BIOTECHNOLOGY (HS)

Plasmid modeling and bacterial transformation

Focus question	How does genetic modification work? How does DNA work in bacteria? How might we use that to aid in genetic modification?
Vocabulary	Restriction enzymes, plasmid vectors, nucleotides, DNA ligase

This lesson uses Bio-Rad's pGLO Bacterial Transformation Kit.

Genetic modification uses plasmid DNA from bacteria to move specific genes from one organism to another. In this lesson, you will model the creation of a plasmid.

Research what a plasmid is. Be sure you can explain how **restriction enzymes** and **DNA ligase** are used in creating plasmids.

Materials

Part 1

- Pop beads
- Mini marshmallows and toothpicks
- Blocks
- Or other similar materials for making sets of nucleotides and a strand of DNA

Part 2

- 1 foam microtube holder/float with the following 4 microtubes:
  - 1 empty microtube labeled +
  - 1 empty microtube labeled –
  - 1 microtube with 1mL of transformation solution labeled TS
  - 1 microtube with 1mL of LB broth labeled LB
- 1 permanent marker
- Package of sterile pipettes
- Package of sterile loops
- Gloves (optional)
- 4 agar plates:
  - 1 LB
  - 2 LB/Amp
  - 1 LB/Amp/Ara
- Cup of crushed ice

Procedure

Part 1: Plasmid modeling

Model construction

In this activity, you will create a model to show, 1) how a gene may be removed from a strand of DNA, then 2) inserted into a plasmid, to be taken up by bacteria.

1. Use beads or other materials (mini marshmallows, blocks, toothpicks, etc.) to create a model to show the process of genetic modification. Share your model with other groups in the class by explaining your model and how it represents the creation of a plasmid. Be sure to explain what it doesn't show as well as what it does.
2. Use your model to explain how genetic modification (by inserting a trait from another organism) may allow for the species that is modified to have better survival chances.
3. Check with your teacher to see that your model is complete before moving on.

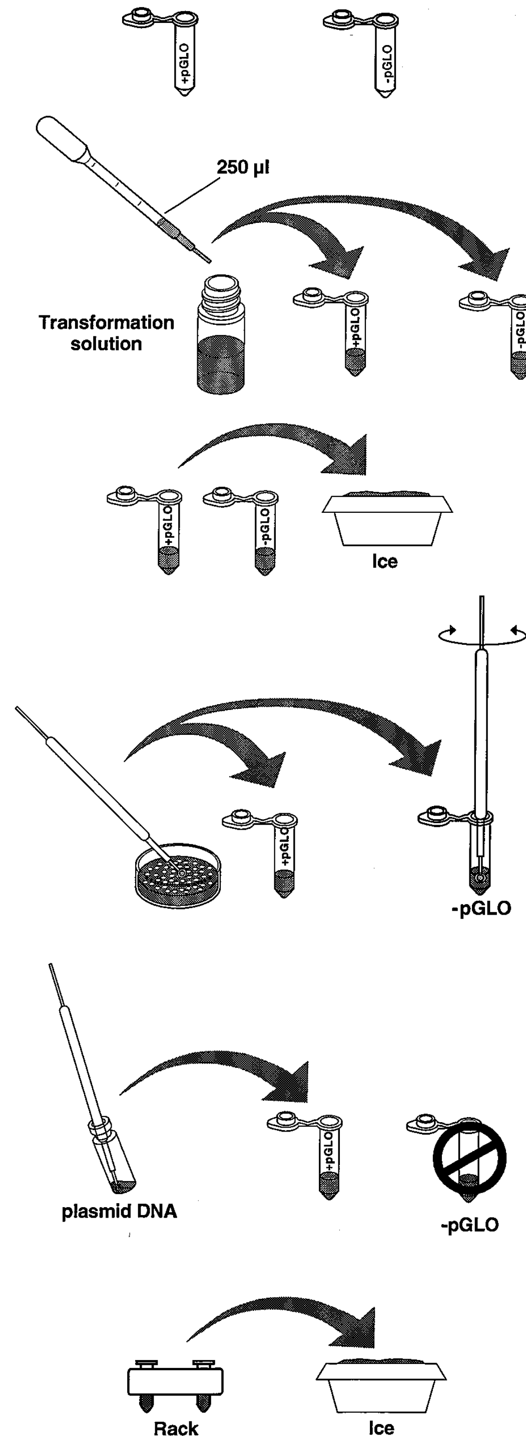
Plasmid uptake

Now that you have your model, how might we get the bacteria to uptake the plasmid you made?

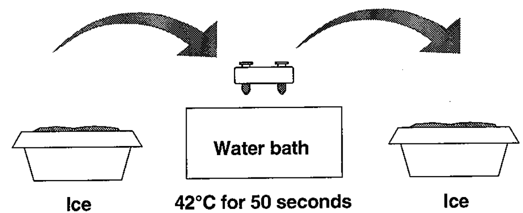
1. If a plastic bottle or balloon acts as the bacteria model, how might we get the genes inside the bottle?
2. What will happen to the cell membrane if we heat the bacteria a bit? (Think of yourself in a hot tub or sauna.) What will happen to the cell membrane if we put it in ice water? (Think of yourself on a chilly day without a coat on.)
3. How could the conditions in question 2 help us get the plasmid in the bottle or balloon?

Part 2: Bacterial transformation

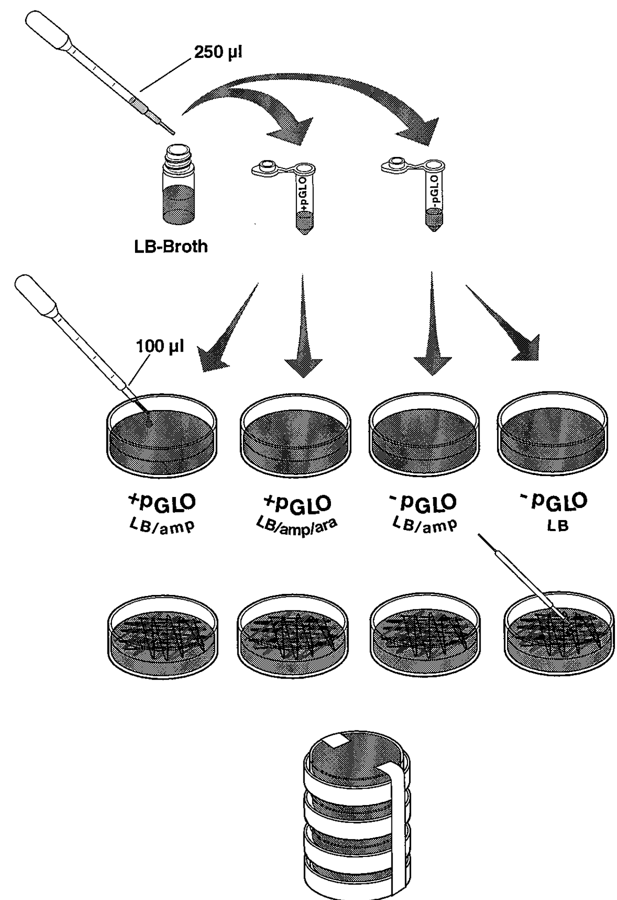
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.
2. Open the tubes and using a sterile transfer pipet, transfer 250 μl of transformation solution ($CaC1_2$).
3. Place the tubes on ice.
4. Use a sterile loop to pick 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 a commodity crop 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, wire worms, corn seed maggot, etc.)
- Decrease of herbicide use
- Changes in the nutritional composition (protein, moisture, starch, etc.)

- pGLO	+ pGLO
LB Amp Prediction: Reason: Observed result:	LB Amp Ara Prediction: Reason: Observed result:
LB Prediction: Reason: Observed result:	LB Amp Prediction: Reason: Observed result:

Rubric for self-assessment

Skill		Yes	No	Unsure
Part 1	My model thoroughly explained the creation of a plasmid.			
	I was able to explain how my model operated in writing or orally to my classmates.			
Part 2	My group completed the lab and had glowing colonies.			
	I can explain the pattern of growth that we observed in our plates.			
	I can explain the process used in this lab to transform bacteria.			
	Extension: I can calculate the transformation efficiency we obtained during this lab.			