

## **Title: GFP Transformation of *E. coli* Bacteria**

### **Approvals:**

Preparer: LM  
Reviewer: AL

Date: 12FEB2021  
Date: 23FEB2021

### **1. Purpose:**

- 1.1. To transform the pGLO plasmid into *E. coli* BL21, creating recombinant *E. coli* with ampicillin resistance and GFP.

### **2. Scope:**

- 2.1. This method is to be used for teaching purposes utilizing small volume bacterial transformations. Transformation volume should not exceed 1mL for this SOP. This SOP also utilizes pre-prepped transformation solution and pGLO plasmid provided by a third party source. This method is only applicable for live cells, and is optimized specifically for *E. coli* cultures.

### **3. Responsibilities:**

- 3.1. It is the responsibility of the students performing this SOP to follow all the instructions to the best of their ability, and to seek help whenever questions or concerns may rise. Students should document any deviations that they experience while following this SOP.
- 3.2. It is the responsibility of the instructor to oversee the proper application of this SOP, answer any questions the students may have, and update the SOP whenever necessary.

### **4. References:**

- 4.1. BioRad Student Manual: pGLO Transformation

### **5. Precautions:**

- 5.1. UV light can cause damage when exposed to unshielded eyes. Ensure that any UV light source is pointed away from the eyes, and install/wear extra eye protection as necessary to decrease UV exposure.
- 5.2. When handling bacteria wear a lab coat, gloves, and eye protection at all times. Always wash hands before and after handling bacteria. Change PPE as necessary if contamination is believed to have occurred.

### **6. Materials:**

- 6.1. *E. coli* starter plate
- 6.2. Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)
- 6.3. Transformation Solution
- 6.4. LB nutrient broth
- 6.5. Rehydrated pGLO plasmid (0.08 ug/uL)
- 6.6. Inoculation loops
- 6.7. Foam microcentrifuge tube holder/float
- 6.8. Ice bucket plus crushed ice
- 6.9. Microcentrifuge tubes
- 6.10. Marking pen
- 6.11. UV light
- 6.12. 20-200µL micropipette, plus tips
- 6.13. 1-10µL micropipette, plus tips

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- 6.14. 42°C water bath with thermometer
- 6.15. 37°C incubator
- 6.16. Timer

### **7. Procedure:**

- 7.1. Disinfect workspace and let dry before retrieving reagents.
- 7.2. Using the lab marker, label one microcentrifuge tube as “+pGLO” and another microcentrifuge tube as “-pGLO”.
- 7.3. Using a sterile transfer pipette, add 250µL of transformation solution to both of the labeled microcentrifuge tubes.
- 7.4. Place both tubes on ice, ensuring that the bottom half of both tubes is adequately covered.
- 7.5. Retrieve the *E. coli* starter plate.
  - 7.5.1. Check to ensure that there is no contamination present before moving forward.
- 7.6. Depending on the size of the colonies present, select 2-4 colonies for each labeled microcentrifuge tube. Ensure that each of the selected colonies are isolated (not touching any other colony) and are uniformly round.
- 7.7. Using a sterile loop, pick up the colonies selected for the +pGLO tube. Quickly open the +pGLO tube and transfer the colonies to liquid inside.
  - 7.7.1. In order to ensure that the colonies have been properly transferred, submerge the inoculation loop completely within the transformation solution, and quickly swirl the loop back and forth with your fingers. Continue swirling until there are no visible floating chunks.
  - 7.7.2. Discard the inoculation loop in biohazard if using a disposable model. Otherwise, sterilize the loop before moving on.
- 7.8. Using a fresh, sterile inoculation loop, repeat steps 7.6, 7.6.1, and 7.6.2 with the colonies selected for the -pGLO tube.
- 7.9. Place tubes back on ice before moving forward.
- 7.10. Retrieve the rehydrated pGLO plasmid. Visually inspect the tube with the UV light, and note any visual changes.
- 7.11. Using a p10 micropipette, transfer 10µL of pGLO plasmid to the +pGLO tube.
  - 7.11.1. Note: you can also use a sterile loop to transfer pGLO. Submerge the loop into the pGLO plasmid and withdraw the loop. Ensure that there is a film of liquid across the loop's surface. Submerge the loop in the solution of the +pGLO tube, spinning a few times to ensure that the pGLO has been properly mixed.
- 7.12. Incubate the tubes on ice for **10 minutes**. Ensure that the bottoms of the tubes are adequately covered in ice.
- 7.13. During the incubation on ice, retrieve the required plates, and label them as follows. Include the date, initials, and bacterial type on every plate. Keep plates covered and inverted until inoculation.
  - 7.13.1. LB plate, with -pGLO.
  - 7.13.2. LB/amp plate, with -pGLO.
  - 7.13.3. LB/amp plate, with +pGLO.
  - 7.13.4. LB/amp/ara plate, with +pGLO.

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- 7.14. After the incubation on ice is complete, bring both the –pGLO and +pGLO tubes over to the 42°C for heat shock.
  - 7.14.1. Place the tubes in a foam microcentrifuge float while ensuring that the bottoms of the tubes are sticking out from the other side. This ensures the solution in the tubes will be completely submerged.
  - 7.14.2. After the tubes are secured, double check the temperature of the water bath to ensure that it is 42°C.
  - 7.14.3. Place the float + tubes into the water bath for exactly **50 seconds**.
- 7.15. Once the heat shock is complete, immediately move the tubes back into the ice. Let them rest on ice for **2 minutes**.
- 7.16. After being allowed to rest, remove the tubes from ice and add 250µL of LB nutrient broth to both of the tubes. Incubate the tubes room temperature for **10 minutes**.
- 7.17. Gently flick the tubes to ensure that the bacteria are resuspended.
- 7.18. Using a micropipette, add the following:
  - 7.18.1. 100µL of –pGLO to the LB plate.
  - 7.18.2. 100µL of –pGLO to one of the LB/amp plates.
  - 7.18.3. 100µL of +pGLO to the other LB/amp plate.
  - 7.18.4. 100µL of +pGLO to the LB/amp/ara plate.
- 7.19. Using a fresh, sterile inoculation loop for each plate, spread the added solution across the surface of each plate.
  - 7.19.1. Drag the sterile loop across the surface in a back and forth pattern, turn the plate 45° and repeat.
  - 7.19.2. Be careful not to gouge the surface of the plate with the inoculation loop.
- 7.20. Tape each plate lid down before storing them, inverted, in the 37°C incubator.
- 7.21. Disinfect the work station and dispose of any spent reagents and –pGLO and +pGLO tubes.
- 7.22. Allow plates to incubate for 24 hours before retrieving plates and recording results.

### **8. History:**

Name	Date	Amendment
RR	18AUG2021	Changed Document number from 1 to GFP002. Revised purpose statement to be more succinct.
FK	19MAR2022	7.18.4. Revised unit typo