Transformation Laboratory Procedure

After discussing your experimental design with the class, it is time to conduct the actual transformation to determine the identity of the plasmids. Each group will be assigned one plasmid to work with.

Remember the following:

- One plasmid has a kanamycin-resistance gene.
- Two plasmids have an ampicillin-resistance gene.
- In addition to having an antibiotic-resistance marker, one plasmid also codes for the gene for green fluorescent protein (GFP), a protein from the bioluminescent jellyfish *Aequorea victoria*.

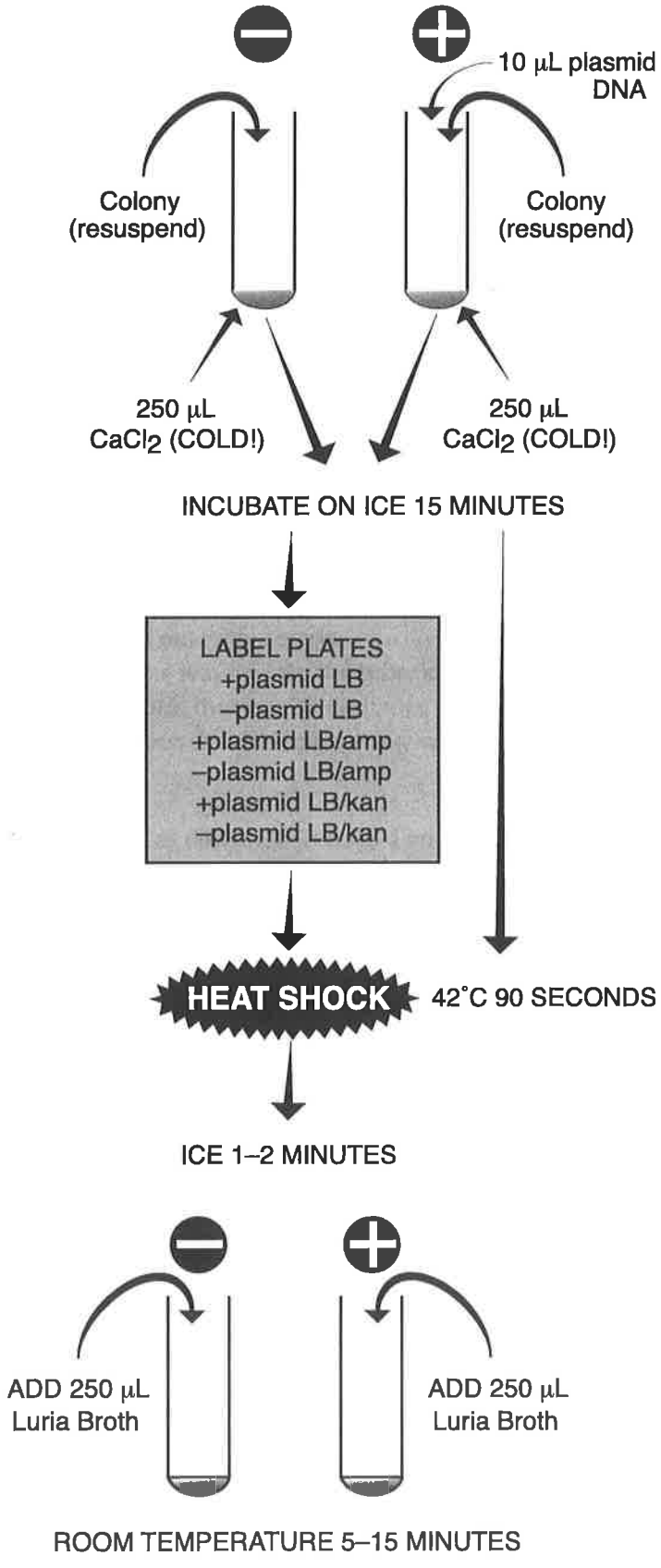
Materials

For your group:

2 LB plates
2 kanamycin plates
2 ampicillin plates
2 sterile transformation tubes
container with crushed ice
rack for holding transformation tubes
3 sterile inoculating loops
6 sterile transfer pipets
waste container
3-mL vial LB
3-mL vial $CaCl_2$ (on ice)

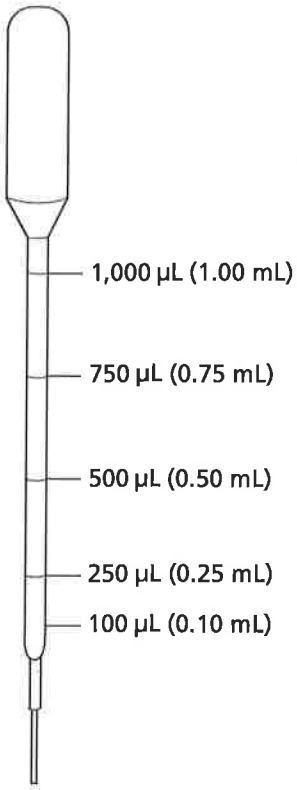
To share:

glass beads for spreading
water bath (42°C)
incubator (if used)
starter plate (shared between two groups)
plasmids (will be marked "Plasmid 1," "Plasmid 2," or "Plasmid 3")



Procedure

When performing all of the following steps use sterile technique.

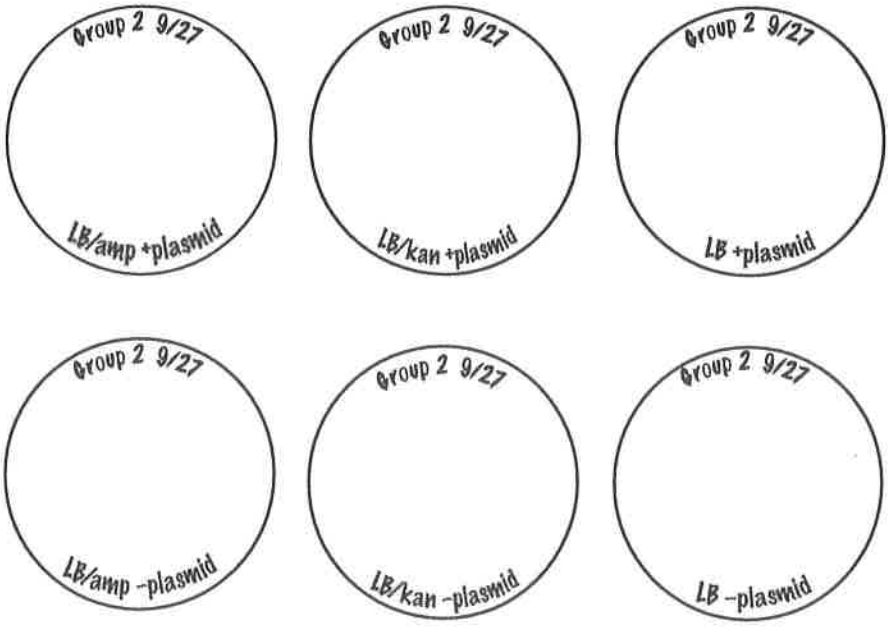


1. Mark one sterile 15-mL tube "+plasmid." Mark another "–plasmid." Plasmid DNA will be added only to the +plasmid tube.
2. Use a sterile transfer pipet to add 250 µL of ice-cold calcium chloride to each tube. See the figure at the right for reference. **Note:** Pressing the conical area between the stem and bulb of the pipet provides better control of the amount of liquid being aspirated.
3. Place both tubes on ice.
4. Use a sterile disposable inoculating loop to transfer isolated colonies of *E. coli* from the starter plate to the +plasmid tube. The total area of the colonies picked should be about half the size of the top of a pencil eraser (the ball of cells on the loop should be approximately 1–2 mm in diameter.)

a. Do not transfer any agar from the plate along with the cell mass.

b. Immerse the cells on the loop in the calcium chloride solution in the +plasmid tube and vigorously spin the loop in the solution to dislodge the cell mass. Hold the tube up to the light to verify that the cell mass has fallen off the loop.

5. **Immediately** suspend the cells by repeatedly pipetting in and out with a sterile transfer pipet. It is not necessary to draw the suspension all the way up into the tube; keep the solution in the stem of the pipet. Examine the tube against the light to confirm that no visible clumps of cells remain in the tube or are lost in the bulb of the transfer pipet. The suspension should appear milky white.
6. Return the +plasmid tube to ice.
7. Transfer a mass of cells to the –plasmid tube and resuspend as described in steps 4 and 5 above.
8. Return the –plasmid tube to ice. Both tubes should now be on ice.
9. Use a new sterile disposable inoculating loop to add one loopful of plasmid DNA to the +plasmid tube **only**. When the DNA solution forms a film across the loop opening, its volume is 10 µL. If you do not see this film, you do not have enough DNA on the loop. Immerse the loopful of plasmid DNA *directly into* the cell suspension and spin the loop to mix the DNA with the cells.
10. Return the +plasmid tube to ice and incubate both tubes on ice for **15 minutes**.
11. While the tubes are incubating, label your media plates as indicated in the diagram and with your lab group name and date. Think about why you are labeling these plates as you are.



12. Following the 15-minute incubation on ice, heat shock the cells as follows. Remove both tubes directly from ice and immediately immerse them in the 42°C water bath for 90 seconds. Gently agitate the tubes while they are in the water bath. Return both tubes directly to ice for 1 minute or more. The abrupt transfer of the tubes from ice to 42°C and back again is critical.
13. Use a sterile transfer pipet to add 250 µL Luria broth (LB) to each tube. Gently agitate the tubes with your finger to mix the LB with the cell suspension. Allow the tubes to sit at room temperature for a 5–15 minute recovery. During this incubation your instructor may have you add the glass beads for spreading your bacteria to your plates. See 14a. for instructions on how to do this.
14. Using the procedure below, spread the cells in the +plasmid tube onto the +plasmid plates and the cells in the –plasmid tube onto the –plasmid plates.
    a. Place the plates lid side down. Slightly open the plate on one side and carefully pour 4–6 glass beads onto each plate. Flip the plate over so that the beads are now resting on the agar.
    b. Using a sterile transfer pipet add 100 µL of cells from the –plasmid transformation tube to each of the appropriate plates.
    c. Using a new sterile transfer pipet add 100 µL of cells from the +plasmid transformation tube to each of the appropriate plates.
    d. Spread the bacteria evenly across the plate by using a back-and-forth motion (not swirling round and round) to move the glass beads across the entire surface of each plate. Shake each plate for several minutes.
    e. When you finish spreading, let the plates sit for several minutes to allow the agar to absorb the cell suspension.
    f. To remove the glass beads, hold each plate vertically over a waste container, slightly open the lower edge, and tap out the beads into the container.
15. Stack your plates together and incubate them as instructed by your instructor.

Laboratory Questions

1. Record your results and conclusions using the organizational method you devised in the Pre-laboratory Inquiry Activity.

2. Did you observe what you expected to? If not, how would you explain your observations?

3. The results from three different experiments are as described in a, b, and c. Something has gone wrong with each of these experiments. Use the controls to figure out what has gone wrong in each experiment.

   a. Results from Experiment 1:

Plate	Results
LB/amp+plasmid plate	(no lawn, no colonies)
LB/amp–plasmid plate	(no lawn, no colonies)
LB/kan+plasmid plate	(no lawn, no colonies)
LB/kan–plasmid plate	(no lawn, no colonies)
LB+plasmid plate	(no lawn, no colonies)
LB–plasmid plate	(no lawn, no colonies)

   Explanation:

   b. Results from Experiment 2:

Plate	Results
LB/amp+plasmid plate	(lawn)
LB/amp–plasmid plate	(lawn)
LB/kan+plasmid plate	(clean plate)
LB/kan–plasmid plate	(clean plate)
LB+plasmid plate	(lawn)
LB–plasmid plate	(lawn)

   Explanation:

   c. Results from Experiment 3:

Plate	Results
LB/amp+plasmid plate	(colonies)
LB/amp–plasmid plate	(colonies)
LB/kan+plasmid plate	(clean plate)
LB/kan–plasmid plate	(clean plate)
LB+plasmid plate	(lawn)
LB–plasmid plate	(lawn)

   Explanation:

4. Having a way to measure transformation efficiency helps in discussing results or in comparing transformations that were not done at the same time. Transformation efficiency is expressed as the number of transformed colonies (in this case those that are antibiotic-resistant) per microgram of plasmid used in the transformation.

   a. Figure out how you would calculate transformation efficiency (i.e., number of colonies/µg of plasmid used). You used 10 µL of plasmid at a concentration of 0.005 µg/µL.

   b. Now use the method you devised above to determine the transformation efficiency for the transformation performed by your group.

   c. What might be sources of error in calculating this number?

5. You are making ampicillin plates. Before pouring the plates, you add 2 mL of 10 mg/mL to the 400-mL bottle of LB agar that you use to pour the plates. What is the final concentration of the ampicillin in the plates? Express your answer as µg/mL. Show your work.

6. Again, you are making ampicillin plates using LB agar. You are given a vial of ampicillin that is labeled as a 1% solution and told that you need to make 40 plates using this solution. Assuming that you will need 25 mL per plate, what volume of LB agar solution should you make to prepare the 40 plates? What volume of the 1% ampicillin solution do you need to add to this volume of LB agar if the final concentration of ampicillin in the plates should be 50 µg/mL? Hint: Percentage is an expression of weight of solute per volume of solution.

Big Idea Assessments

1. Give a specific example of how the introduction of a gene into a bacterium can change the phenotype of the bacterium. Also explain the specific role of the protein expressed by the gene in changing the bacterium's phenotype.

2. Griffin performed experiments demonstrating that when live, nonpathogenic, *S. pneumoniae* (which produce rough-surfaced colonies) are mixed with killed smooth-surfaced *S. pneumoniae* (which are pathogenic when alive) and are then injected into mice, the mice become ill. Bacteria isolated from these sick mice form the smooth colonies characteristic of the pathogenic strain. What happened to the bacteria to make them pathogenic to the mice?

3. Briefly describe how the experiments of Avery, McCarty, and MacLeod, building on the work of Griffith, demonstrated that DNA was the molecule that passed on traits.

4. You have isolated one of the genes for producing one of the blood-clotting proteins needed by some hemophiliacs. Briefly describe how you could create bacteria that would produce this protein.

5. Name the two ways in which bacteria can acquire new genetic material. Both ways are examples of lateral (or horizontal) gene transfer.

6. Assume that you transform bacteria with a plasmid containing an ampicillin-resistance gene. Instead of directly plating the transformed population as you did in this lab, you set up two liquid cultures of them, one that contains ampicillin and one that does not. You will then assay these cultures on plates at two different times: immediately after you set up the cultures, and then again after the bacteria have been in culture for an extended period. The assays will demonstrate the number of ampicillin-resistant vs. ampicillin-sensitive bacteria in each culture at each time. To perform each of the two assays, you prepare serial dilutions of the two cultures and plate them onto LB plates with and without ampicillin (the dilution is simply to ensure that you will get some plates on which you can distinguish separate colonies).

   Describe what you expect to observe in the initial assay and in the second assay. What, if any, differences might you expect in terms of the ratios of ampicillin-resistant and nonresistant bacteria?

Appendix

Data Tables

1. Count the number of colonies on each plate and record your results in the table below. If there is a continuous, or nearly continuous, lawn of bacterial growth write "lawn." Use the data presented by your classmates to fill in the parts of the table for which you have no data. There may be multiple sets of data for each plasmid. Circle the data from your group's experiment.

	LB AMPICILLIN PLATES		LB KANAMYCIN PLATES		LB PLATES		
	+ plasmid	– plasmid	+ plasmid	– plasmid	+ plasmid	– plasmid	Color of colonies (where they appear)
Plasmid 1							
Plasmid 2							
Plasmid 3							

2. Use the data in the table above to fill in the table below.

Does the plasmid below contain	an ampicillin-resistance gene?	a kanamycin-resistance gene?	a green fluorescent protein gene?
Plasmid 1			
Plasmid 2			
Plasmid 3			