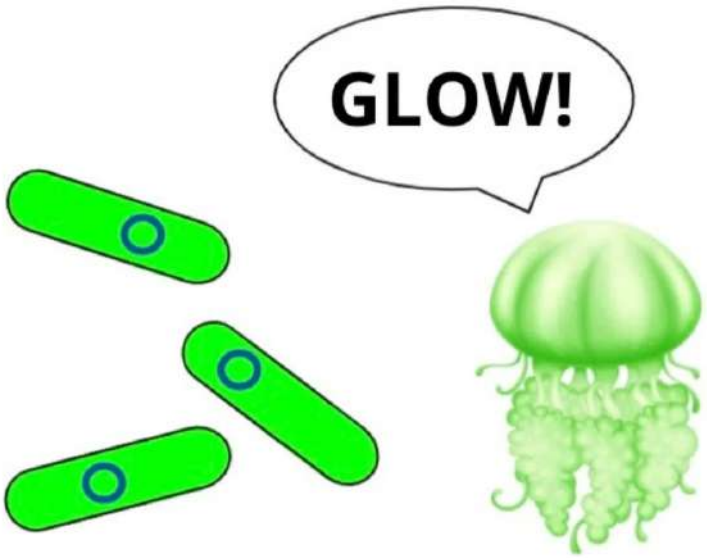
Let it Glow Bacterial Transformation MiniLab

Student Guide

Cat# M6300

Version v122323



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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.

Modules 1 + 2: transform and plate bacteria

Experimental timing

Modules 1 + 2 can be completed in a 45 minute classroom period, or split into two classroom periods by storing the cell suspension overnight at 4°C.

Module 1:

- Apply lactose to Lane 4 of the gloTray® - 5 minutes
- Centrifuge cell suspension - 5-10 minutes
- Resuspend bacteria in $CaCl_2$ and add to DNA and H_2O - 5-10 minutes
- If running modules 1 &2 on separate days, store the bacterial suspension at 4°C until use.

Module 2:

- Run heat shock transformation protocol - 15 minutes
- Spread transformed bacteria on gloTray® - 10 minutes

Before the lab

- Complete the teacher prep prior to starting the lab
- Remove gloTray® from the refrigerator and allow them to come to room temperature.
- Set out the materials for the common workstation and each student group

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 with aliquoted overnight culture (approx 400 µL)
- 1 x yellow PCR tube with 10 µL eGFP plasmid DNA
- 1 x blue PCR tube with 10 µL dH_2O
- 1 x clear 0.65 mL tube with 100 µL $CaCl_2$
- 1 x green 0.65 mL tube with 50 µL Lactose
- 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. Identify all of your reagents and make note of the sizes and colors of the tubes.
2. Use the Visual Protocol worksheet (page 10) to annotate the details from this written protocol. Make notes about when to switch tips, being careful with the pellet, re-suspending and mixing etc. You can always refer back to this written protocol.
3. Remove the lid and sealing film from your gloTray®. Discard the film but **keep the lid.**
4. 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

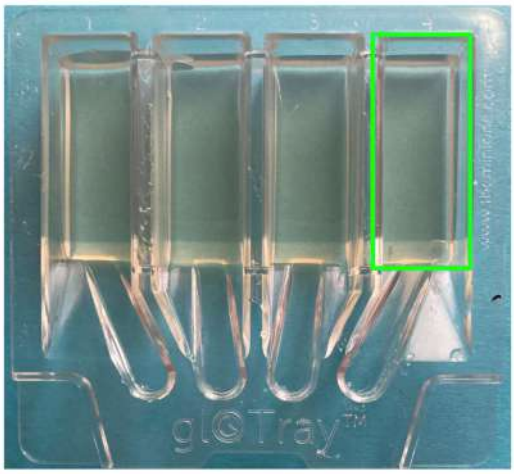
Lane 2: LB Agar + Ampicillin

Lane 3: LB Agar + Ampicillin

Lane 4: LB Agar + Ampicillin

5. Using your 2-20 uL pipette, **add 25 uL of lactose from the green PCR tube to Lane 4 ONLY**

Lane 4



Tilt the gloTray® back and forth in the direction of the arrows to spread out the lactose

Slide lid back on and set aside until you are ready to spread the bacteria after transformation.

6. The yellow PCR tube contains the eGFP plasmid DNA, label this "P" for plasmid. Label the blue PCR tube "H_20" for sterile H_2O. Label both tubes with your group ID.

7. Use a benchtop centrifuge to spin down the liquid in the tubes containing eGFP plasmid DNA, $CaCl_2$, and dH_2O if necessary.
8. Label your overnight cell culture tube with your group ID.
9. Centrifuge your cell culture tube for 2 minutes in your benchtop microcentrifuge (minimum 6,000 RPM). Make sure your centrifuge is balanced with tubes from other groups.
10. After removing the tube from the centrifuge, you should see a cell pellet at the bottom of the tube. See image at right.

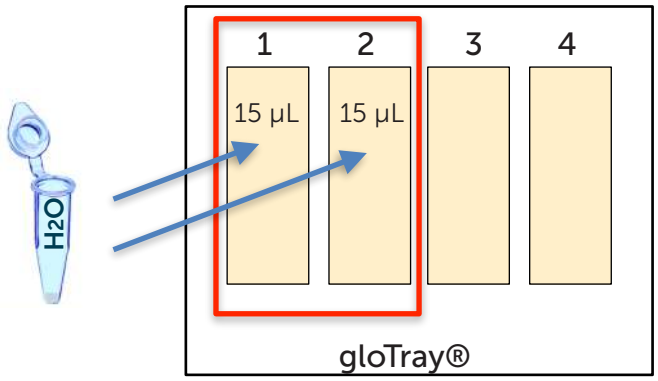
11. Carefully remove the supernatant with a 20-200 µL pipette and discard, being careful not to disturb the pellet.
12. Add **60 µL $CaCl_2$ to the clear PCR tube with the pellet** and gently pipette up and down to resuspend the pellet. This is your cell suspension.
13. Add **30 µL of the cell suspension to the "P" yellow tube.** Gently pipette up and down to mix, being careful not to make air bubbles.
14. Change to 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.
15. Turn on the PCR machine and connect with your tablet.
16. Place your tubes in the PCR machine and close the lid.
17. 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.

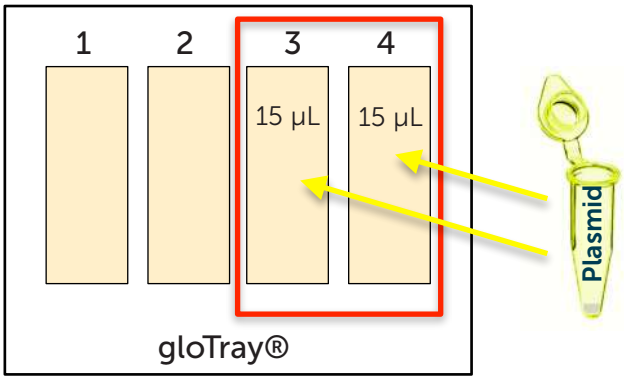
To Link protocols go to your constant temperature protocol library, select the 600 sec/4°C protocol, swipe and tap the "Attach to Link" message (swipe right on Android and left on iOS). From the same protocol library screen, select the 45 sec/42°C protocol, swipe and tap the "Attach to Link" message. 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.

18. 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.



19. Use a 2-20 µL pipette to drop **15 µL of your "H_20"** cell suspension from the **blue tube** 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. Do not overspread towards the edges of the lanes.

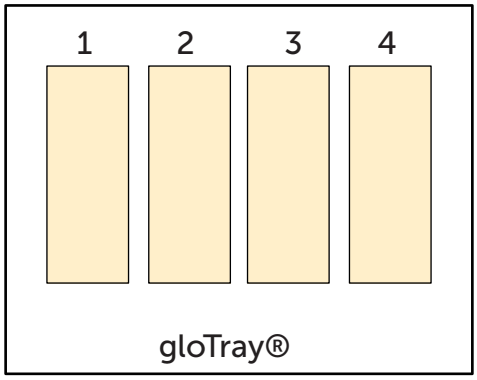


Note: Hold on the pointed end and use the rounded end of the spreader to spread out the liquid. Position the rounded end parallel to the surface of the agar and gently skate across the surface of the whole lane to spread out the liquid, being careful not to puncture the surface of the agar.

20. Use a clean 2-20 µL pipette to drop **15 µL of your "P"** cell suspension from the **yellow tube** 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. Do not overspread towards the edges of the lanes.

21. Label your trays with your group ID. Cover your gloTray® with the lid and incubate overnight at 33°C +/- 2°C.. When placing your trays in the incubator, make sure that the bottom of the gloTray® is facing up to prevent any condensation from dropping onto the surface of the media. **Do not incubate at 37°C or above - cells may grow but not glow in lane 4!**

22. Use the diagram below to sketch what you think your gloTray® will look like after overnight incubation.



Module 3: Document Results

Experimental timing

Documenting the transformation results should take less than 10 minutes. The rest of the class period can be spent answering the analysis questions, or discussing the mechanisms of transformation and regulation of gene expression.

Before the lab

- Remove gloTray® from the incubator.
- Set out the materials for the common workstation and each student group
- Make sure Winston Fluorescence Readers are charged

Materials for each group

- gloTray® from previous lab session

Common workstation

- MiniOne Winston Fluorescence Readers
- MiniOne photo hoods

Experimental procedures

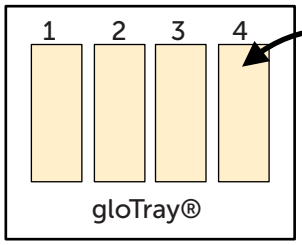
1. Retrieve your group's gloTray® from the incubator.

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.

Visual Protocol Worksheet

Prepare gloTray®



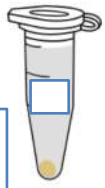
Add ___ µL lactose to lane 4 of unsealed gloTray®, tilt to spread.

→ Cover and set aside until ready to streak

Start Lab

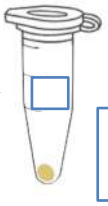
Starter Culture

Harvest Cells →

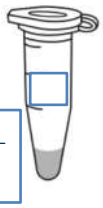


Group Label ___
Spin starter culture for ____min

Remove supernatant →



→ Add ___ µL $CaCl_2$



Divide culture into 2 PCR tubes, ___µL per tube

	Blue Tube	Yellow Tube
Contents		
Label		
Group #		

Heat Shock →

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

Labels the lanes #'s on the gloTray®

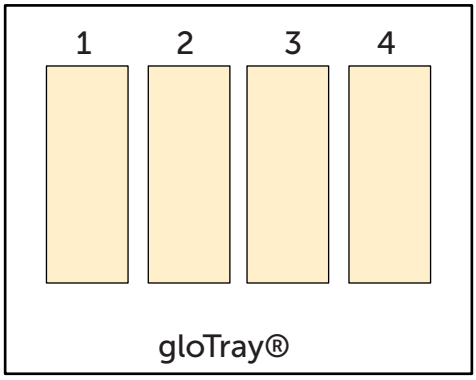
Group label ____

Plate and spread bacteria →

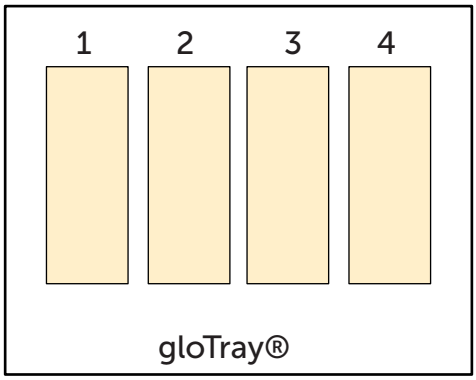
Lane	What's in the lane?	Tube Color to use	Volume to add (µL)
1	LB Agar		
2	LB Agar + Ampicillin		
3	LB Agar + Ampicillin		
4	LB Agar + Ampicillin + Lactose		

Incubate gloTray® upside down at 33° +/-2°C for at least 20 hours

	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

Module 4 (Optional): Flip the Genetic Switch!

Materials for each group

- 1 x gloTray®from the day before
- 1 pipette tip
- p20 or p200 pipette
- 25 µL of lactose supplement

Once you have transformed your bacteria and observed the various behaviors in each lane, you can see about turning the expression of the eGFP on in the transformed and uninduced samples in Lane 3.

1. Using a fresh tip, pipette 25 µL of the lactose supplement onto lane 3. Tilt the gloTray® to get the lactose to spread.
2. Cover your gloTray® with the lid and incubate overnight at 33°C +/- 2°C. When placing your trays in the incubator, make sure that gloTray® is upside down so that the surface of the agar is facing down to prevent any condensation from dropping onto the surface of the agar.
3. The next day, retrieve your group's gloTray® from the incubator.
4. 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. Place your cell phone camera directly over the top of the photo hood and take a picture of the gloTray®.
5. Describe what you see in lane 3 compared to the previous day.

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.

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