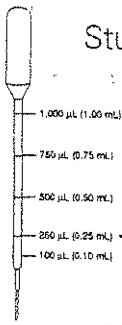
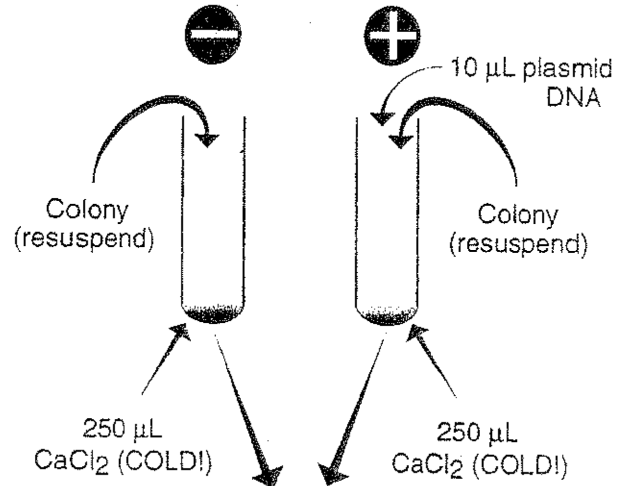
Laboratory Procedure

Name:

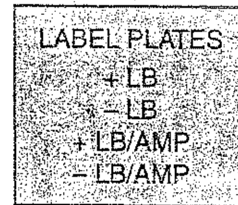
Date:



1. Mark one sterile 15-mL tube "+." Mark another "–." (Plasmid DNA will be added to the "+" tube; none will be added to the "–" tube.)
2. Use a sterile transfer pipet to add 250 μL of ice-cold calcium chloride to each tube.
3. Place both tubes on ice.
4. Use a sterile plastic inoculating loop to transfer a cell mass about the diameter of a pencil eraser from isolated colonies of *E. coli* cells from the starter plate into the + tube.
   a. Be careful not to 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 + tube and vigorously spin the loop in the solution to dislodge the cell mass. Hold the tube up to the light to observe 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. Examine the tube against 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 + tube to ice. Transfer a mass of cells to the – tube and *suspend as described in steps 4 and 5 above.*
7. Return the – tube to ice. Both tubes should now be on ice.
8. Use a sterile plastic inoculating loop to add one loopful of plasmid DNA to the + tube. When the DNA solution forms a bubble across the loop opening, its volume is 10 μL. Immerse the loopful of plasmid DNA *directly into* the cell suspension and spin the loop to mix the DNA with the cells.
9. Return the + tube to ice and incubate both tubes on ice for 15 minutes.
10. While the tubes are incubating, label your media plates with your lab group name and date.
    a. Label one LB/Amp plate "+." This is an experimental plate.
    b. Label the other LB/Amp plate "–." This is a negative control.
    c. Label your LB plate either "+" or "–," according to your teacher's instructions. This is a positive control to test the viability of the cells after they have gone through the transformation procedure.

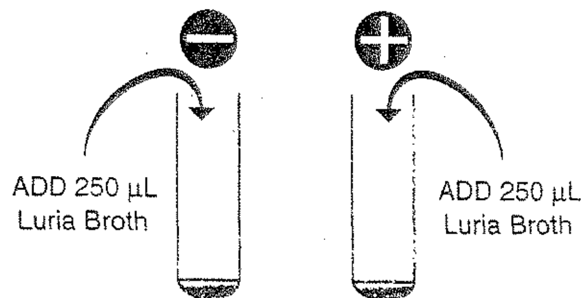


INCUBATE ON ICE 15 MINUTES



HEAT SHOCK 42°C 90 SECONDS

ICE 1–2 MINUTES



ROOM TEMPERATURE 5–15 MINUTES

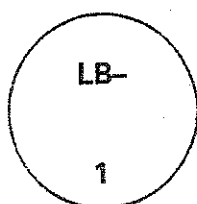
11. Following the 15-minute incubation on ice, "heat shock" the cells. Remove both tubes *directly from ice and immediately* immerse them in the 42°C water bath for 90 seconds. Gently agitate the tubes while they are in the water bath. Return both the tubes *directly* to ice for 1 or more minutes.
12. Use a sterile transfer pipet to add 250 µL Luria broth (LB) to each tube. Gently tap the tubes with your finger to mix the LB with the cell suspension. Place the tubes in a test-tube rack at room temperature for a 10-minute recovery.
13. Now you will remove some cells from each transformation tube and spread them on the plates. Do one plate at a time, from start to finish. Cells from the – tube should be spread on the – plates, and cells from the + tube should be spread on the + plates.
14. Use a sterile transfer pipet to add 100 µL of cells from the – transformation tube to the appropriate plate(s). Using the procedure below, immediately spread the cells over the surface of a plate.
    a. "Clam shell" (slightly open) the lids and carefully pour 4–6 glass beads onto each plate.
    b. Use a back-and-forth and up-and-down shaking motion (not swirling round and round) to move the glass beads across the **entire** surface of the plate. This should evenly spread the cell suspension all over the agar surface.
    c. When finished spreading, let the plates rest for several minutes to allow the cell suspensions to become absorbed into the agar.
    d. To remove the glass beads, hold each plate vertically over a container, clam shell the lower part of the plate, and tap out the glass beads into the container.
15. Use another sterile transfer pipet to add 100 µL of cell suspension from the +DNA tube onto the appropriate plate(s).
16. Immediately spread cell suspension(s) as described in step 14.
17. Wrap the plates together with tape and place the plates upside down either in the incubator or at room temperature. Incubate them for approximately 24–36 hours in a 37°C incubator or 48–72 hours at room temperature.

Transformations
Student Sheet

Name:
Date:

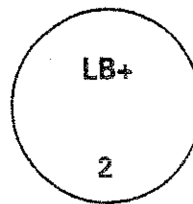
Data and Analysis

1. Observe the colonies through the bottom of the petri plate. Do not open the plates.
2. Predict your results. Write "yes" or "no," depending on whether you think the plate will show growth. Give the reason(s) for your predictions.



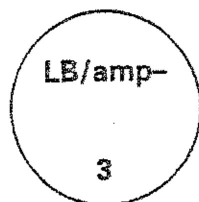
Prediction:
Reason:

Observed Result:



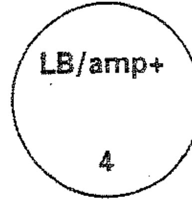
Prediction:
Reason:

Observed Result:



Prediction:
Reason:

Observed Result:



Prediction:
Reason:

Observed Result:

3. Record your observed results in the spaces above. If your observed results differed from your predictions, explain what you think may have occurred.

Light-Producing genes

GTAGCACATAAAGGACGAAAGCATTTGCTGAAAGGAGTTCCCCCAGGCATCCA

CATCGTGTATTTCCTGCTTTCGTAAACGACTTTCCTCAAGGGGGTCCGTAGGT

pVIB PLASMID DNA

With Light-Producing and Ampicillin-resistance genes

** Coding strand = **Inside** **

GGCTACTTGACCTTCTCGA

CGATGACACTGGAGGAGCT

Ampicillin-Resistance Gene

Bacterial TRANSFORMATION Lab: Bead Souvenirs

Directions:

1. **Transcribe** the pVIB plasmid coding strand (INside letters) for the light-producing and antibiotic-resistance genes to determine the corresponding mRNA.
2. **Translate** each mRNA using the CODON TABLE to determine the amino acid sequence for the light-producing and antibioitic resistance proteins.
3. Then use the bead color-coding tables to build a bead **necklace** model of the pVIB plasmid mRNA strands and a bead **bracelet** of the light-producing and antibiotic-resistance proteins which were used to TRANSFORM your *E. coli* bacteria into bacteria that make their own light.

1. **Transcription** light-producing mRNA antibiotic-resistance mRNA

mRNA (necklace) ______________________________

____

mRNA N-Base letters	Bead Color / Shape
Guanine	
Cytosine	
Adenine	
Uracil	

2. **Translation** light-producing protein antibiotic-resistance protein

protein (bracelet) ______________________________

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Bead Color Sequence ______________________________

Codons in mRNA					
First base	Second base U	C	A	G	Third base
U	UUU, UUC Phenylalanine; UUA, UUG Leucine	UCU, UCC, UCA, UCG Serine	UAU, UAC Tyrosine; UAA, UAG Stop	UGU, UGC Cysteine; UGA – Stop; UGG – Tryptophan	U C A G
C	CUU, CUC, CUA, CUG Leucine	CCU, CCC, CCA, CCG Proline	CAU, CAC Histidine; CAA, CAG Glutamine	CGU, CGC, CGA, CGG Arginine	U C A G
A	AUU, AUC, AUA Isoleucine; AUG – Start	ACU, ACC, ACA, ACG Threonine	AAU, AAC Asparagine; AAA, AAG Lysine	AGU, AGC Serine; AGA, AGG Arginine	U C A G
G	GUU, GUC, GUA, GUG Valine	GCU, GCC, GCA, GCG Alanine	GAU, GAC Aspartic Acid; GAA, GAG Glutamic Acid	GGU, GGC, GGA, GGG Glycine	U C A G

Amino Acid	Bead color
Val	YELLOW-glow
Ala	ORANGE-glow
His	PINK-glow
Lys	GREEN-glow
Gly	BLUE-glow
Arg	WHITE-glow
Leu	Orange-regular
Pro	Purple-regular
Ser	Dark Red-regular