
GFP TEACHER LAB SET-UP INSTRUCTIONS

LESSON 1: **(NOTE: Plates must be prepared at least 2 day in advance)**

NOTE: If you are starting with transformed bacteria from the pGLO lab, ignore #1-4 below. Go directly to #5)

1. Prepare LB **Agar** tablets for pouring 20 plates: Add 260 ml of distilled water to a 1L flask. Heat in a microwave to boiling. Add 5 LB agar tablets and let them soak in the hot water for 20 minutes. Heat the flask again to boiling, then swirl. Repeat heating and swirling until all the agar is dissolved and the solution is clear. The end volume should be about 250 ml. (DO THIS STEP ONLY IF YOU ARE NOT USING PLATES FROM THE pGLO LAB)
2. Cool agar from #2 to 50°C (DO NOT add ampicillin if the temperature is above 50). Add 2.5 ml of the arabinose solution and 1.5 ml of the ampicillin solution to the agar. Swirl to mix. Pour plates immediately. (Save the rest of the ampicillin and arabinose—0.5ml each-- to use in the liquid culture.
3. After plates have cured for 2 days at room temperature, they can be stacked and slipped back into their plastic sleeve. Invert and store in the refrigerator.
4. Rehydrate the bacterial library (if you are using it) with 250 µl of TE buffer.
5. Prepare liquid LB media (for lesson 3): Add 55 ml distilled water to a flask and heat to boiling. Add a single LB **Broth** tablet and follow the directions as in #2 and 3 above. Fill culture tubes with 2 ml of the completed broth/ampicillin/arabinose (need 2 tubes per student group). (NOTE: If you used plates from pGLO to start this lab, use one whole vial each of frozen ampicillin and arabinose to add to the LB broth)

LESSON 2:

1. Set up student workstations with 2 culture tubes each containing 2 ml of liquid culture.

LESSON 3:

1. Rehydrate the vial of lysozyme (if necessary) with 1 ml of TE buffer. Mix gently. Refrigerate until ready to use.

This activity takes 5 days of lab time. During the 5 days, however, there is quite a bit of “wait time” and you could start discussions or the Marketing activity during this time.

Pre lab	Streak bacterial libraries on plates. (You can eliminate this step by using the cultures from the pGLO lab). Grow overnight in incubator. (Lots of extra time)
Lab Day 1	Set up liquid cultures of selected bacterial colonies. Incubate overnight. (This takes about 15 minutes at most)
Lab Day 2	Concentrate bacteria by centrifugation. Add lysozyme and freeze. (This takes about 15-20 minutes)
Lab Day 3	Lyse cells and prepare columns. (takes about 20 minutes)
Lab Day 4	Run the protein separation columns (takes the whole period and more)

Workstations Daily inventory list

Prelab day

Inoculation loops
Previously poured agar plates

Bacterial Library--rehydrated
37° incubator

Marking pen

Lab Day 1

Streaked bacterial plates
Inoculation loops
2 Culture tubes with LB broth/ group
Marking pen
Test tube holder

Shaking platform
UV light

Lab Day 2

Microtubes (white)
Pipette
Microtube rack
Marker
Water for rinsing pipette

TE buffer (10 mM Tris/EDTA)
Lysozyme (rehydrated as necessary)
Centrifuges
UV light

Lab Day 3

Microtubes
Pipettes

Binding Buffer (4 M $(\text{NH}_4)_2\text{SO}_4$)
Equilibration Buffer (2 M $(\text{NH}_4)_2\text{SO}_4$)

Microtube rack

Marker
Water for rinsing pipettes
HIC chromatography columns
Column end cap (yellow)

Centrifuges

UV light

Lab Day 4

Collection tubes
Pipettes
Microtube racks
Water for rinsing pipettes
Previously prepared HIC columns

Wash Buffer (1.3 M $(\text{NH}_4)_2\text{SO}_4$)
Equilibration Buffer (2 M $(\text{NH}_4)_2\text{SO}_4$)
TE Buffer (10 mM Tris/EDTA)
UV light

Column end caps

Instructors answer guide (GFP protein separation lab)

Introduction:

1. A protein is a macromolecule which consists of chains of amino acids
2. Antibodies, digestive enzymes, hair proteins, hormones, hemoglobin, etc.
3. Genes contain the genetic code which determine the amino acid composition of a protein.
There is a unique sequence of DNA (gene or part of a gene) for each protein within all of the cells of the body.
4. The duplication and propagation of a cell or organism.
5. A bacterial library contains a diverse mixture of bacterial types which contain a diverse mixture of genes. A single colony of bacteria originates from an individual clone which only contains a single gene from the original organism.
6. One can isolate a single fluorescent green colony of bacteria and grow large amounts of the bacteria in a liquid growth media. Bacteria in liquid media can be concentrated by centrifugation. After the bacterial cells are lysed to release the protein, the protein can be isolated by passage through a chromatography column which has an affinity for the protein.

Lesson 1:

1. A bacterial colony is a large group or cluster of bacterial cells that originated from a single, clonal cell.
2. A green colony was picked in order to produce large amounts of GFP. A white colony was chosen as a “negative control” to show that only the green colonies contain and produce the GFP.
3.
 - a. The GFP fluoresces, and is thus visible, when exposed to the UV light.
 - b. The incubator provides a warm temperature which encourages bacteria to grow.
 - c. Provides aeration which oxygenates the bacterial cultures. Increased oxygen accelerates the growth rate of bacteria
4. Cloned cells of bacteria which contain the GFP must first be grown on a larger scale to produce adequate amounts of GFP which can be purified. When a large amount of GFP-containing bacteria is available, the cells can be concentrated and the GFP can be released and purified from the concentrated pellet.

Lesson 2:

1.
 - a. functions to pellet the bacteria and separate the bacteria from the growth media.
 - b. functions to enzymatically digest the bacterial cell wall, which in turn weakens it so that it will rupture upon freezing
 - c. functions to freeze the bacteria which causes the cytoplasm to expand, which completely ruptures the weakened cell.
2. The supernatant contains the bacterial growth media and does not contain the desired GFP
3. The white culture of bacteria does not contain the GFP and is not needed for the subsequent purification step.
4. When a bacterial cell freezes, the volume of the cytoplasm expands. The expansion puts pressure on the weakened cell wall, which then ruptures from the pressure.
5. The bacteria need to be ruptured in order to release the GFP, which can then be purified using column chromatography.

Lesson 3:

1. The pellet should be a whitish or pale green color. The supernatant should fluoresce bright green.
2. The fluorescent green color of the supernatant indicates that the GFP was released from the bacteria and remained in the supernatant. The much lighter color of the bacterial pellet suggests that the GFP was released from the bacteria upon lysis.
3. The pellet contains unwanted bacterial debris—bacterial cell walls, membranes, and chromosomal DNA. The pellet contains little, if any, GFP and can be discarded.
4. Protein chromatography is a technique which can be used to separate or purify proteins from other molecules. This lab used hydrophobic interaction chromatography to purify GFP based upon its hydrophobic properties.

Lesson 4:

1.

Collection Tube Number	Prediction	Observation under UV Light (column and collection tube)
TUBE 1 Sample in Binding Buffer	GFP should stick to the column	GFP resides as a band at the top of the column
TUBE 2 Sample with Wash Buffer	GFP should stick to the column	GFP remains as a broad band on top of the column
TUBE 3 Sample with Elution Buffer	GFP should elute from the column (should come off the column)	GFP travels down the column as a ring and elutes into tube 3

2.
 - a. GFP binds to the top of the chromatography column
 - b. GFP remained bound to the top of the column
 - c. GFP is eluted from the column
3.
 - a. This buffer prepares the column for the application of GFP. Equilibration buffer raises the salt concentration of the column to match that of the bacterial GFP lysate.
 - b. This buffer raises the salt concentration of GFP which causes a conformational change in GFP, exposing the hydrophobic regions.
 - c. Wash buffer functions to wash away less hydrophobic, contaminating proteins from the column.
 - d. TE (elution) buffer functions to remove GFP from the column.
4. Binding buffer>>Equilibration buffer>>Wash buffer>>Elution buffer

Binding buffer has the highest concentration of salt because it is needed to raise the salt concentration of the GFP lysate. The hydrophobic patches of proteins are exposed in high salt buffer. TE elution buffer has the lowest salt concentration because it causes GFP to elute from the column. The hydrophobic patches of proteins re-orient to the interior, and the hydrophobic regions are hidden in low salt buffer.

5. If tube 3 fluoresces green, the student was successful in purifying GFP. If GFP is not present in tube 3, examine the column---application of an incorrect buffer would prevent the elution. Alternatively, if the student did not start with a bright green culture, tube 3 will not be extremely bright.