezing causes _____.

Purification phase 2: bacterial _____

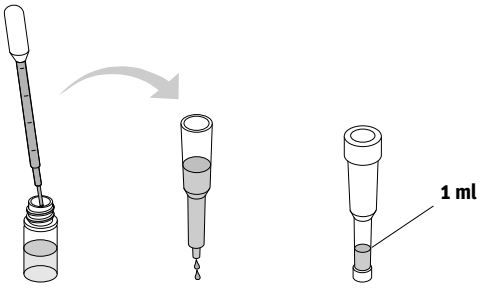
1. Remove the microtube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.



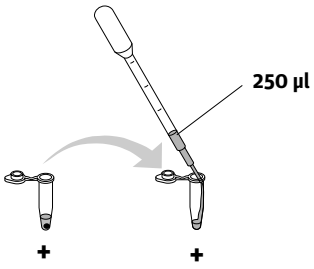
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



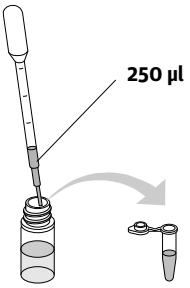
3. Prepare the column by adding 2 ml of _____ Buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipette. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



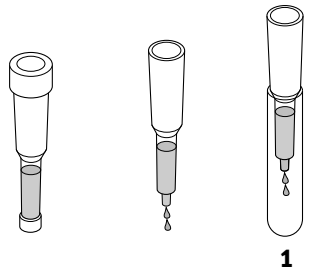
4. After the 10 minute spin, immediately remove your tube from the centrifuge. Examine the tube with the UV light. Using a new pipette, transfer 250 µl of the "+" supernatant into a new microtube labeled "+". Again, rinse the pipette well for the rest of the steps of this lab period.



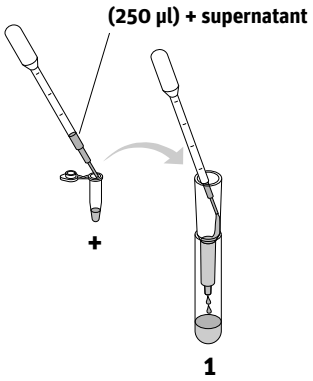
5. Using a well-rinsed pipette, transfer 250 µl of _____ buffer to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.



- **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

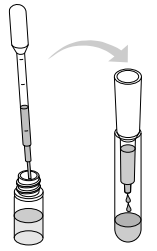


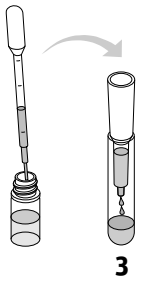
Collection tube number	Prediction	Observations under UV light (column and collection tube)
Tube 1 Sample in binding buffer		
Tube 2 Sample with wash buffer		
Tube 3 Sample with elution buffer		

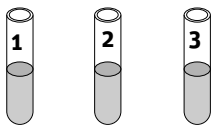


Purification phase 3: protein chromatography

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 _____ 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 _____ 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.







Procedure

Remove the GFP protein from the bacteria that made it.

1. The materials you will use include the following: centrifuge, freezer, lysozyme, various buffers (see list below)
2. Determine how to break down the bacteria to release the GFP protein, then use the column to help you purify it. Take notes on your process of thinking and completing the extraction and purification.
3. Research Hydrophobic Interaction Column Chromatography (see **bio-rad.com/en-us/applications-technologies/introduction-hydrophobic-interaction-chromatography-hic?ID=MWHB53MNI**)

Buffers

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

1. What were the biggest challenges in determine your protocol?

2. What properties of the GFP protein did you use to purify it from the bacteria?

Rubric for self-assessment

Skill	Yes	No	Unsure
I was able to break down the process of extracting GFP from bacteria and purifying the protein into smaller steps to accomplish the goal.			
My group worked together to determine the process for extracting and purifying the GFP.			
I am able to explain the function/role of each buffer in the process of chromatography.			