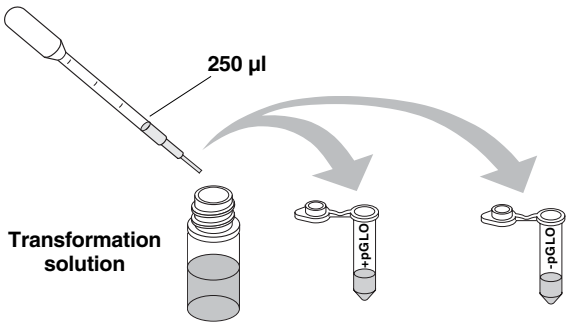
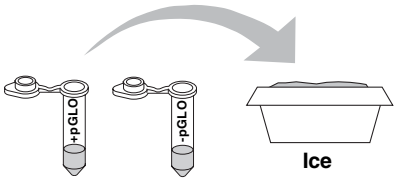
Transformation Kit—Quick Guide

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.

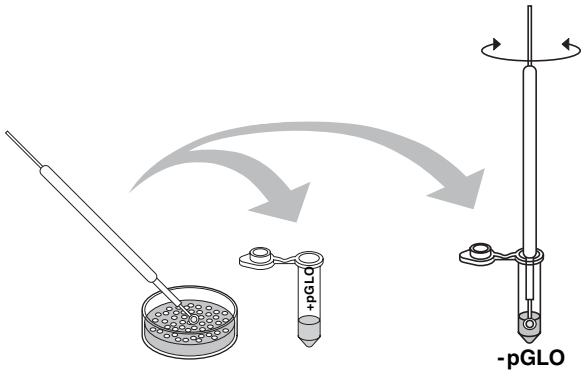
2. Open the tubes and using a sterile transfer pipet, transfer 250 µl of transformation solution ($CaC1_2$).



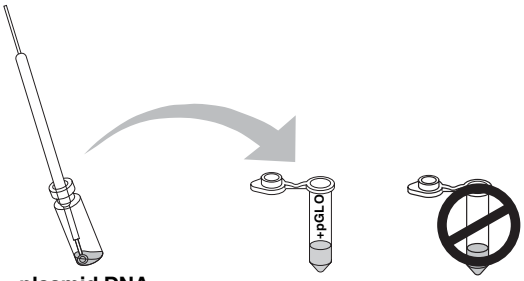
3. Place the tubes on ice.



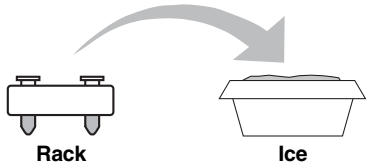
4. Use a sterile loop to pick up a single colony of bacteria from your starter plate. Pick up the +pGLO tube and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the transformation solution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

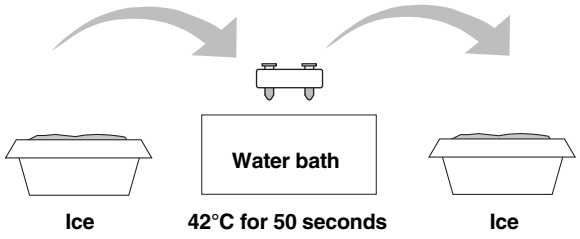


6. Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.



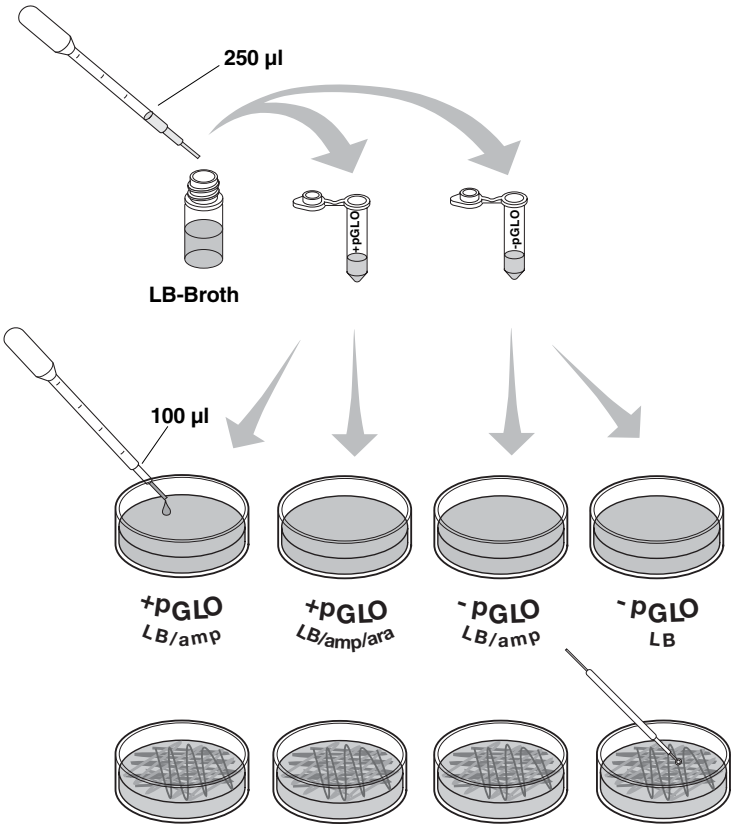
7. While the tubes are sitting on ice, label your four agar plates on the bottom (not the lid) as follows: Label one LB/amp plate: +pGLO; Label the LB/amp/ara plate: +pGLO; Label the other LB/amp plate: -pGLO; Label the LB plate: -pGLO.

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.



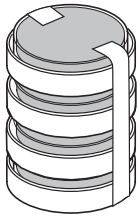
9. Remove the rack containing the tubes from the ice and place on the bench top. Open a tube and, using a new sterile pipet, add 250 µl of LB nutrient broth to the tube and reclose it. Repeat with a new sterile pipet for the other tube. Incubate the tubes for 10 minutes at room temperature.

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.



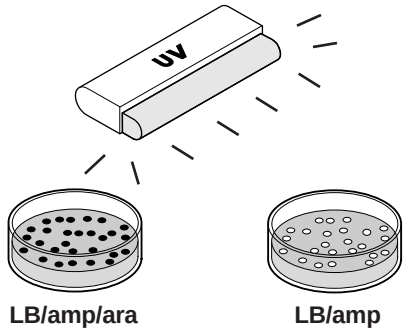
11. Use a new sterile loop for each plate. Spread the suspensions evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.

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.

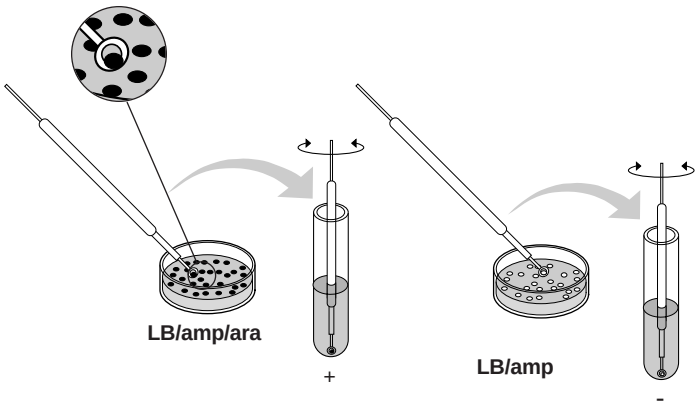


Lesson 2 Inoculation
Growing Cell Cultures

1. Remove the transformation plates from the incubator and examine using the UV light. Identify several green colonies that are not touching other colonies on the LB/amp/ara plate. Identify several white colonies on the LB/amp plate.



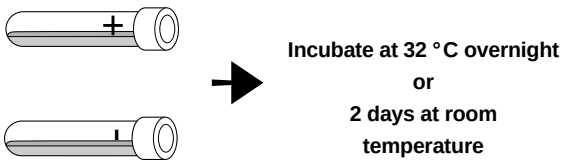
2. Obtain two culture tubes containing the growth media LB/amp/ara. Label one "+" and one "-". Using a sterile loop, lightly touch the loop to a green colony and immerse it in the "+" tube. Using a new sterile loop, repeat for a white colony and immerse it in the "-" tube (it is very important to pick only a single colony). Spin the loop between your index finger and thumb to disperse the entire colony.



3. Cap the tubes and place them in the shaking incubator or on the shaking platform and culture overnight at 32 °C or 2 days at room temperature.

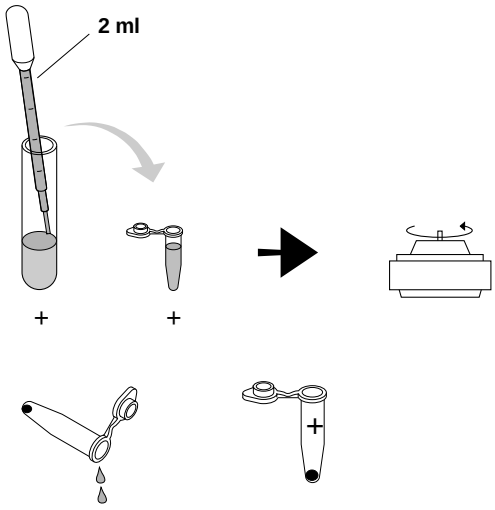
   or

   Cap the tubes and shake vigorously by hand. Place in the incubator horizontally at 32 °C for 24–48 hours. Remove and shake by hand periodically when possible.



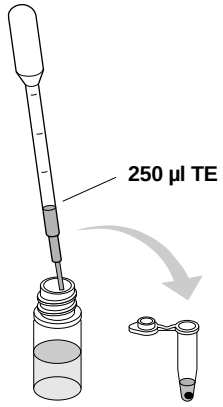
Lesson 3 Purification Phase 1
Bacterial Concentration

1. Label one microtube "+" with your name and class period. Remove your liquid cultures from the shaker and observe with the UV light. Note any color differences between the two cultures. Using a new pipette, transfer 2 ml of "+" liquid culture into the "+" microtube. Spin the microtube for 5 minutes in the centrifuge at maximum speed. The pipette used in this step can be repeatedly rinsed in a beaker of water and used for all following steps of this laboratory period.

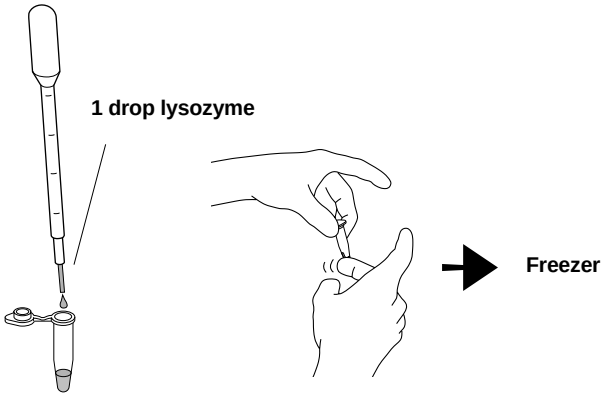


2. Pour out the supernatant and observe the pellet under UV light.

3. Using a rinsed pipette, add 250 µl of TE solution to the tube. Resuspend the pellet thoroughly by rapidly pipetting up and down several times.



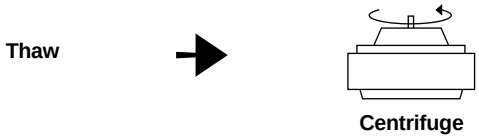
4. Using a rinsed pipette, add 1 drop of lysozyme to the resuspended bacterial pellet to initiate enzymatic digestion of the bacterial cell wall. Mix the contents gently by flicking the tube. Observe the tube under the UV light.



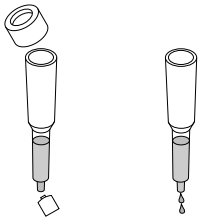
5. Place the microtube in the freezer until the next laboratory period. The freezing causes the bacteria to rupture completely.

Lesson 4 Purification Phase 2
Bacterial Lysis

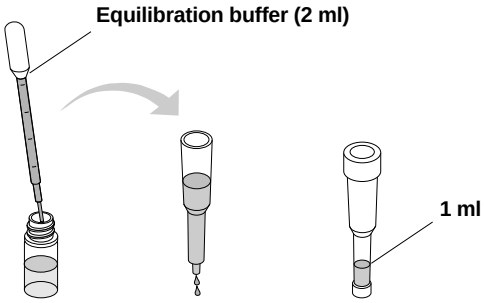
1. Remove the microtube from the freezer and thaw using hand warmth. Place the tube in the centrifuge and pellet the insoluble bacterial debris by spinning for 10 minutes at maximum speed.



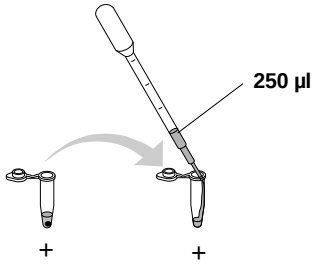
2. While your tube is spinning, prepare the chromatography column. Remove the cap and snap off the bottom from the prefilled HIC column. Allow all of the liquid buffer to drain from the column (~3–5 minutes).



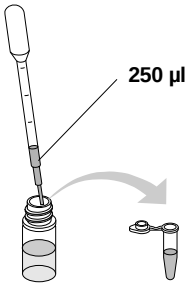
3. Prepare the column by adding 2 ml of Equilibration Buffer to the top of the column. This is done by adding two 1 ml aliquots with a rinsed pipette. Drain the buffer to the 1 ml mark on the column. Cap the top and bottom and store the column at room temperature until the next laboratory period.



4. After the 10 minute spin, immediately remove your tube from the centrifuge. Examine the tube with the UV light. Using a new pipette, transfer 250 µl of the "+" supernatant into a new microtube labeled "+". Again, rinse the pipette well for the rest of the steps of this lab period.



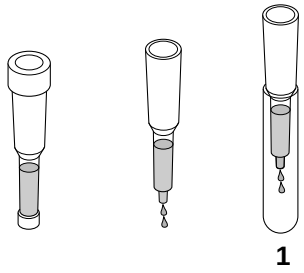
5. Using a well rinsed pipette, transfer 250 µl of binding buffer to the "+" supernatant. Place the tube in the refrigerator until the next laboratory period.



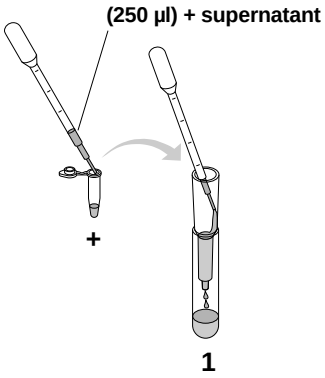
Lesson 5 Purification Phase 3

Protein Chromatography

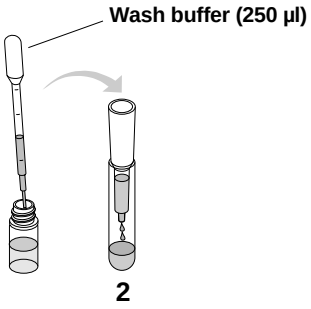
1. Label 3 collection tubes 1–3 and place the tubes in the foam rack or in a rack supplied in your laboratory. Remove the caps from the top and bottom of the column and place the column in collection tube 1. When the last of the buffer has reached the surface of the HIC matrix proceed to the next step below.



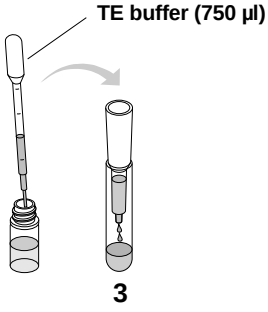
2. Using a new pipette, carefully and gently load 250 µl of the "+" supernatant onto the top of the column. Hold the pipette tip against the side of the column wall, just above the upper surface of the matrix and let the supernatant drip down the side of the column wall. Examine the column using a UV light. Note your observations. After it stops dripping transfer the column to collection tube 2.



3. Using the rinsed pipette, add 250 µl of wash buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations. After the column stops dripping, transfer it to tube 3.



4. Using the rinsed pipette, add 750 µl of TE Buffer and let the entire volume flow into the column. Examine the column using the UV light. Note your observations.



5. Examine all three collection tubes and note any differences in color between the tubes. Parafilm or Saran Wrap the tubes and place in the refrigerator until the next laboratory period.