

Transformation

Bacterial Transformation and Green Fluorescent Protein

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In this activity, you will learn about the process of moving genes from one organism to another using genetic transformation. Genetic transformation literally means a change caused by genes, and it involves the insertion of a foreign gene into an organism. This process is also referred to as genetic engineering or gene splicing. Recall that genes are pieces of DNA that provide the instructions for making proteins. Proteins are essential molecules for all organisms and cause the expression of a trait, or phenotype, which is associated with that gene.

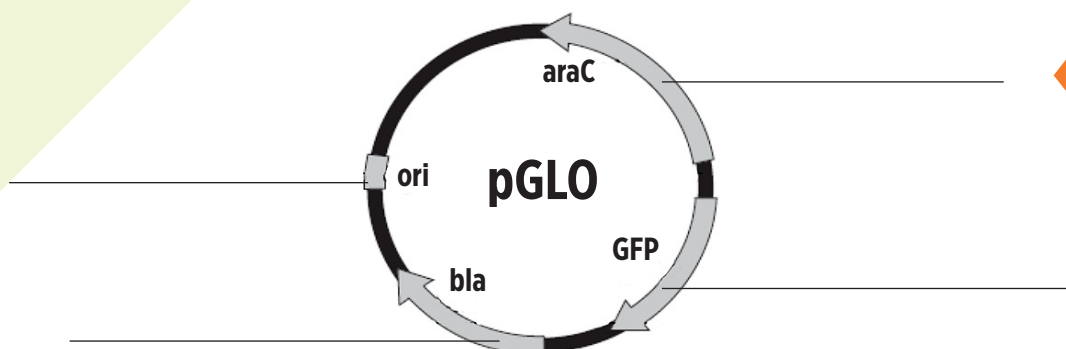
In this specific procedure, we will perform genetic transformation by inserting a plasmid into the bacteria, E. Coli. In addition to having one large chromosome, most bacteria contain small, self-replicating, circular pieces of DNA called plasmids. Plasmids have their own **origin of replication** (ori), which allows them to replicate independently of the chromosomal DNA, as well as 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 beneficial genes and rapidly adapt to new environments. The recent occurrence of bacterial resistance to antibiotics, for instance, is due to the transmission of plasmids.

The plasmid we are inserting into E.Coli contains a gene that codes for **green fluorescent protein** (GFP). Green fluorescent protein is found naturally in jellyfish and allows them to fluoresce. Following the transformation procedure, the bacteria can express their newly acquired jellyfish gene and, in the presence of sugar, will produce the fluorescent protein. This causes the bacteria to glow a brilliant green color under ultraviolet light.

The plasmid we will use is Bio-Rad's pGLO plasmid that contains both the gene for GFP and a gene for **resistance to the antibiotic, ampicillin** (bla). Since the transformed bacteria will be resistant to ampicillin, we can use nutrient plates that contain this antibiotic to select for the engineered bacteria (The antibiotic kills any non-transformed bacteria).

Finally, the pGLO plasmid also contains a gene regulation region that controls the expression of the fluorescent protein in transformed cells. This **regulation region** (araC) relies on the sugar arabinose to control GFP expression.. When present in the cell, arabinose binds to the protein transcribed by the araC gene, which in turn promotes the expression of the GFP gene. Therefore, arabinose must be present for the GFP gene to be switched on and for GFP to be produced. This means that transformed cells will appear white (wild-type phenotype) on plates not containing arabinose and fluorescent green (mutant phenotype) when arabinose is included in the nutrient agar medium.

Fill in the blanks using the bold words above, and note how each of these regions helps us visualize our transformed bacteria.

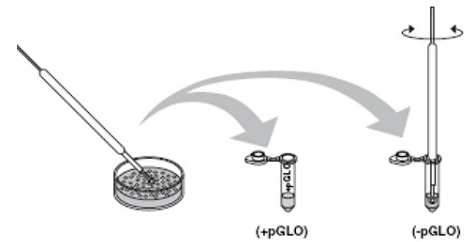


MATERIALS

- pGLO plasmid
- Calcium chloride (CaCl₂)
- Micropipette and tips
- Cold block
- Agar plates
- LB nutrient broth
- 37°C incubator
- *E. coli* starter plate
- Inoculation loop
- 42°C heat block

PART I — Transformation procedure

1. Label one closed microcentrifuge tube **(+)pGLO** and another **(-)pGLO**.
2. Open the tubes and transfer 250 μ L of CaCl₂ into each tube.
3. Place the tubes into the cold block.
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the **(+)pGLO** tube and immerse the loop into the CaCl₂ 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. Place the tube back in the cold block. Using a new sterile loop, repeat this step for the **(-)pGLO** tube.



QUICK CHECK:

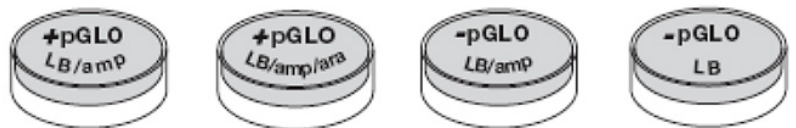
? Would the pGlo plasmid solution in the tube glow under UV light? Why or why not?

5. Add 10 μ L of pGLO plasmid to the cell suspension in the **(+)pGLO** tube only. Close both tubes and return them to the ice block. **DO NOT** add plasmid DNA to the **(-)pGLO** tube.
6. Incubate the tubes in the cold block for 5 minutes making sure to push them all the way down into the block.
7. Transfer both the **(+)pGLO** and **(-)pGLO** tubes into the 42°C heat block for exactly 50 seconds. When the 50 seconds have elapsed, place both tubes back into the ice block.

QUICK CHECK:

? What will heat-shocking do to the bacteria?

8. While the tubes are sitting in the cold block, label your four LB nutrient agar plates on the bottom (not the lid) as follows:
 - Label one **LB/amp** plate: **+ pGLO**
 - Label the **LB/amp/ara** plate: **+ pGLO**
 - Label the other **LB/amp** plate: **- pGLO**
 - Label the **LB** plate: **- pGLO**

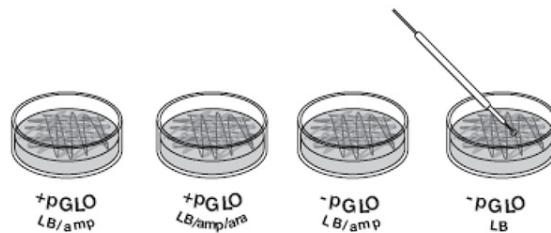


- 9. Remove the tubes from the ice block, and place them on the bench top. Open a tube and, using a new sterile pipette, add 250 μ L of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipette for the other tube. Hold the tube in hand to incubate for 5 minutes.

QUICK CHECK:

? Why is this incubation period, called the expression time, critical for the growth of the transformed bacteria?

- 10. Tap the closed tubes with your finger to mix. Using a new sterile pipette for each tube, pipette 100 μ L of the transformation or control suspensions (+/- tubes) onto the appropriate nutrient agar plates.
- 11. **Use a new sterile loop for each plate.** Spread the suspensions evenly around the surface of the LB nutrient agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.



- 12. Stack up your plates, with the LB/Amp/Ara plate on the bottom, and tape them together. Put your group name and class period on the bottom of the stack. Place the stack of plates upside down in the 37°C incubator until the next day.

PART II — Hypothesize if the bacteria will grow and/or glow on each plate.

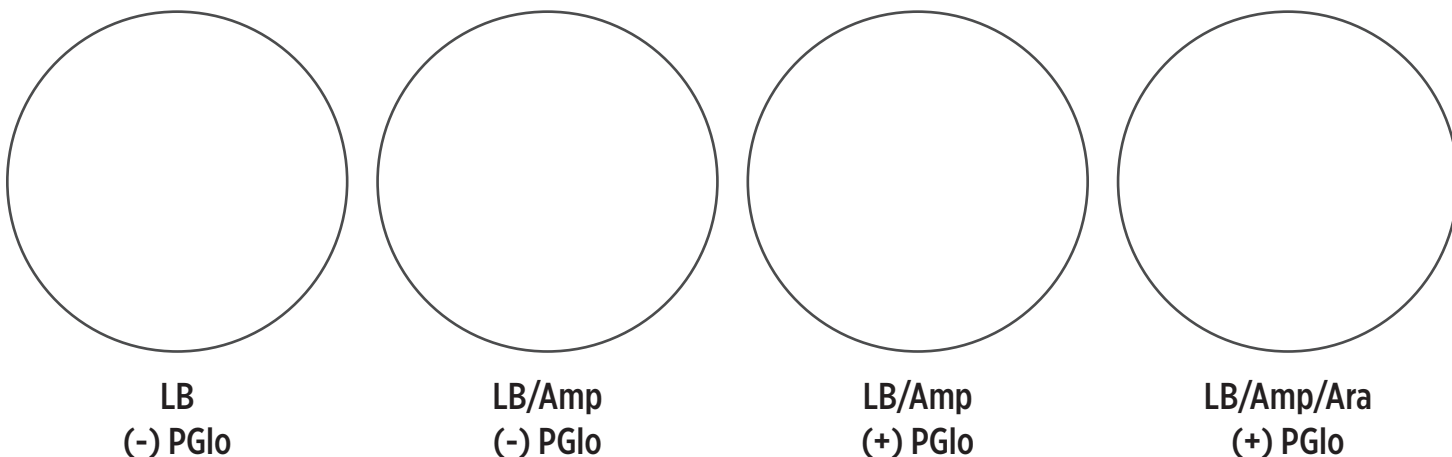
Think about the genes contained in the pGlo plasmid as well as the regulatory system for transcribing those genes. Hypothesize what the results will be by checking whether or not the bacteria will grow and whether or not it will fluoresce.

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LB (-) PGlo	LB/Amp (-) PGlo	LB/Amp (+) PGlo	LB/Amp/Ara (+) PGlo

PART III — Data analysis

13. Observe the results you obtained from the transformation lab under normal room lighting. Then turn out the lights and hold the ultraviolet light over the plates.

Carefully observe and draw what you see on each of the four plates.



PART IV — Conclusion

Now that you have completed the transformation process, you can calculate the transformation efficiency, the efficiency at which the cells picked up the PGlo plasmid, for transformed cells. This measurement is dependent on the amount of pGlo plasmid that was added to the cell culture. The calculation of transformation efficiency allows us to quantify how well the bacteria expressed the green fluorescent protein.

To calculate efficiency we need:

1. The total number of green fluorescent colonies on your LB/AMP/ARA plate.
 2. The total amount of pGlo plasmid DNA in the bacterial cells spread on the LB/AMP/ARA plate.
14. Count the number of colonies on your LB/AMP/ARA plate and record below on line 1. Each colony on the plate can be assumed to have been derived from a single cell.
15. Divide this number by 0.16 μg of pGlo plasmid.

$$\frac{(1)}{0.16 \mu\text{g pGlo}} = \text{_____} \text{ transformants}/\mu\text{g DNA}$$