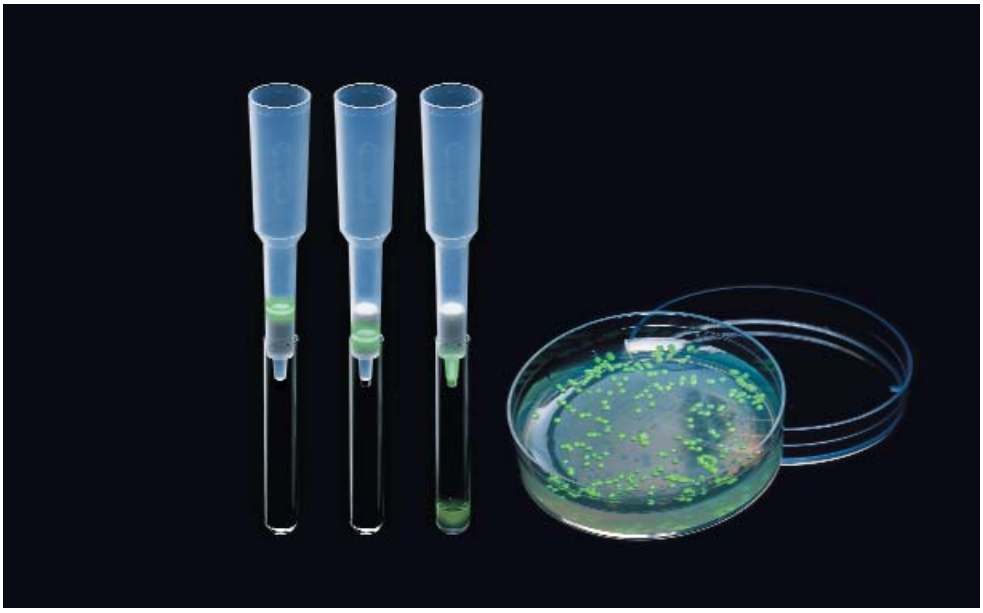


Biotechnology Explorer™

Green Fluorescent Protein (GFP) Purification Kit

Instruction Manual

Catalog #166-0005EDU

explorer.bio-rad.com

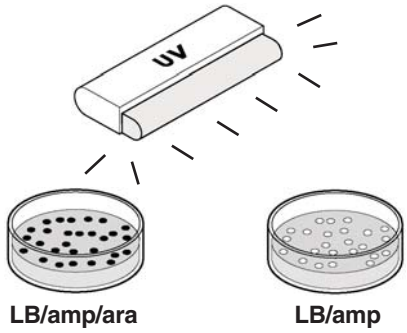
Please visit explorer.bio-rad.com to access our selection of language translations for Biotechnology Explorer kit curriculum.

BIO-RAD

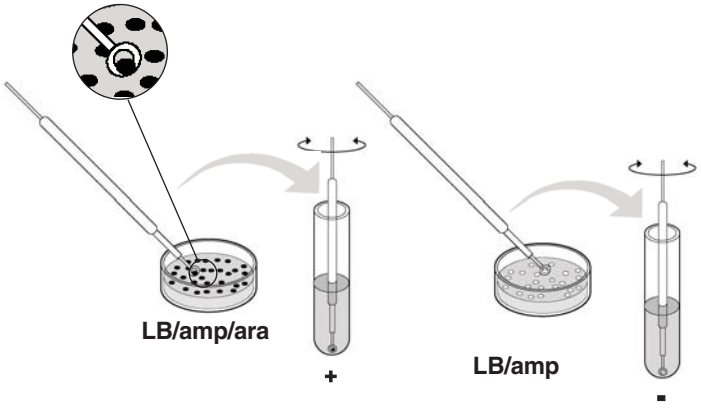
GFP Purification—Quick Guide

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.



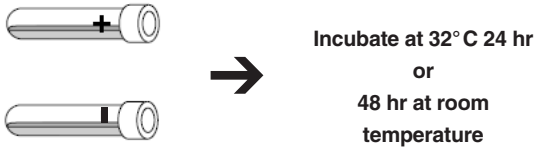
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick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

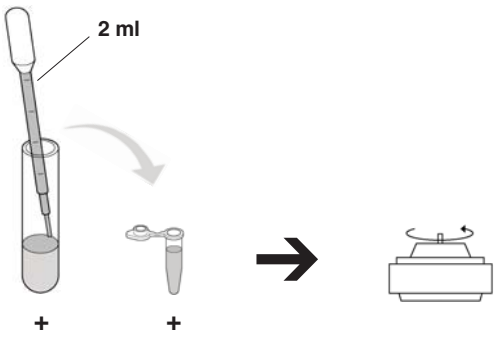
   or

   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.



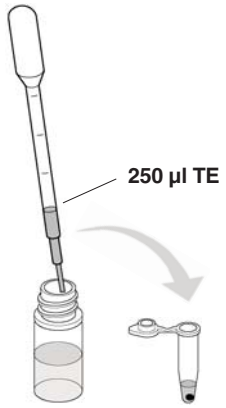
Lesson 3 Purification Phase 1
Bacterial Concentration

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.

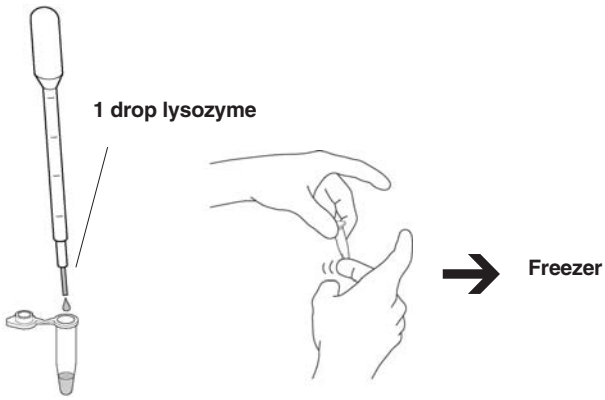


2. Pour out the supernatant and observe the pellet under UV light.

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.

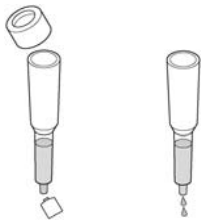


5. Place the microcentrifuge tube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

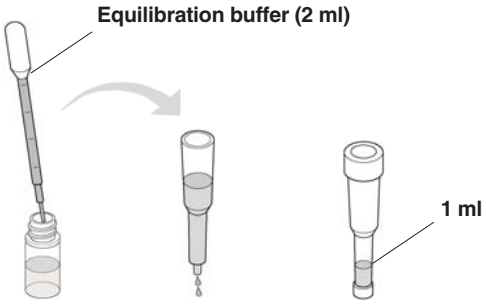
Lesson 4 Purification Phase 2
Bacterial Lysis

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.

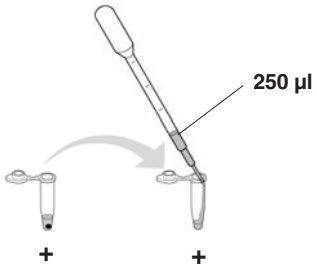
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



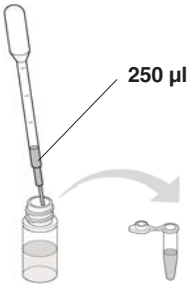
3. Prepare the column by adding 2 ml of Equilibration Buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipet. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



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.

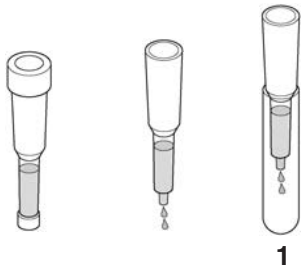


5. Using a well rinsed pipet, transfer 250 µl of binding buffer to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.

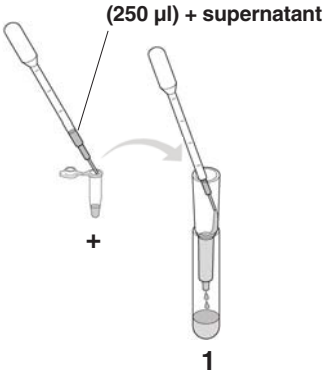


Lesson 5 Purification Phase 3
Protein Chromatography

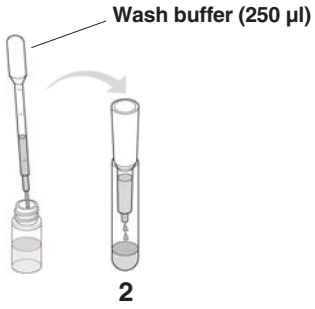
1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix proceed to the next step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



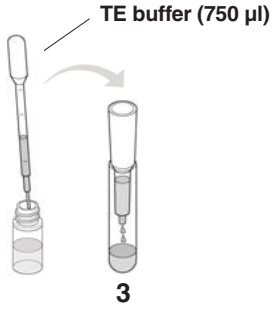
2. Using a new pipet, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipet tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light.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



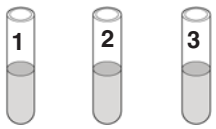
3. Using the rinsed pipet, add 250 µl of wash buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.



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.



Green Fluorescent Protein (GFP) Purification

Student Manual

"Bioengineered DNA was, weight for weight, the most valuable material in the world. A single microscopic bacterium, too small to see with the human eye, but containing the gene for a heart attack enzyme, streptokinase, or for "ice-minus" which prevented frost damage to crops, might be worth 5 billion dollars to the right buyer."

Michael Crichton - Jurassic Park

Contents

Lesson 1 Finding the Green Fluorescent Molecule

Genetic Transformation Review

With the pGLO Bacterial Transformation kit, you performed a genetic transformation of *E. coli* bacterial cells. The results of this procedure were colonies of cells that fluoresced when exposed to ultraviolet light. This is not a normal phenotype (characteristic) for *E.coli*. You were then asked to figure out a way to determine which molecule was becoming fluorescent under UV light. After determining that the pGLO plasmid DNA was not responsible for the fluorescence under the UV light, you concluded that it was not the plasmid DNA that was fluorescing in response to the ultraviolet light within the cells. This then led to the next hypothesis that if it is not the DNA fluorescing when exposed to the UV light, then it must be a protein that the new DNA produces within the cells.

1. Proteins.
   a. What is a protein?

   b. List three examples of proteins found in your body.

   c. Explain the relationship between genes and proteins.

2. Using your own words, describe cloning.

3. Describe how the bacterial cloned cells on your LB/amp plate differ from the cells on your LB/amp/ara plate. Can you design an experiment to show that both plates of cloned cells behave similarly and do contain the same DNA?

4. Describe how you might recover the cancer-curing protein from the bacterial cells.

Laboratory Procedure for Lesson 2

Picking Colonies and Growing a Cell Culture

Examine your two transformation plates under the ultraviolet (UV) lamp. On the LB/amp plate pick out a single colony of bacteria that is well separated from all the other colonies on the plate. Use a magic marker to circle it on the bottom of the plate. Do the same for a single green colony on the LB/amp/ara plate. Theoretically both white and green colonies were transformed with the pGLO plasmid? How can you tell?

Both colonies should contain the gene for the Green Fluorescent Protein. To find out, you will place each of the two different bacterial colonies (clones) into two different culure tubes and let them grow and multiply overnight.

Your Task

In this lab, you will pick one white colony from your LB/amp plate and one green colony from your LB/amp/ara plate for propagation in separate liquid cultures. Since it is hypothesized that the cells contain the Green Fluorescent Protein, and it is this protein we want to produce and purify, your first consideration might involve thinking of how to produce a large number of cells that produce GFP.

You will be provided with two tubes of liquid nutrient broth into which you will place cloned cells that have been transformed with the pGLO plasmid.

Workstation Daily Inventory Check (✔) List

Your Workstation. Materials and supplies that should be present at your student workstation site prior to beginning this lab activity are listed below.

Instructors (Common) Workstation. Materials, supplies, and equipment that should be present at a common location that can be accessed by your group during each lab activity are also listed below.

Your workstation	**Number**	**(✔)**
Transformation plates from pGLO Bacterial Transformation kit (LB/amp/ara and LB/amp)	2	❑
Inoculation loops	2	❑
Culture tubes, containing 2 ml growth media	2	❑
Marking pen	1	❑
Test tube holder	1	❑

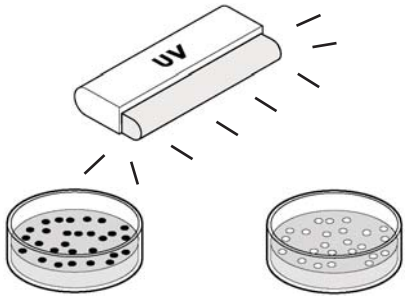
Instructors workstation

Shaking incubator, shaking water bath, tube roller or rocking platform (optional)	1	❑
UV light	1	❑

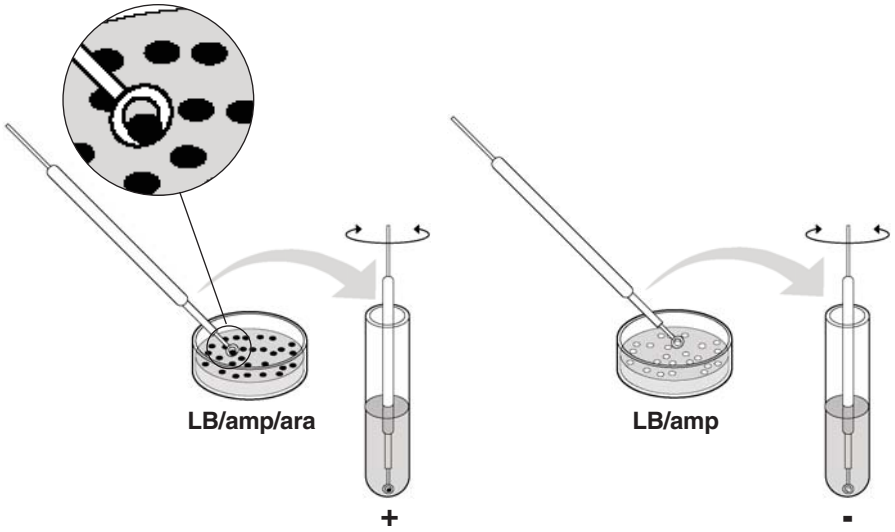
Laboratory Procedure for Lesson 2

1. Examine your LB/amp and LB/amp/ara plates from the transformation lab. First use normal room lighting, then use an ultraviolet light in a darkened area of your laboratory. Note your observations.

   To prevent damage to your skin or eyes, avoid exposure to the UV light. Never look directly into the UV lamp. Wear safety glasses whenever possible.



2. Identify several green colonies that are not touching other colonies on the LB/amp/ara plate. Turn the plate over and circle several of these green colonies. On the other LB/amp plate identify and circle several white colonies that are also well isolated from other colonies on the plate.
3. Obtain two culture tubes containing 2 ml of nutrient growth media and label one tube "+" and one tube "–". Using a sterile inoculation loop, lightly touch the "loop" end to a circled single green colony and scoop up the cells without grabbing big chunks of agar. Immerse the loop in the "+" tube. Spin the loop between your index finger and thumb to disperse the entire colony. Using a new sterile loop, repeat for a single white colony and immerse it in the "–" tube. It is very important to pick cells from a single bacterial colony.



4. Cap your tubes and place them in the shaking incubator, shaking water bath, tube roller or rocker. Let the tubes incubate for 24 hr at 32°C or for 2 days at room temperature. If a shaker is not available, shake your two tubes vigorously, like you would shake a can of spray paint, for about 30 sec. Then place them in an incubator oven for 24 hr. Lay the tubes down horizontally in the incubator. (If a rocking table or tube roller is available, but no incubator, tape the tubes to the platform or insert in tube roller and let them rock or spin at maximum speed for 24 hr at 32°C or at room temperature for 48 hr. We do not recommend room temperature incubation without rocking or shaking.)

Culture Condition	Days Required
32°C—shaking or rolling	1 day
32°C—no shaking	1–2 days*
Room temperature—shaking or rolling	2 days
Room temperature—no shaking	Not recommended

* Periodically shake by hand and lay tubes horizontally in incubator.

Lesson 2 Name_____

Review Questions

1. What is a bacterial colony?

2. Why did you pick one green colony and one white colony from your agar plate(s)? Why do you think you picked one of each color? What could this prove?

3. How are these items helpful in this cloning experiment?
   a. ultraviolet (UV) light -

   b. incubator -

   c. shaking incubator -

4. Explain how placing cloned cells in nutrient broth to multiply relates to your overall goal of purifying the fluorescent protein.

Lesson 3

Purification Phase 1
Bacterial Concentration and Lysis

So far you have mass produced living cultures of two cloned bacterium. Both contain the gene which produces the green fluorescent protein. Now it is time to extract the green protein from its bacterial host. Since it is the bacterial cells that contain the green protein, we first need to think about how to collect a large number of these bacterial cells.

A good way to concentrate a large number of cells is to place a tube containing the liquid cell culture into a centrifuge and spin it. As you spin the cell culture, where would you expect the cells to concentrate, in the liquid portion or at the bottom of the tube in a pellet?

Workstations Check (✔) List

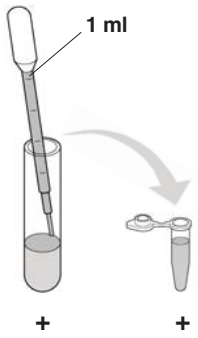
Your Workstation. Make sure the correct materials listed below are present at your workstation prior to beginning this lab experiment.

Instructors (Common) Workstation. Materials that should be present at a common location to be accessed by your group are also listed below.

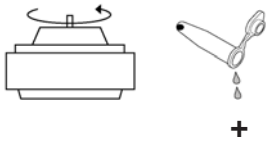
Your workstation	**Number**	**(✔)**
Microcentrifuge tubes	1	❑
Pipets	1	❑
Microcentrifuge tube rack	1	❑
Marking pen	1	❑
Beaker of water for rinsing pipets	1	❑
Instructors workstation		
TE buffer	1 bottle	❑
Lysozyme (rehydrated)	1 vial	❑
Centrifuge	1	❑
UV light	1–4	❑

Laboratory Procedure for Lesson 3

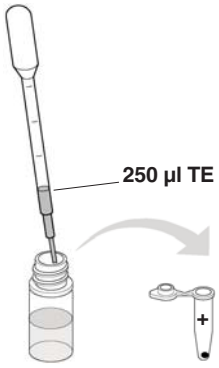
1. Using a marker, label one new microcentrifuge tube with your name and period.
2. Remove your two liquid cultures from the shaker or incubator and observe them in normal room lighting and then with the UV light. Note any color differences that you observe. Using a clean pipet, transfer the entire contents of the (+) liquid culture into the 2 ml microcentrifuge tube also labeled (+), then cap it. You may now set aside your (–) culture for disposal.



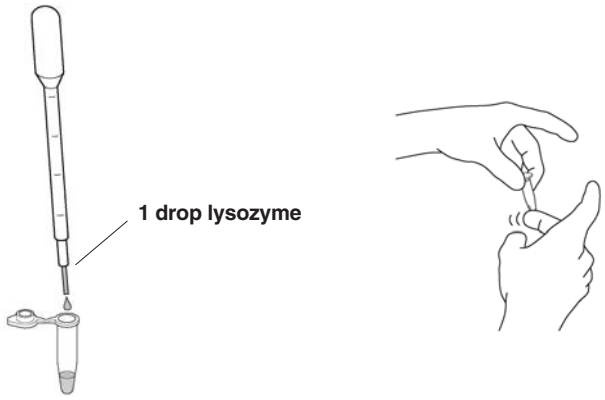
3. Spin the (+) microcentrifuge tube for 5 minutes in the centrifuge at maximum speed. Be sure to balance the tubes in the machine. If you do not know how to balance the tubes, do not operate the centrifuge.
4. After the bacterial liquid culture has been centrifuged, open the tube and slowly pour off the liquid supernatant above the pellet. After the supernatant has been discarded, there should be a large bacterial pellet remaining in the tube.



5. Observe the pellet under UV light. Note your observations.
6. Using a new pipet, add 250 µl of TE buffer to each tube. Resuspend the bacterial pellet thoroughly by rapidly pipetting up and down several times with the pipet.



7. Using a rinsed pipet, add 1 drop of lysozyme to the resuspended bacterial pellet. Cap and mix the contents by flicking the tube with your index finger. The lysozyme will start digesting the bacterial cell wall. Observe the tube under the UV light. Place the micro-centrifuge tube in the freezer until the next laboratory period. The freezing will cause the bacteria to explode and rupture completely.



Lesson 3 Name _____

Review Questions

1. You have used a bacterium to propagate a gene that produces a green fluorescent protein. Identify the function of these items you need in Lesson 3.
   a. Centrifuge -
   b. Lysozyme -
   c. Freezer -

2. Can you explain why both liquid cultures fluoresce green?

3. Why did you discard the supernatant in this part of the protein purification procedure?

4. Can you explain why the bacterial cells' outer membrane ruptures when the cells are frozen. What happens to an unopened soft drink when it freezes?

5. What was the purpose of rupturing or lysing the bacteria?

Lesson 4

Purification Phase 2
Removing Bacterial Debris

The bacterial lysate that you generated in the last lab contains a mixture of GFP and endogenous bacterial proteins. Your goal is to separate and purify GFP from these other contaminating bacterial proteins. Proteins are long chains of amino acids, some of which are very hydrophobic or "water-hating". GFP has many patches of hydrophobic amino acids, which collectively make the entire protein hydrophobic. Moreover, GFP is much more hydrophobic than most of the other bacterial proteins. We can take advantage of the hydrophobic properties of GFP to purify it from the other, less hydrophobic (more hydrophilic or "water-loving") bacterial proteins.

Chromatography is a powerful method for separating proteins and other molecules in complex mixtures and is commonly used in biotechnology to purify genetically engineered proteins. In chromatography, a column is filled with microscopic sperical beads. A mixture of proteins in a solution passes through the column by moving downward through the spaces between the beads.

You will be using a column filled with beads that have been made very hydrophobic—the exact technique is called hydrophobic interaction chromatography (HIC). When the lysate is applied to the column, the hydrophobic proteins that are applied to the column in a high salt buffer will stick to the beads while all other proteins in the mixture will pass through. When the salt is decreased, the hydrophobic proteins will no longer stick to the beads and will drip out the bottom of the column in a purified form.

Workstations Check (✔) List

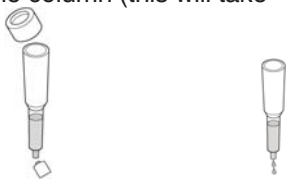
Student Workstations. Make sure the materials listed below are present at your workstation prior to beginning this lab experiment.

Instructors (Common) Workstation. Materials that should be present at a common location to be accessed by your group are also listed below.

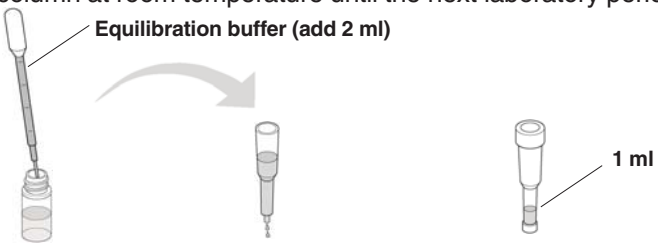
Student workstation items	**Quantity**	**(✔)**
Microcentrifuge tubes	1	❑
Pipets	1	❑
Microcentrifuge tube rack	1	❑
Marking pen	1	❑
Beaker of water for rinsing pipets	1	❑
HIC chromatography column	1	❑
Column end cap	1	❑
Waste beaker or tube	1	❑
Instructors workstation items		
Binding buffer	1 bottle	❑
Equilibration buffer	1 bottle	❑
Centrifuge	1	❑
UV light	1–4	❑

Laboratory Procedure for Lesson 4

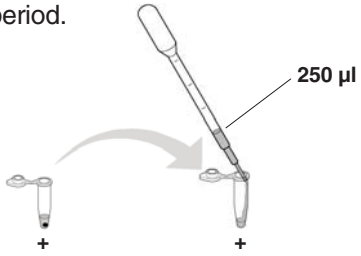
1. Remove your microcentrifuge tube from the freezer and thaw it out using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed. Label a new microcentrifuge tube with your team's initials.
2. While you are waiting for the centrifuge, prepare the chromatography column. Before performing the chromatography, shake the column vigorously to resuspend the beads. Then shake the column down one final time, like a thermometer, to bring the beads to the bottom. Tapping the column on the table-top will also help settle the beads at the bottom. Remove the top cap and snap off the tab bottom of the chromatography column. Allow all of the liquid buffer to drain from the column (this will take ~3–5 minutes).



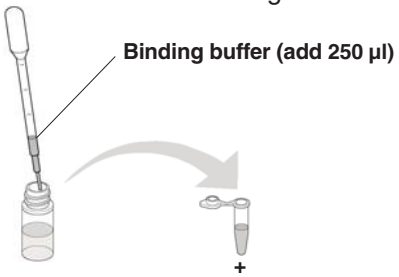
3. Prepare the column by adding 2 ml of equilibration buffer to the top of the column, 1 ml at a time using a well rinsed pipet. Drain the buffer from the column until it reaches the 1 ml mark which is just above the top of the white column bed. Cap the top and bottom of the column and store the column at room temperature until the next laboratory period.



4. After the 10 min centrifugation, immediately remove the microcentrifuge tube from the centrifuge. Examine the tube with the UV light. The bacterial debris should be visible as a pellet at the bottom of the tube. The liquid that is present above the pellet is called the supernatant. Note the color of the pellet and the supernatant. Using a new pipet, transfer 250 µl of the supernatant into the new microcentrifuge tube. Again, rinse the pipet well for the rest of the steps of this lab period.



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



Lesson 4 Name _____

Review Questions

1. What color was the pellet in this step of the experiment? What color was the supernatant? What does this tell you?

2. Why did you discard the pellet in this part of the protein purification procedure?

3. Briefly describe hydrophobic interaction chromatography and identify its purpose in this lab.

Lesson 5

Purification Phase 3
Protein Chromatography

In this final step of purifying the Green Fluorescent Protein, the bacterial lysate you prepared will be loaded onto a hydrophobic interaction column (HIC). Remember that GFP contains an abundance of hydrophobic amino acids making this protein much more hydrophobic than most other bacterial proteins. In the first step, you will pass the supernatant containing the bacterial proteins and GFP over an HIC column in a highly salty buffer. The salt causes the three-dimensional structure of proteins to actually change so that the hydrophobic regions of the protein move to the exterior of the protein and the hydrophilic ("water-loving") regions move to the interior of the protein.

The chromatography column at your workstation contains a matrix of microscopic hydrophobic beads. When your sample is loaded onto this matrix in very salty buffer, the hydrophobic proteins should stick to the beads. The more hydrophobic the proteins, the tighter they will stick. The more hydrophilic the proteins, the less they will stick. As the salt concentration is decreased, the three-dimensional structure of proteins change again so that the hydrophobic regions of the proteins move back into the interior and the hydrophilic ("water-loving") regions move to the exterior.

You will use these four solutions to complete the chromatography:

Equilibration buffer—A high salt buffer (2 M $(NH_4)_2SO_4$)

Binding buffer—A very high salt buffer (4 M $(NH_4)_2SO_4$)

Wash buffer—A medium salt buffer (1.3 M $(NH_4)_2SO_4$)

Elution buffer—A very low salt buffer (10 mM Tris/EDTA)

Workstation Check (✔) List

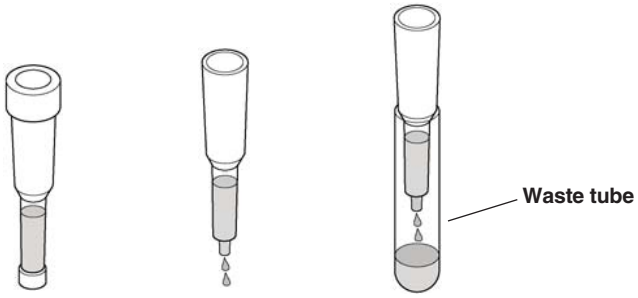
Your Workstation. Make sure the materials listed below are present at your workstation prior to beginning this lab experiment.

Instructors (Common) Workstation. Materials that should be present at a common location to be accessed by your group are also listed below.

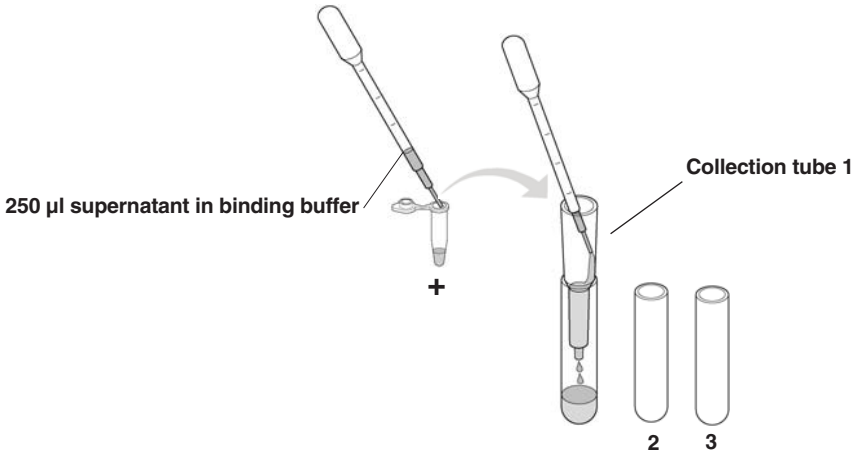
Your workstation	Number	(✔)
Collection tubes	3	❑
Pipets	1	❑
Microcentrifuge tube rack	1	❑
Marking pen	1	❑
Beaker of water for rinsing pipets	1	❑
HIC chromatography column	1	❑
Column end cap	1	❑
Beaker to collect waste	1	❑
Instructors workstation		
Wash buffer	1 vial	❑
Equilibration buffer	1 vial	❑
TE buffer	1 vial	❑
UV light	1–4	❑

Lesson 5 Laboratory Procedure

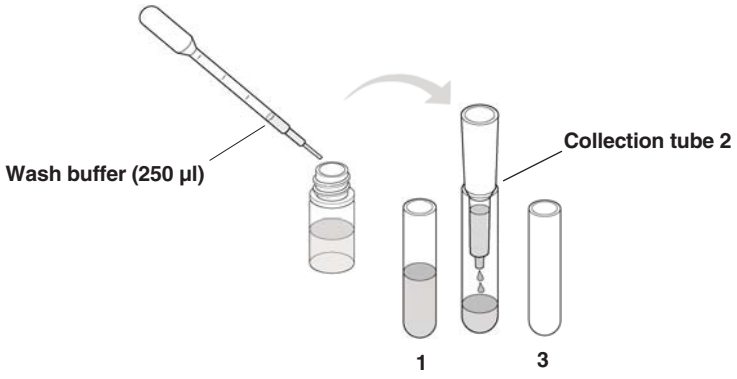
1. Obtain 3 collection tubes and label them 1, 2, and 3. Place the tubes in a rack. Remove the cap from the top and bottom of the column and let it drain completely into a liquid waste container (an extra test tube will work well). When the last of the buffer has reached the surface of the HIC column bed, gently place the column on collection tube 1. Do not force the column tightly into the collection tubes—the column will not drip.



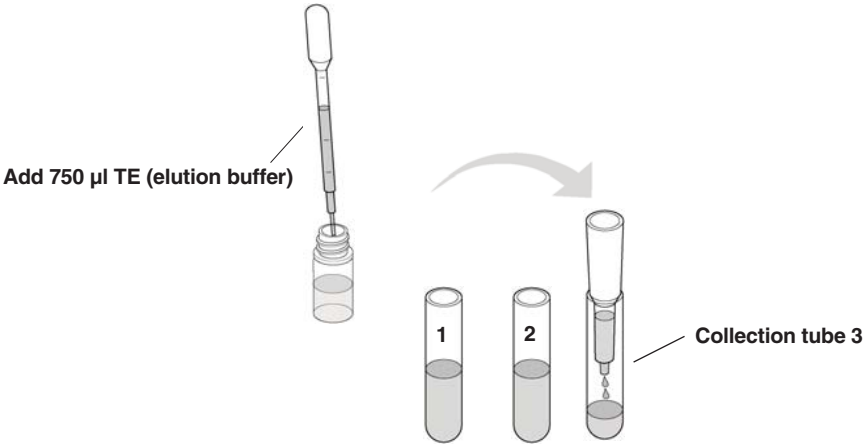
2. Predict what you think will happen for the following steps and write it along with your actual observations in the data table on page 42.
3. Using a new pipet, carefully load 250 µl of the supernatant (in binding buffer) into the top of the column by resting the pipet tip against the side of the column and letting the supernatant drip down the side of the column wall. Examine the column using the UV light. Note your observations in the data table. Let the entire volume of supernatant flow into tube 1.



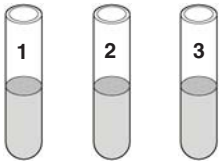
4. Transfer the column to collection tube 2. Using the rinsed pipet and the same loading technique described above, add 250 µl of wash buffer and let the entire volume flow into the column. As you wait, predict the results you might see with this buffer. Examine the column using the UV light and list your results on page 42.



5. Transfer the column to tube 3. Using the rinsed pipet, add 750 µl of TE (elution buffer) and let the entire volume flow into the column. Again, make a prediction and then examine the column using the UV light. List the results in the data table on page 42.



6. Examine all of the collection tubes using the UV lamp 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.



Lesson 5 Name _____

Review Questions

1. List your predictions and observations for the sample and what happens to the sample when the following buffers are added to the HIC 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		

2. Using the data table above, compare how your predictions matched up with your observations for each buffer.
   a. Binding buffer-
   b. Wash buffer-
   c. Elution buffer-

3. 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)-

4. Which buffers have the highest salt content and which have the least? How can you tell?

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

Appendix A Glossary of Terms

Agar
Provides a solid matrix to support bacterial growth. Contains nutrient mixture of carbohydrates, amino acids, nucleotides, salts, and vitamins.

Antibiotic Selection
The plasmid used to move the genes into the bacteria also contain the gene for beta-lactamase which provides resistance to the antibiotic ampicillin. The beta-lactamase protein is produced and secreted by bacteria which contain the plasmid. The secreted beta-lactamase inactivates the ampicillin present in the LB/agar, which allows for bacterial growth. Only bacteria which contain the plasmids, and express beta-lactamase can survive on the plates which contain ampicillin. Only a very small percentage of the cells take up the plasmid DNA and are transformed. Non-transformed cells, cells that do not contain the plasmid, can not grow on the ampicillin selection plates.

Arabinose
A carbohydrate, normally used as source of food by bacteria.

Bacterial Library
A collection of *E. coli* that has been transformed with recombinant plasmid vectors carrying DNA inserts from a single species.

Bacterial Lysate
Material released from inside a lysed bacterial cell. Includes proteins, nucleic acids, and all other internal cytoplasmic constituents.

Beta-Lactamase
Beta-lactamase is a protein which provides resistance to the antibiotic ampicillin. The beta-lactamase protein is produced and secreted by bacteria which have been transformed with a plasmid containing the gene for beta-lactamase (*bla*). The secreted beta-lactamase inactivates the ampicillin present in the growth medium, which allows for bacterial growth and expression of newly acquired genes also contained on the plasmid *i.e.* GFP.

Biotechnology
Applying biology in the real world by the specific manipulation of living organisms, especially at the genetic level, to produce potentially beneficial products.

Chromatography
A process for separating complex liquid mixtures of proteins or other molecules by passing a liquid mixture over a column containing a solid matrix. The properties of the matrix can be tailored to allow for the selective separation of one kind of molecule from another. Properties include solubility, molecular size, and charge.

Cloning

When a population of cells is prepared by growth from a single cell, all the cells in the population will be genetically identical. Such a population is called “clonal”. The process of creating a clonal population is called “cloning”. Identical copies of a specific DNA sequence, or gene, can be accomplished following mitotic division of a transformed host cell.

Colony

A clump of genetically identical bacterial cells growing on an agar plate. Because all the cells in a single colony are genetically identical, they are called clones.

Centrifugation

Spinning a mixture at very high speed to separate heavy and light particles. In this case, centrifugation results in a “pellet” found at the bottom of the tube, and a liquid “supernatant” that resides above the pellet.

Culture Media

The liquid and solid media are referred to as LB (named after Luria-Bertani) broth and agar are made from an extract of yeast and an enzymatic digest of meat byproducts which provides a mixture of carbohydrates, amino acids, nucleotides, salts, and vitamins, all of which are nutrients for bacterial growth. Agar, which is from seaweed, polymerizes when heated to form a solid gel (very analogous to Jell-O), and functions to provide a solid support on which to culture the bacteria.

DNA Library

When DNA is extracted from a given cell type, it can be cut into pieces and the pieces can be cloned en masse into a population of plasmids. This process produces a population of hybrid *i.e.* recombinant DNAs. After introducing these hybrids back into cells, each transformed cell will have received and propagated one unique hybrid. Every hybrid will contain the same vector DNA but a different “insert” DNA.

If there are 1,000 different DNA molecules in the original mixture, 1,000 different hybrids will be formed; 1,000 different transformant cells will be recovered, each carrying one of the original 1,000 pieces of genetic information. Such a collection is called a DNA library. If the original extract came from human cells, the library is a human library.

Individual DNAs of interest can be fished out of such a library by screening the library with an appropriate probe.

Genetic Engineering

The manipulation of an organism’s genetic material (DNA) by introducing or eliminating specific genes.

Gene Regulation	Gene expression in all organisms is carefully regulated to allow for differing conditions and to prevent wasteful overproduction of unneeded proteins. The genes involved in the transport and breakdown of food are good examples of highly regulated genes. For example, the simple sugar, arabinose, can be used as a source of energy and carbon by bacteria. The bacterial enzymes that are needed to breakdown or digest arabinose for food are not expressed in the absence of arabinose but are expressed when arabinose is present in the environment. In other words when arabinose is around the genes for these digestive enzymes are turned on. When arabinose runs out these genes are turned back off. See Appendix D for a more detailed explanation of the role that arabinose plays in the regulation and expression of the green fluorescent protein gene.
Green Fluorescent Protein	Green Fluorescent Protein (GFP) was originally isolated from the bioluminescent jellyfish, *Aequorea victoria*. The gene for GFP has recently been cloned. The unique three-dimensional conformation of GFP causes it to resonate when exposed to ultraviolet and give off energy in the form of visible green light.
Lysozyme	Enzyme needed to lyse, or break open bacteria cell walls. The enzyme occurs naturally in human tears, acting as a bactericidal agent to help prevent bacterial eye infections. Lysozyme gets its name from its ability to lyse bacteria.
Pellet	In centrifugation, the heavier particles such as bacteria or the cellular membranes and other debris of lysed bacteria are found at the bottom of a microfuge tube in a pellet.
Plasmid	A circular DNA molecule, capable of autonomous replication, carrying one or more genes for antibiotic resistance proteins.
pGLO	Plasmid containing the GFP sequence and ampicillin resistance gene which codes for Beta-lactamase.
Recombinant DNA Technology	The process of cutting and recombining DNA fragments as a means to isolate genes or to alter their structure and function.
Screening	Process of identifying wanted bacteria from a bacterial library.
Sterile Technique	Minimizing the possibility of outside bacterial contamination during an experiment through observance of cleanliness and using careful laboratory techniques. See Appendix C.
Streaking	Process of passing an inoculating loop with bacteria on it across an agar plate.
Supernatant	Liquid containing cellular debris that are lighter than the debris in the pellet formed after centrifugation.