Let it Glow Bacterial Transformation MiniLab

Student Guide

Cat# M6300

Version 021822

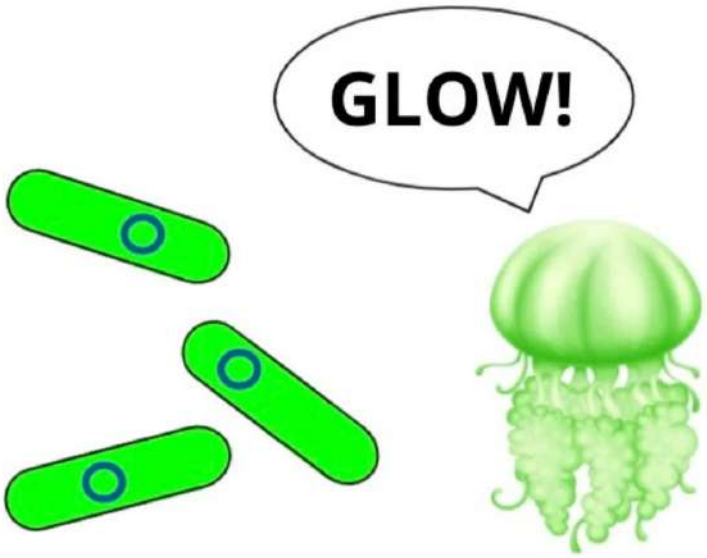


Table of Contents

Laboratory Safety

1. Wear lab coats, gloves, and eye protection whenever possible.
2. Use caution with all electrical equipment such as PCR machines and centrifuges.
3. The PCR machine has surfaces that can be extremely hot. Use caution when opening and closing the lid and when placing and removing tubes.
4. Wash your hands thoroughly after handling biological materials and chemicals.
5. Dispose of all materials in a biohazard bag or in a wash tub containing a 10% bleach solution.

Pre-lab questions

1. Explain the difference between constitutive and conditional expression of a gene.

2. How is plasmid DNA different from genomic DNA in bacteria?

3. How are antibiotics used to select for just bacteria that have been transformed?

4. How is heat shock used to introduce plasmids into bacterial cells?

5. What is the purpose of treating the bacterial cells with $CaCl_2$ before heat shocking them?

6. Describe one application of bacterial transformation in biotechnology.

Module 1: prepare E. coli starter broth culture

Please check with your teacher if this step is necessary. This may already be prepared for you.

Materials

1 x BL21 stock culture on gloTray
1 x 15 mL tube with 5 mL LB broth
1 x sterile inoculating loop
Incubator or heated water bath set to 30-37°C

Experimental procedures

1. Use a 10 µL plastic inoculating loop to scrape bacteria off the surface of the agar in the stock culture. Running it in a single line along the length of the lane should collect a visible blob of bacteria that will be sufficient for inoculating the starter culture.
2. Dip the loop into one of the plastic tubes of LB medium. Swirl the loop around until the *E. coli* are dislodged from the loop. If there are large chunks you can pipette up and down gently with a 1 mL pipette to break them up.
3. Incubate the starter culture tube at 30-37°C for minimum 3 hours, maximum 24 hours in a water bath or incubator.
4. After the incubation, invert the tube to make sure that the cells are well suspended and evenly mixed.

Modules 2 + 3: transform and plate bacteria

Materials for each group

- 1 x gloTray™ with LB agar culture media
- 1 pack of sterile wooden spreaders
- 1 x 0.65 mL microcentrifuge tube
- 1 x PCR tube with 10 µL eGFP plasmid DNA (yellow tube labeled "P")
- 1 x PCR tube with 10 µL dH_2O (blue tube labeled "H_20")
- 1 x 0.65 mL tube with 100 µL $CaCl_2$ (clear tube labeled "$CaCl_2$")
- 1 x 20-200 µL adjustable volume micropipette
- 1 x 2-20 µL adjustable volume micropipette
- 1 x rack 2-200 µL pipette tips
- Racks for PCR tubes and 0.65 mL microcentrifuge tubes
- Fine point permanent marker
- Waste container
- Visual Protocol Worksheet to Annotate

Common workstation

- Starter culture in LB broth
- MiniOne® PCR Systems
- Tablets with MiniOne® PCR App
- Benchtop microcentrifuges
- Incubator set to 30°C
- Gloves, lab coats, and goggles

Experimental procedures

1. Read the entire protocol first. Check that you have all reagents and equipment before starting.
2. Use the Visual Protocol worksheet (page 9) to annotate the details from this written protocol. Make notes about about switching tips, being careful about the pellet, resuspending and mixing etc. You can always refer back to this written protocol.
3. You should have one yellow PCR tube labeled "P" that contains the eGFP plasmid DNA and one blue PCR tube labeled "H_20" that contains the sterile H_2O. Label both tubes with your group ID.
4. Use a benchtop centrifuge to spin down the liquid in the tubes containing eGFP plasmid DNA, $CaCl_2$, and dH_2O.
5. Pipette 400 µL of the cell suspension into an **empty** 0.65 mL microcentrifuge tube. You can set your 20-200 µL micropipette to 150 µL and add this volume three times. Label your tube with your group ID.

6. Centrifuge your cell suspension for 2 minutes in your benchtop microcentrifuge (minimum 6,000 RPM). Make sure your centrifuge is balanced with tubes from other groups.
7. After removing the tube from the centrifuge, you should see a cell pellet at the bottom of the tube. See image at right.
8. Carefully remove the supernatant with a 20-200 µL pipette and discard, being careful not to disturb the pellet.
9. Add 60 µL $CaCl_2$ to the tube and gently pipette up and down to resuspend the pellet.
10. Add 30 µL of the cell suspension to the "P" yellow tube. Gently pipette up and down to mix, being careful not to make air bubbles.
11. Change a new pipette tip. Add 30 µL of the cell suspension to the "H_20" blue tube. Gently pipette up and down to mix, being careful not to make air bubbles.
12. Turn on the PCR machine and connect with your tablet.
13. Place your tubes in the PCR machine and close the lid.
14. You will use the link protocol function of the PCR app to run 3 consecutive constant temperature protocols. First, go to "Constant Temperature" and set up and save three separate constant temperature protocols.

Protocol 1: 600 seconds incubation at 4°C
Protocol 2: 45 seconds heat shock at 42°C
Protocol 3: 120 seconds incubation at 4°C
For each protocol, use 4°C as the final incubation temperature.

To Link protocols go to your constant temperature protocol library, select the 600 sec/4°C protocol, swipe and tap the "Attach to Link" message (swipe right on Android and left on iOS). From the same protocol library screen, select the 45 sec/42°C protocol, swipe and tap the "Attach to Link" message. Again from the protocol library screen, select your 120 sec/ 4°C protocol, swipe and tap the "Attach to Link" message. You will see a number in the top right corner of each of the protocols you've linked and which order they will run. *Link the protocols in the order listed above.*

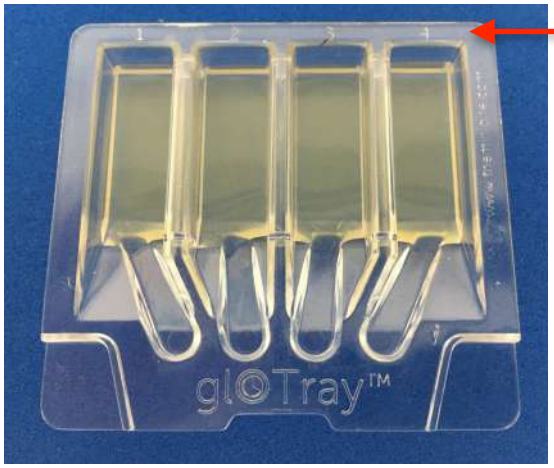
Once you've attached all of your protocols, select **"LINK"** at the bottom of the screen and

then the **"PLAY"** button to start the run. At this point the 3 protocols will run sequentially in the order you programmed in.

15. When the protocol is finished, open the lid and quickly remove the tubes. It is important to do this step as quickly as possible, because if left in the PCR machine, they will start to heat up again on account of hot components in the machine.

16. Remove the lid and sealing film from your gloTray. To keep it sterile, don't peel the film off until just before use. Discard the film but **keep the lid.**

17. Find the numbers on the gloTray that identify the lanes as shown below.



Lane labels

The lanes of the gloTray contain:

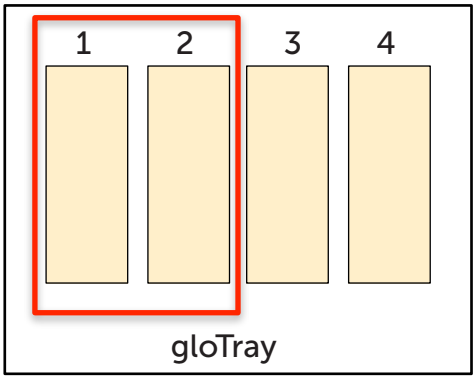
Lane 1: LB Agar

Lane 2: LB Agar + Ampicillin

Lane 3: LB Agar + Ampicillin

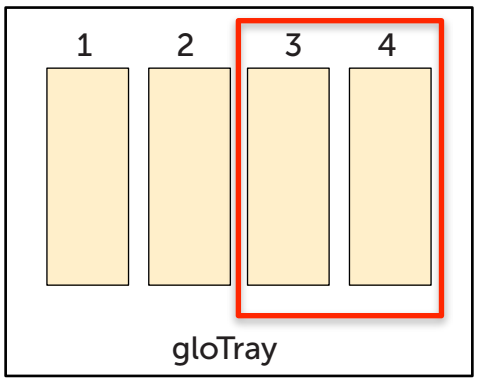
Lane 4: LB Agar + Ampicillin + Lactose

18. Use a 2-20 µL pipette to drop **15 µL of your "H_20"** cell suspension onto each surface of **lanes 1 + 2** of the gloTray. Use the rounded end of the wooden spreaders to carefully spread the bacteria over the surface of the agar in each lane preceding from Lane 1 to Lane 2. Use one clean spreader per lane.

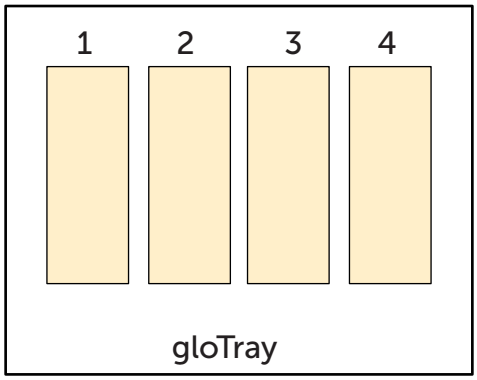


Note: Place the gloTray in your palm. Hold on the pointed end of the wooden spreader and use the rounded end of the spreader to spread out the liquid. Position the rounded end parallel to the surface of the agar and gently skate across the surface of the whole lane to spread out the liquid, being careful not to puncture the surface of the agar.

19. Use a clean 2-20 µL pipette to drop **15 µL of your "P"** cell suspension onto each surface of **lanes 3 + 4** of the gloTray. Use wooden spreaders to carefully spread the bacteria over the surface of the agar in each lane preceding from Lane 3 to Lane 4. Use one clean spreader per lane.



20. Label your trays with your group ID. Cover your gloTray with the lid and incubate overnight at 30°C. When placing your trays in the incubator, make sure that the bottom of the agar trays is facing up to prevent any condensation from dropping onto the surface of the agar.
21. Use the diagram below to sketch what you think your gloTray will look like after overnight incubation.



Visual Protocol Worksheet

Starter culture

Harvest Cells

Transfer ___ µL
Group Label ___
Spin for ____min

Remove supernatant

Add
____ µL
$CaCl_2$

Divide culture into 2 PCR tubes, ____ µL per tube

Blue tube contains _____ and is labelled ____ on top and has your group label ____

Yellow tube contains _____ and is labelled ____ on top and has your group label ____

Heat Shock		
Step	**Temperature (°C)**	**Duration (seconds)**
Cool		
Heat Shock		
Cool		

Labels the lanes #'s on the gloTray below

Group label
____

Plate and spread bacteria

Lane #	What's in the lane	Tube color to use	Volume to add (µL)
1	LB Agar		
2	LB agar + Ampicillin		
3	LB agar + Ampicillin		
4	LB agar + Ampicillin + Lactose		

Module 4: Document Results

Materials for each group

- gloTrays from previous lab session

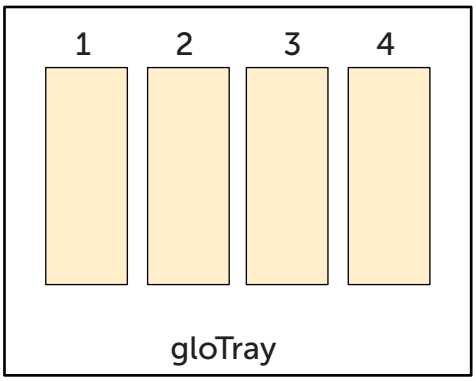
Common workstation

- MiniOne Winston Fluorescence Readers
- MiniOne photo hoods

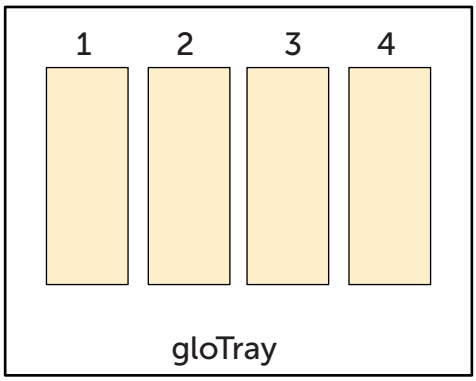
Experimental procedures

1. Retrieve your group's gloTray from the incubator.
2. Remove the lid from the gloTray. Place the gloTray in the Winston Fluorescence Reader with the surface of the agar facing up. Place the photo hood on top of the Winston. You should see glowing colonies in lane 4. Place your cell phone camera directly over the top of the photo hood and take a picture of the gloTray.
3. Describe what you see in each lane and record your observations in the table below. Specify whether the lane has a lawn of bacteria or colonies, and whether those colonies are glowing.
4. Remove the gloTray from the Winston. Describe what you see in each lane and compare to what you see when the gloTray is in the Winston.

	Medium	Plasmid? "–" or "+"	Observations
Lane 1	LB agar		
Lane 2	LB agar + ampicillin		
Lane 3	LB agar + ampicillin		
Lane 4	LB agar + ampicillin + lactose		



Results when visualized in The Winston



Results when visualized in without blue light

Post-lab analysis questions:

1. What was the negative control condition in this experiment? What was the purpose of including a negative control?

2. When you see a uniform coating of bacteria on an agar surface, this is called a "lawn". Did you observe a lawn on any of your lanes? What can you conclude about the bacteria and the growth conditions based on this observation?

3. When you see separated spots growing on an agar surface, these are called colonies. All bacteria in a colony are descended from a single bacterial cell. Did you observe colonies on any of your lanes? Why do you think there were colonies and not a lawn?

4. Are there any lanes where you did not observe any growth? What can you conclude about the bacteria and the growth conditions based on this observation?

5. Did you observe eGFP fluorescence in any of your lanes? Based on your observations, under what conditions is the eGFP expressed?

6. If you were to scrape a glowing colony off your agar, spread it on a lane containing LB agar and ampicillin, and allow these bacteria to grow overnight, would you expect to see fluorescence? Why or why not?

7. If you were to scrape a non-glowing colony off agar containing ampicillin, spread it on a lane containing LB agar, ampicillin, and lactose, then allow these bacteria to grow overnight, would you expect to see fluorescence? Why or why not?

8. Do you expect the bacteria growing on the lane with LB agar + ampicillin to contain the gene that codes for eGFP? Why or why not? Design an experiment to test your prediction.

miniOne®
SYSTEMS

theminione.com (858) 684-3190 info@theminione.com