Student Manual

pGLO Transformation

Lesson 1 Introduction to Transformation

In this lab you will perform a procedure known as genetic transformation. Remember that a gene is a piece of DNA which provides the instructions for making (codes for) a protein. This protein gives an organism a particular trait. Genetic transformation literally means "change caused by genes," and involves the insertion of a gene into an organism in order to change the organism's trait. Genetic transformation is used in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be genetically transformed into plants. In bioremediation, bacteria can be genetically transformed with genes enabling them to digest oil spills. In medicine, diseases caused by defective genes are beginning to be treated by gene therapy; that is, by genetically transforming a sick person's cells with healthy copies of the defective gene that causes the disease.

You will use a procedure to transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this gene is the bioluminescent jellyfish *Aequorea victoria*. Green Fluorescent Protein causes the jellyfish to fluoresce and glow in the dark. Following the transformation procedure, the bacteria express their newly acquired jellyfish gene and produce the fluorescent protein, which causes them to glow a brilliant green color under ultraviolet light.

In this activity, you will learn about the process of moving genes from one organism to another with the aid of a plasmid. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth allowing them to share these beneficial genes. This natural mechanism allows bacteria to adapt to new environments. The recent occurrence of bacterial resistance to antibiotics is due to the transmission of plasmids.

Bio-Rad's unique pGLO plasmid encodes the gene for GFP and a gene for resistance to the antibiotic ampicillin. pGLO also incorporates a special gene regulation system, which can be used to control expression of the fluorescent protein in transformed cells. The gene for GFP can be switched on in transformed cells by adding the sugar arabinose to the cells' nutrient medium. Selection for cells that have been transformed with pGLO DNA is accomplished by growth on ampillicin plates. Transformed cells will appear white (wild-type phenotype) on plates not containing arabinose, and fluorescent green under UV light when arabinose is included in the nutrient agar medium.

You will be provided with the tools and a protocol for performing genetic transformation.

Your task will be to:

1. Do the genetic transformation.
2. Determine the degree of success in your efforts to genetically alter an organism.

Consideration 2: How Can I Tell if Cells Have Been Genetically Transformed?

Recall that the goal of genetic transformation is to change an organism's traits, also known as their phenotype. Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of *E. coli* on your starter plates. List all observable traits or characteristics that can be described:

The following pre-transformation observations of *E. coli* might provide baseline data to make reference to when attempting to determine if any genetic transformation has occurred.

a) Number of colonies

b) Size of : 1) the largest colony
2) the smallest colony
3) the majority of colonies

c) Color of the colonies

d) Distribution of the colonies on the plate

e) Visible appearance when viewed with ultraviolet (UV) light

f) The ability of the cells to live and reproduce in the presence of an antibiotic such as ampicillin

1. Describe how you could use two LB/agar plates, some *E. coli* and some ampicillin to determine how *E. coli* cells are affected by ampicillin.

2. What would you expect your experimental results to indicate about the effect of ampicillin on the *E. coli* cells?

Lesson 2 Transformation Laboratory

Workstation (✔) Checklist

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

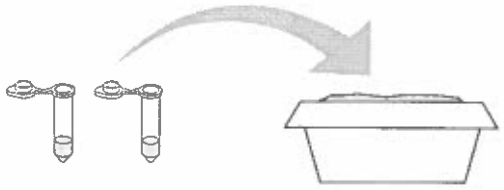
Student workstation

Material	Quantity	(✔)
E. coli starter plate	1	❑
Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)	4	❑
Transformation solution	1	❑
LB nutrient broth	1	❑
Inoculation loops	7 (1 pk of 10)	❑
Pipets	5	❑
Foam microcentrifuge tube holder/float	1	❑
Container (such as foam cup) full of crushed ice (not cubed ice)	1	❑
Marking pen	1	❑
Copy of Quick Guide	1	❑
Microcentrifuge tubes	2	❑

Common workstation. A list of materials, supplies, and equipment that should be present at a common location to be accessed by your team is also listed below.

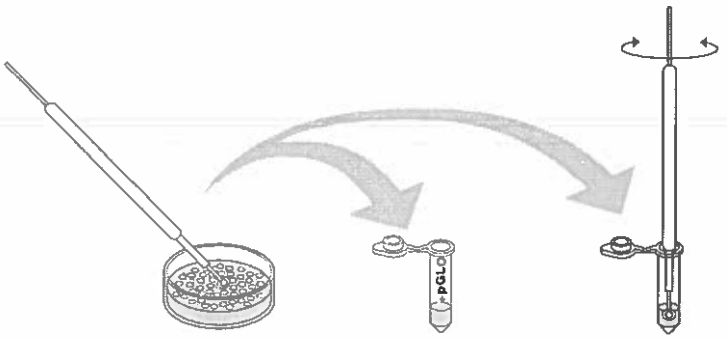
Material	Quantity	
Rehydrated pGLO plasmid	1 vial	❑
42°C water bath and thermometer	1	❑
UV Light	1	❑
37°C incubator	1	❑
(optional, see General Laboratory Skills–Incubation)		
2–20 µl adjustable volume micropipets	1	❑
2–20 µl micropipet tips	1	❑

3. Place the tubes on ice.



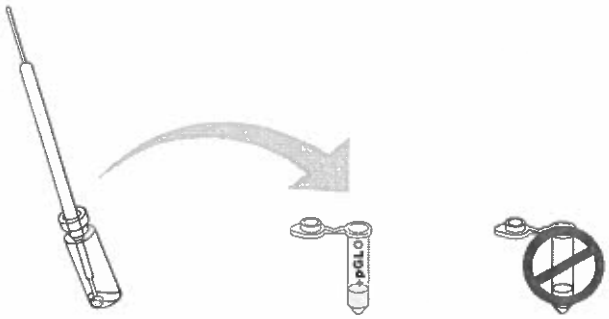
Ice

4. Use a sterile loop to pick up **2–4 large colonies of bacteria** from your starter plate. Select starter colonies that are "fat" (ie: 1–2 mm in diameter). It is important to take individual colonies (not a swab of bacteria from the dense portion of the plate), since the bacteria must be actively growing to achieve high transforation efficiency. Choose only bacterial colonies that are uniformly circular with smooth edges. Pick up the **+pGLO** tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the **-pGLO** tube.



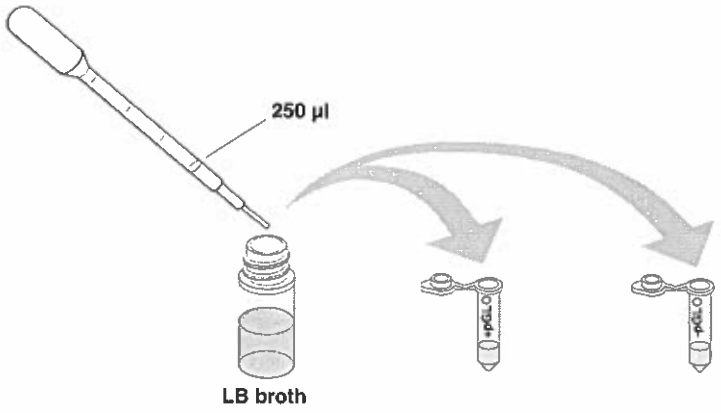
(+pGLO) (-pGLO)

5. Examine the pGLO DNA solution with the UV lamp. Note your observations. Immerse a **new sterile loop** into the pGLO plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the **+pGLO** tube. Optionally, pipet 10 µl of pGLO plasmid into the +pGLO tube & mix. **Do not** add plasmid DNA to the **-pGLO** tube. Close both the **+pGLO** and **-pGLO** tubes and return them to the rack on ice.

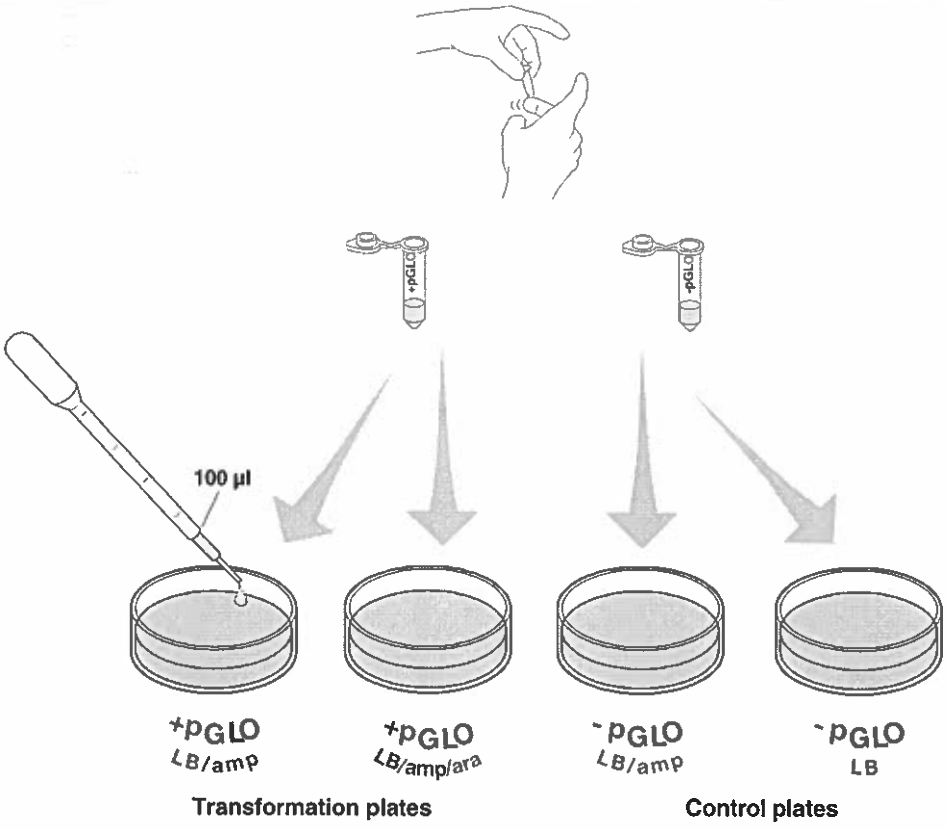


pGLO Plasmid DNA (+pGLO) (-pGLO)

9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 min at room temperature.



10. Gently flick the closed tubes with your finger to mix and resuspend the bacteria. Using a new sterile pipet for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate nutrient agar plates.



Lesson 2 Review Questions Name _____

Before collecting data and analyzing your results answer the following questions.

1. On which of the plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed? Explain your predictions.

2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Explain your predictions.

3. Which plates should be compared to determine if any genetic transformation has occurred? Why?

4. What is meant by a control plate? What purpose does a control serve?

B. Analysis of Results

The goal of data analysis for this investigation is to determine if genetic transformation has occurred.

1. Which of the traits that you originally observed for *E. coli* did not seem to become altered? In the space below list these untransformed traits and how you arrived at this analysis for each trait listed.

Original trait	Analysis of observations

2. Of the *E. coli* traits you originally noted, which seem now to be significantly different after performing the transformation procedure? List those traits below and describe the changes that you observed.

New trait	Observed change

3. If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be inferred about the other genes on the plasmid that you used in your transformation procedure?

4. From the results that you obtained, how could you prove that the changes that occurred were due to the procedure that you performed?

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ATTENTION!

- **Here are some helpful hints to optimize your pGLO™ kit lab experiment!**
- **pGLO Bacterial Transformation Kit Instruction Manual, catalog #166-0033EDU is available online at explorer.bio-rad.com**
- **Protocol for rehydrating *E. coli* HB101 K-12 bacteria has changed. See below.**

Storage
Store the *E. coli* HB101 K-12 vial at 4°C.

Practicing Techniques
Some educators like to do a dry run of the procedures to explain sterile technique, practice using the pipets and loops, and practice streaking and spreading bacteria on agar. Paying close attention to the following experimental points will result in successful transformations.

Rehydration of *E. coli* HB101 K-12 Bacteria
Rehydrating *E. coli* HB101 K-12 bacteria using LB nutrient broth and incubating the bacteria gives greater numbers of starter colonies than rehydrating in transformation solution and plating immediately. Rehydrate the lyophilized *E. coli* HB101 K-12 36–48 hr prior to the lab. Use a sterile pipet to add 250 µl of LB nutrient broth directly to the vial. Recap and gently shake to ensure all bacteria are rehydrated. Incubate the vial at 37°C for 8–24 hr. If an incubator or waterbath is not available, the vial can be placed into a large volume of water heated to 37°C and then left at room temperature for 16–24 hr.

Preparing *E. coli* HB101 K-12 Starter (Agar) Plates
Best results are obtained by using a flask instead of a beaker to prepare the agar. Ensure that the agar is completely dissolved as uneven mixing can result in agar that does not solidify. Follow the directions in "Advance Preparation Step 1" closely to minimize introducing contaminants. After streaking the starter plates with *E. coli* HB101 K-12 and incubating them at 37°C, they should be used within 24–36 hr. Delays of more than 36 hr will compromise transformation.

Transferring Bacterial Colonies from Agar Plates to Microcentrifuge tubes
The process of scraping a single colony off the starter plate leads to the temptation to get more cells than needed. A single colony that is approximately 1 mm in diameter contains millions of bacterial cells. To increase transformation efficiency, students should select 2–4 "fat" colonies that are 1–1.5 mm in diameter. Selecting more than 4 colonies may decrease transformation efficiency. Select individual colonies rather than a swab of bacteria from the dense portion of the plate since the bacteria must be actively growing in order to have a successful transformation.

DNA Transfer
The transfer of plasmid DNA from its stock tube to the transformation suspension is crucial. Students must look carefully at the loop to see if there is a film of plasmid solution across the ring. This is similar to seeing a soapy film across a wire ring for blowing soap bubbles. Optionally, the students may pipet 10 µl of the pGLO plasmid into the tube labeled "+pGLO" and mix well.

Heat Shock
The procedure used to increase the bacterial uptake of foreign DNA is called **heat shock**. It is important that students follow the directions regarding time. Also important is the rapid temperature change and the duration of the heat shock. For optimal results, the tubes containing the cell suspensions must be taken directly from ice, placed into the water bath at 42°C for 50 seconds and returned immediately to the ice. The absence of the heat shock will result in a 10-fold decrease in transformants while a 90 second heat shock will give about half as many as would 50 seconds of heat shock. In either case, the experiment will work, but it has been optimized at a 50 seconds heat shock procedure. Be sure to use crushed ice, not cubed ice for maximum transformation efficiency. Use two thermometers to check the temperature of the incubator to ensure accuracy.

Spreading Transformants and Controls
Delivering more transformed culture to the plates with the disposable transfer pipet is counterproductive as the plates may not absorb the additional liquid and spreading will be uneven. Transferring bacterial suspensions from the microcentrifuge tubes to the Petri dishes requires some care. The bacteria will settle to the bottom, so the students can hold the top of a closed tube between the index finger and thumb of one hand and flick the bottom of the tube with the index finger of the other hand. Be sure that students tap the tube with their finger or stir the suspension with the pipet before drawing it up. Also, make sure that the students cover the Petri dishes with the lid immediately after pipeting in the transformation culture and spreading the cells. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.

Green Fluorescent Protein (GFP) Chromatography Kit
If you plan to follow the pGLO bacterial transformation experiment with the GFP purification kit (166-0005EDU), you must save the pGLO-transformed bacteria grown on the LB/amp/ara plates. The best way to save the plates is to store them media-side up in a cool place, such as a refrigerator. This will keep the cells alive but limit their active growth until you need them to start the next experiment. Storing the plates upside down prevents condensed moisture from smearing the colonies on the media. Ideally, plates should be used within 2–4 weeks. For longer storage, make sure that the plates are wrapped with Parafilm to prevent moisture loss.

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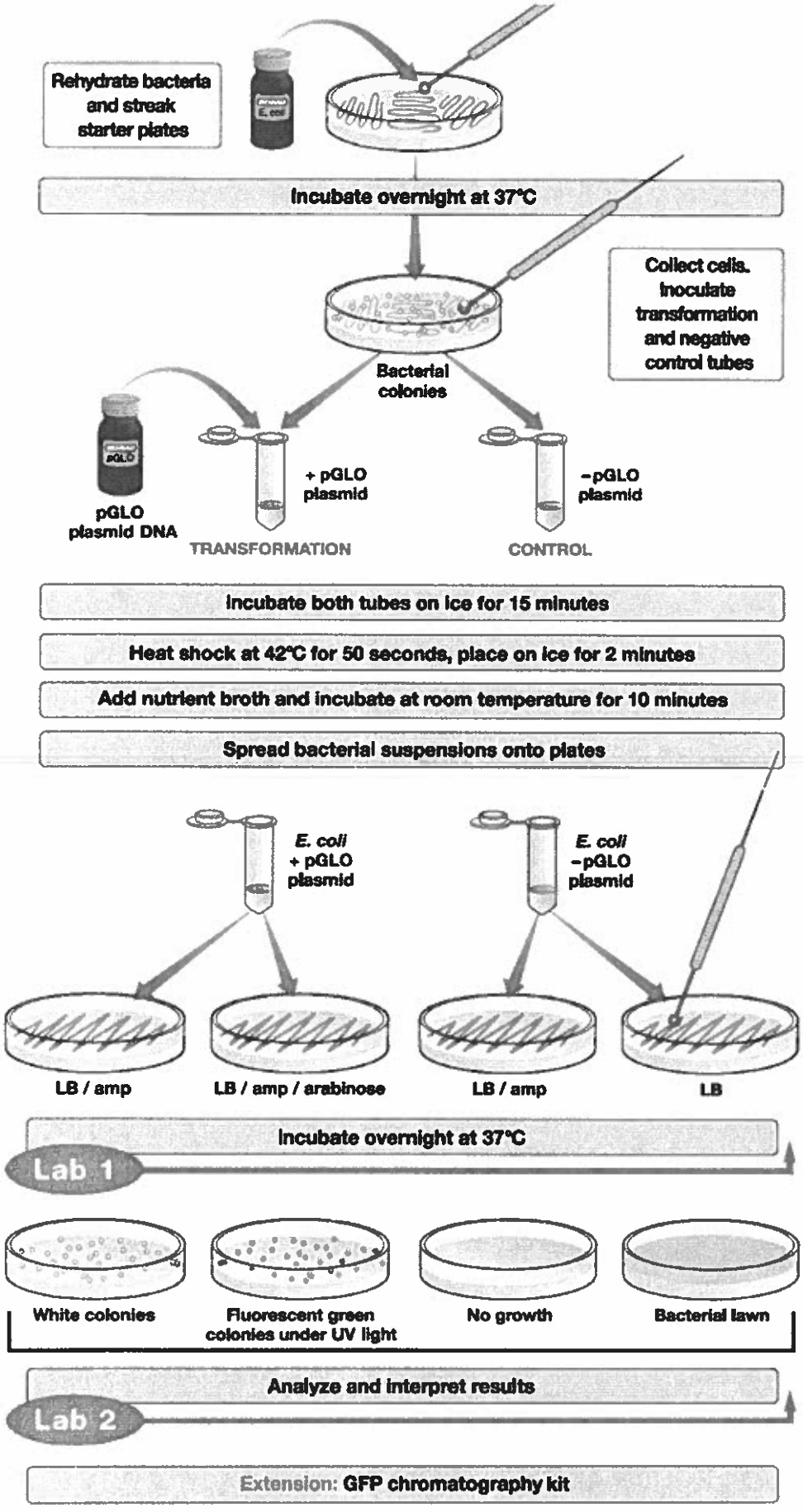
Rehydrate bacteria and streak starter plates
Incubate overnight at 37°C
Collect cells. Inoculate transformation and negative control tubes
Bacterial colonies
pGLO plasmid DNA
+ pGLO plasmid
−pGLO plasmid
TRANSFORMATION
CONTROL
Incubate both tubes on ice for 15 minutes
Heat shock at 42°C for 50 seconds, place on ice for 2 minutes
Add nutrient broth and incubate at room temperature for 10 minutes
Spread bacterial suspensions onto plates
E. coli + pGLO plasmid
E. coli −pGLO plasmid
LB / amp
LB / amp / arabinose
LB / amp
LB
Incubate overnight at 37°C
Lab 1
White colonies
Fluorescent green colonies under UV light
No growth
Bacterial lawn
Analyze and interpret results
Lab 2
Extension: GFP chromatography kit