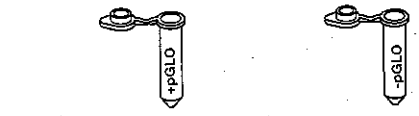
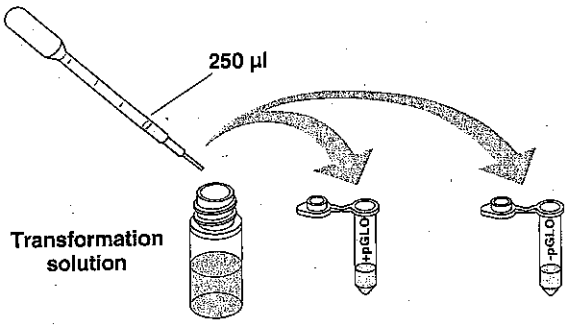
Transformation Kit—Quick Guide

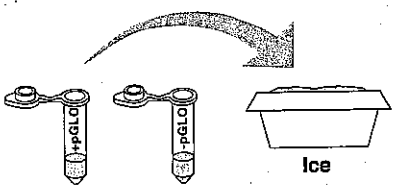
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.



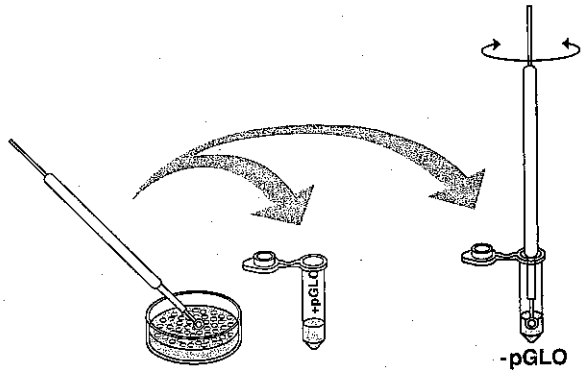
2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaC1_2$) into each tube.



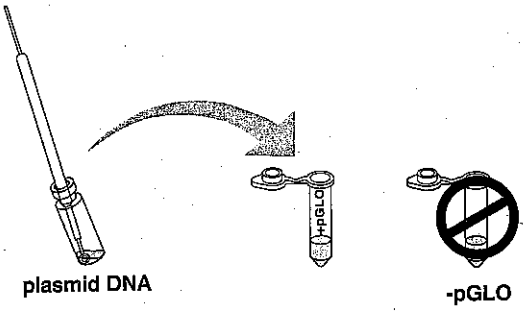
3. Place the tubes on crushed ice. Do not use cubed ice.



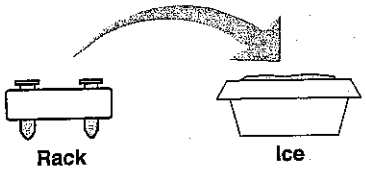
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution (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.



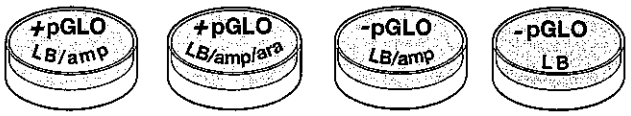
5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles. Mix the loopful into the cell suspension of the +pGLO tube. Optionally, pipet 10 µl of pGLO plasmid into the +pGLO tube and mix. Close the -pGLO tube and return it to the rack on ice. Do not add plasmid DNA to the -pGLO tube. Why not? Close the -pGLGO tube and return it to the rack on ice.



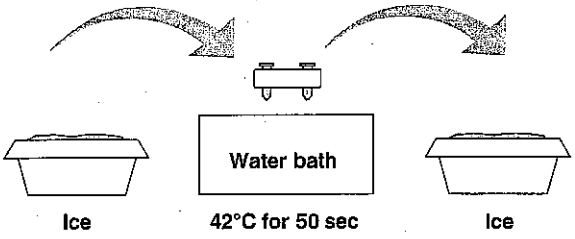
6. Incubate the tubes on ice for 10 min. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



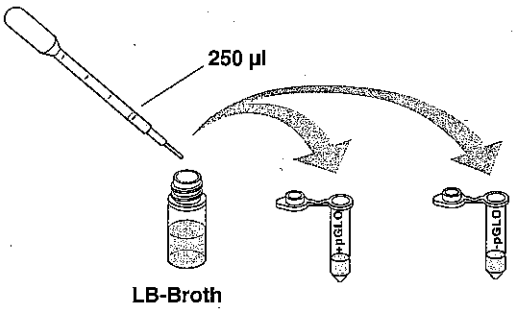
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as shown on the diagram.



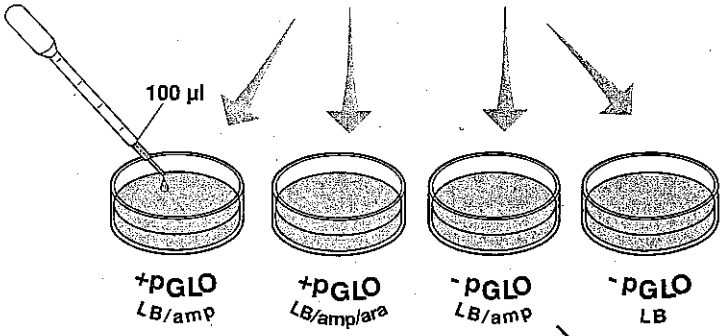
8. Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42°C, for **exactly** 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds have passed, 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 min.



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 min at room temperature.



10. Gently flick the closed tubes with your finger to mix. Using a **new sterile pipet for each tube**, pipet 100 µl from each of the tubes to the corresponding plates, as shown on the diagram 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.