Transform bacteria to glow

How can we genetically modify bacteria?
What are the results of gene insertion?

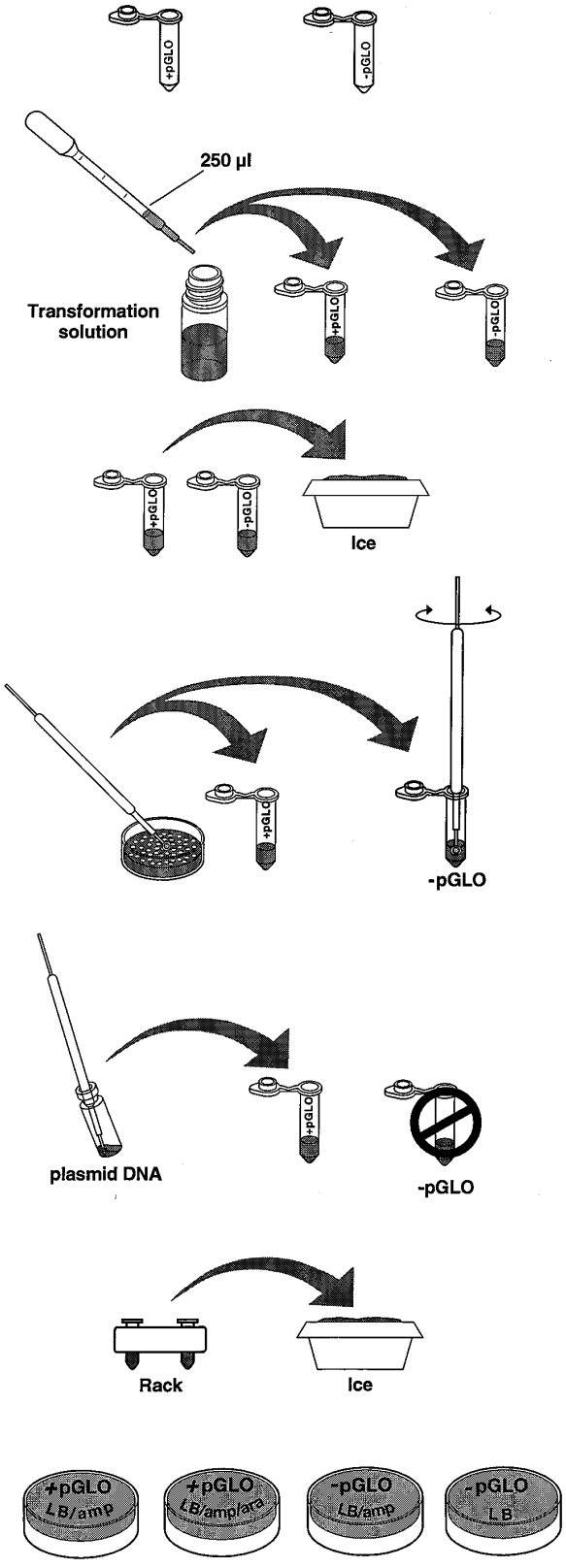
This activity shows in one lab period how bacteria can be genetically modified. Genetic modification is a powerful tool wherein DNA from one organism is added to the DNA of a different species. The results of genetic modification in agriculture have resulted in plants that can make their own pesticides and resist herbicides. In this lesson, green fluorescent protein (GFP) is inserted or added to non-virulent *E. coli* bacteria.

The source of GFP is a jellyfish, *Aequorea victoria.* This protein allows the jellyfish to fluoresce and glow in the dark. The mechanism to transform the bacteria is by the use of a plasmid. The kit comes with a plasmid that contains the GFP, antibiotic resistance and a gene regulation system that turns the production of the protein on in the presence of arabinose, a monosaccharide. The plasmid is added to the bacteria in solution, then conditions are created to encourage the bacteria to uptake the plasmid. The bacteria is then grown overnight on various media that act as controls, and/or select for transformed cells.

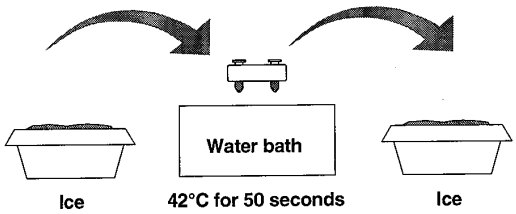
As always when using bacteria in the lab, **sterile technique** should be used when transferring materials by sterile pipette or sterile loops. The condition for uptake of the plasmid by the bacteria is critical for success in this lab. The process, called **heat shock,** can be compared to how skin pores close in extreme cold, open in warmth and close again quickly when exposed to cold once more. Bacteria also have pore spaces that will be closed in the cold, opened in the heat and closed again tightly to hold in the plasmid if it crossed the membrane. The transformation solution used with the bacteria also sets up a charge differential across the membrane that attracts the plasmid to cross the membrane. The colonies in suspension will be held on ice, then in a water bath at 42° C for 50 seconds, then immediately back on ice.

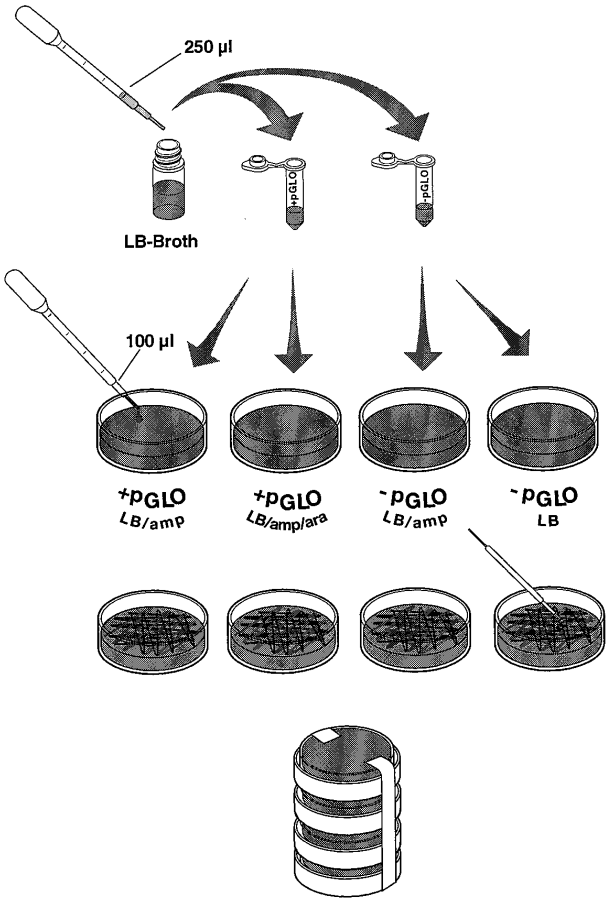
Once students complete the procedure, have them complete the table with their predictions about bacteria growth and fluorescence.

1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.
2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaC1_2$).
3. Place the tubes on ice.
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 transformation solution 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 (with no floating chunks). Place the tube back in the tube rack in the ice. Using a new sterile loop, repeat for the -pGLO tube.
5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Close the tube and return it to the rack on ice. Also close the -pGLO tube. Do not add plasmid DNA to the -pGLO tube. Why not?
6. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.
7. While the tubes are sitting on ice, label your four 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.

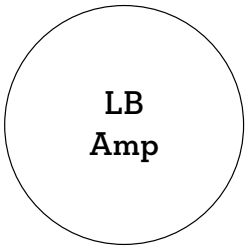
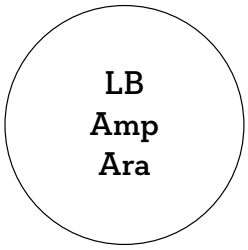
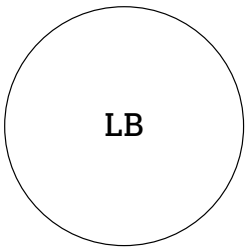
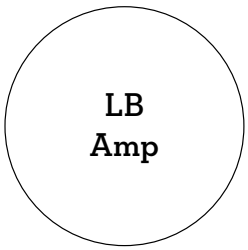


8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds are done, place both tubes back on ice. For the best transformation results, the change from the ice (0°C) to 42°C and then back to the ice must be rapid. Incubate tubes on ice for 2 minutes.
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at room temperature.
10. Tap the closed tubes with your finger to mix. Using a new sterile pipet for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate plates.
11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.
12. Stack up your plates and tape them together. Put your group name and class period on the bottom of the stack and place the stack upside down in the 37°C incubator until the next day.





- Research one of the genetic modifications that have been made to dent corn in the past 30 years. Create a presentation with data and photographs that explains how this genetic modification has improved any of the following areas:
  - Yield increase
  - Decrease of nutrient requirements for growth
  - Increase of insect resistance
  - Drought Tolerance
  - Resistance to soil pests (nematodes)
  - Decrease of herbicide use
  - Changes in the nutritional composition (protein, moisture, starch, etc.)

– pGLO	+ pGLO
 Prediction: Reason: Observed result:	 Prediction: Reason: Observed result:
 Prediction: Reason: Observed result:	 Prediction: Reason: Observed result: