

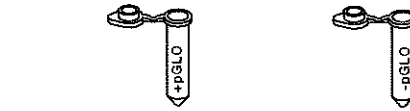
You will be using your pre-lab, not this paper on Friday so
 make sure you do an adequate pre-lab

Transformation Kit—Quick Guide

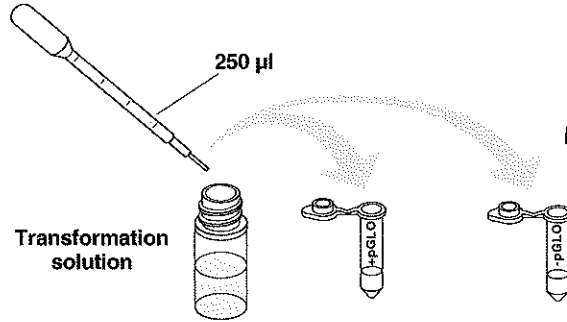
also write
 "+", "0", or "-"
 on the
 lid

- put your group # on tube, too

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.

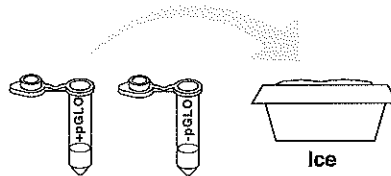


2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution (CaCl₂).

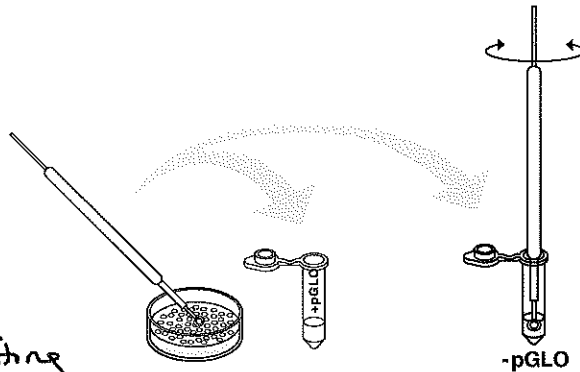


use a micropipettor instead of a plastic transfer pipette.

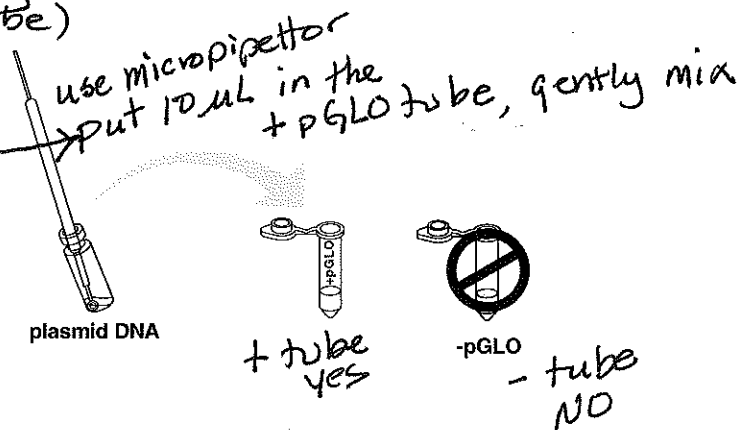
3. Place the tubes on ice. (crushed ice in a beaker...)



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. (you are putting bacteria in each tube)

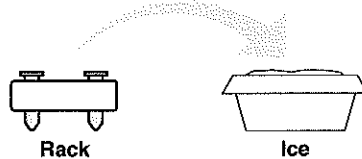


5. Examine the pGLO plasmid DNA solution with the UV lamp. Note your observations. Immerse a new sterile loop into the plasmid DNA stock tube. Withdraw a loopful. There should be a film of plasmid solution across the ring. This is similar to seeing a soapy film across a ring for blowing soap bubbles.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



(in the closet?)
 does plasmid by itself glow?

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.

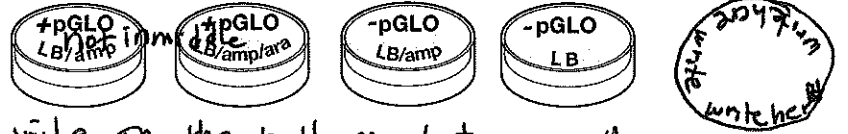


or don't use rack and just stick tubes in ice

We are using heating blocks so it may make more sense to take the tubes out of the racks and just hold them at 42° for 50 sec.

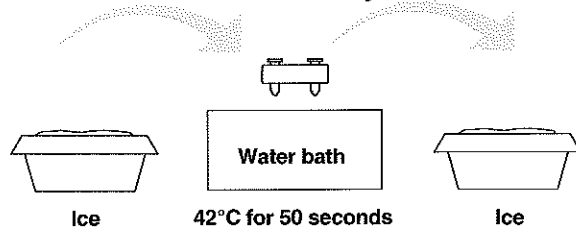
write near edges, not through middle

- 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.



write on the bottom plate on the bottom (outside) of it - not the short edge part

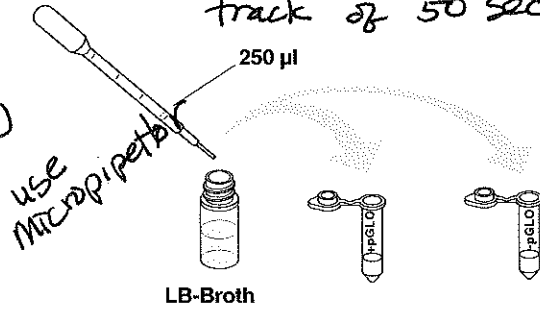
- Heat shock. Using the foam rack as a holder, transfer both the (+) pGLO and (-) pGLO tubes into the water bath, set at 42 °C, for exactly 50 seconds. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the warm water. When the 50 seconds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



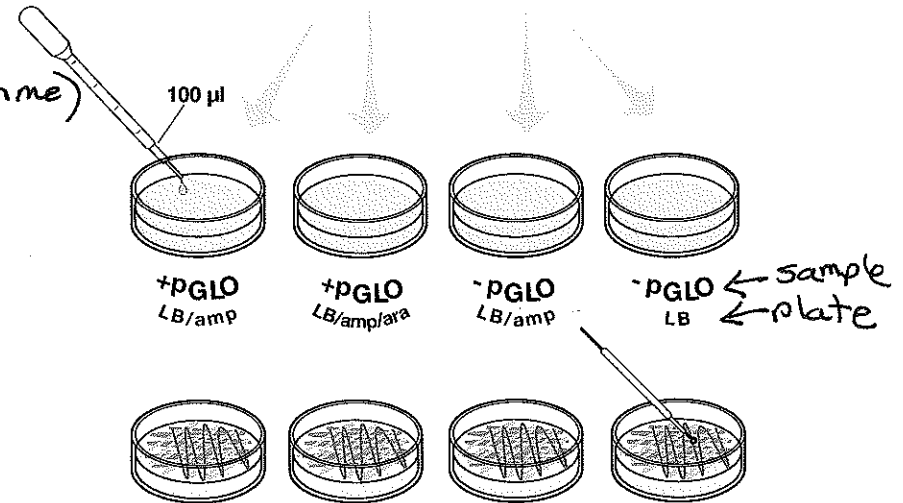
one lab partner holds the tubes - the other lab partner keeps track of 50 seconds

- look at your starter plate as an example

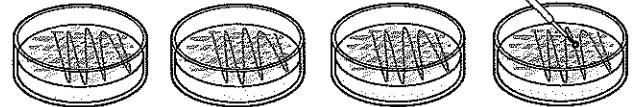
- Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at room temperature. (more recovery time)



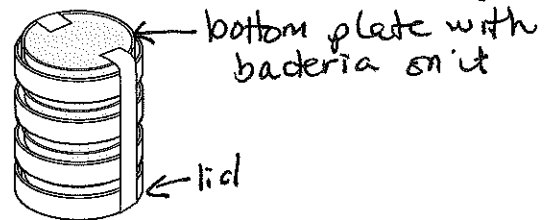
- Tap the closed tubes with your finger to mix. Using a new sterile pipet for each tube, pipet 100 µl of the transformation and control suspensions onto the appropriate plates.



- 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.



- 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.



We may actually use cell spreaders instead of loops - or just shake to distribute.

Don't forget to include the data table with explanations in your pre-lab.