Glowing Bacteria!

**Purpose:** To use your knowledge about genetic transformation to successfully transform *E. coli* cells to produce green fluorescent protein.

**Background:**

The gene encoding green fluorescent protein (GFP) was originally isolated from *Aequora victoria*, a bioluminescent jellyfish. The pGLO plasmid contains both the gene for GFP as well as a gene for resistance to the antibiotic ampicillin. Once pGLO has been inserted into a bacterial cell, adding the sugar arabinose can control expression of GFP. Transformed cells will appear white under incandescent light and fluorescent green under UV light.

**Hypothesis:** Which plate(s) will have bacterial growth and why? Which plate(s) do you expect to fluoresce (glow) and why?

**Materials:** *E. coli*, pGLO plasmid, transformation solution, nutrient broth, inoculation loops, pipettes, pipette tips, micro-tubes, tube rack, ice bath, 42°C water bath, UV light, incubator, agar plates, ampicillin, arabinose

**Procedures:**

*ALWAYS USE ASEPTIC TECHNIQUE WHEN HANDLING BACTERIA- WEAR GLOVES, CLEAN THE COUNTER, AND WASH YOUR HANDS.*

1. Your instructor will illuminate the pGLO plasmid solution with UV light. Record the results under Data & Observations.

2. Label one closed tube (already containing 250 µL transformation solution) +pGLO and another –pGLO. Label both with your group’s name and place them in the foam tube rack.

3. Place tubes on ICE.

4. Use a sterile loop to pick up 2-4 colonies of bacteria from your starter plate.

5. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Gently swizzle the loop between your index finger and thumb to dislodge the cells. Close the tube and discard the loop into the appropriate waste container.
6. Repeat steps 4-5 using a new sterile loop for the -pGLO tube. Return both tubes to ICE.

7. Add 10 µl of pGLO plasmid solution into the +pGLO tube and mix. DO NOT ADD PLASMID TO THE –pGLO TUBE!

8. Incubate the tubes on ice for 10 min. Make sure the tubes are all the way down in the rack so the bottom makes contact with the ice.

9. Label your plates as shown here →

10. **Heat shock.** Keeping both tubes in the foam rack, immerse the tubes into the 42°C water bath for exactly 50 seconds.

11. Immediately return the tubes to ICE for 2 minutes.

12. Place the tube rack on the bench top.

13. Open the +pGLO tube, add 250 µl nutrient broth (LB) and reclose it.

14. Repeat step 13 with the -pGLO tube.

15. Incubate the tubes for 10 min. at room temp.

16. Gently flick the closed tubes with your finger to resuspend the bacteria.

17. Using a clean, sterile tip each time, transfer 100 µl of the +pGLO solution and the -pGLO solution to the appropriate plates.

18. Use a new sterile loop for each plate to spread the solution evenly over the entire surface of the nutrient agar. DO NOT PRESS INTO THE AGAR!

*Uncover only one plate at a time and re-cover immediately to reduce contamination. Discard loops into the appropriate waste container.

19. Stack your plates and tape them together. Label the stack with your group name and class period.

20. Incubate the plates upside-down at 37°C overnight.

21. Observe the plates under normal room lighting and UV light. Record your results in **Table 1** under Data and Observations.
**Data & Observations:**
Did the pGLO solution fluoresce under UV light? *Yes / No* (Circle answer)

**TABLE 1**

<table>
<thead>
<tr>
<th>Plate</th>
<th>pGLO</th>
<th>Drawing</th>
<th># Colonies</th>
<th>Morphology*</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB/Amp</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB/Amp/Arabinose</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Describe what the colonies look like (size, shape, color, etc.)*
**Analysis Questions:**
1. Why didn’t the pGLO solution fluoresce (glow) under UV light?

2. What special compound is needed to cause expression of the GFP gene?

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

4. What is the purpose of a control?

5. Describe 2 traits of *E. coli* that were NOT changed by transformation.

6. If the transformed cells have gained the ability to live in the presence of the antibiotic ampicillin, what can we infer about the plasmid gene that is controlling ampicillin resistance (what is happening it to)?

**Conclusions:** Describe the evidence that indicates whether your genetic transformation experiment was successful for not successful.