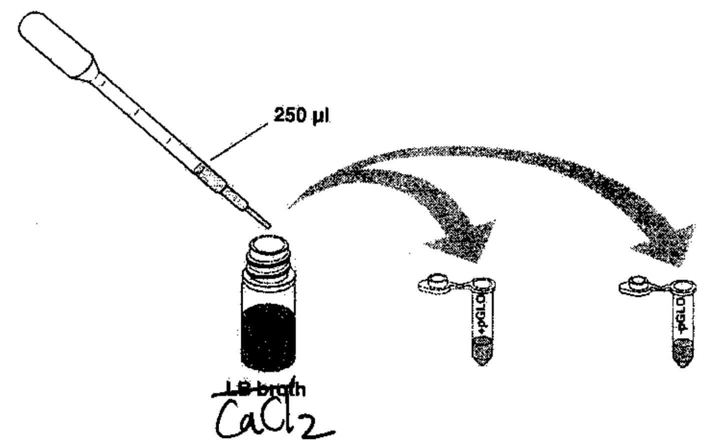
pRed Lab Materials and Methods – Day 2 Protocol

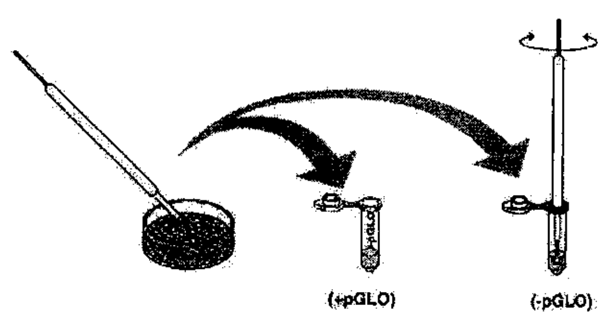
1. Label one closed micro test tube +pGLO and another -pGLO. Label both tubes with your group's name. Place them in the foam tube rack.

2. Open the tubes and, using a sterile transfer pipette, transfer 250 µl (microliters) of transformation solution (CaCl2) into each test tube.

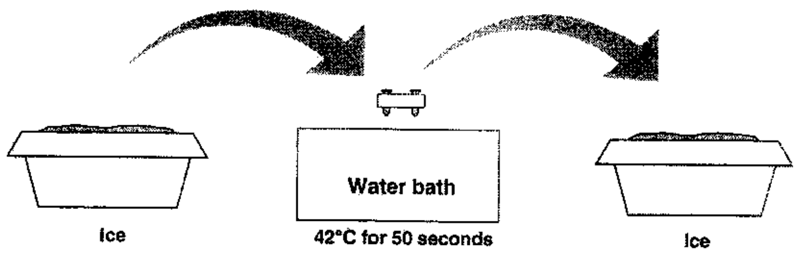


3. Place the tubes on ice (beaker with ice and water)

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.

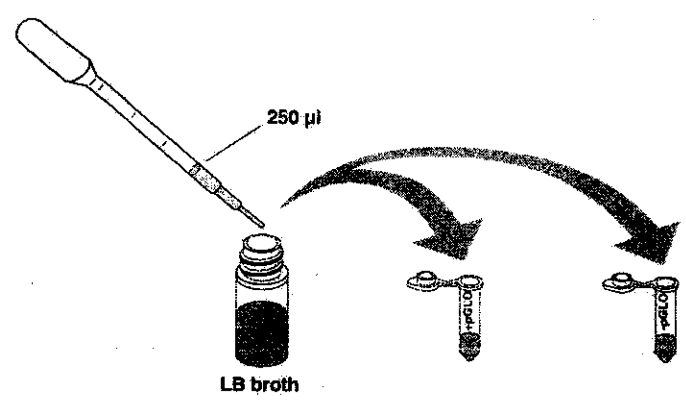


5. Examine the pGLO DNA with the UV lamp. Note your observations. Immerse a new sterile loop into the pGLO plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across the ring. Mix the loopful into the cell suspension of the +pGLO



toss
2.5ml use pippett

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 nutrient agar plates.