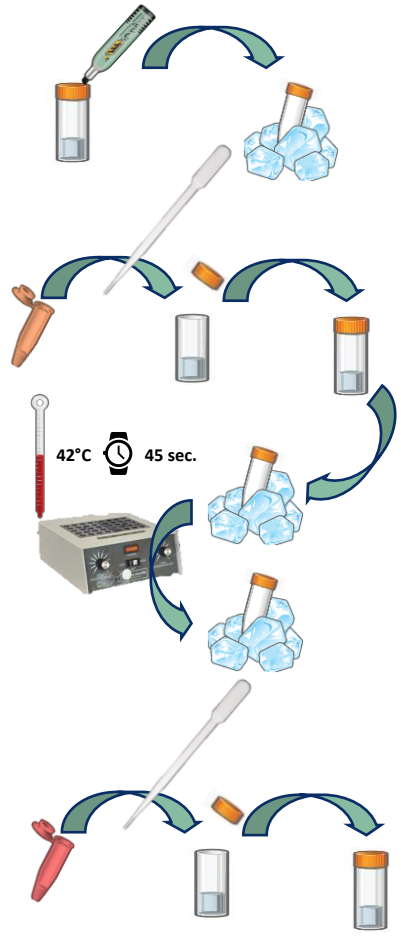
BioPharmaceutical Technology Center
I N S T I T U T E

Genetic Transformation & Bioluminescence

Procedure

1. Each person or team should label the top of a screw-capped tube containing bacteria with their initials. Return the tube to the ice bucket.

   This is the reaction tube.



2. Using a disposable pipette, take up the plasmid DNA solution from the small tube and add it to your screw-capped tube. Screw the cap back on and mix by inverting the tube two or three times.

3. Place the screw-capped tube on ice for 5 minutes.

4. Heat shock the bacteria by placing the screw-capped tube in a 42°C heat block for 45 sec.

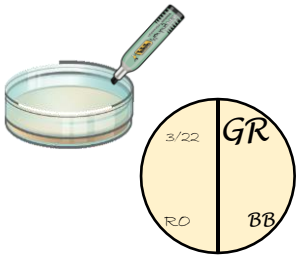
5. Return the screw-capped tube to the ice for 25-30 seconds.

6. Use the pipette to add growth media from the pink tube to the screw-capped tube.

7. Incubate your screw-capped tube at room temperature for about 5 minutes.

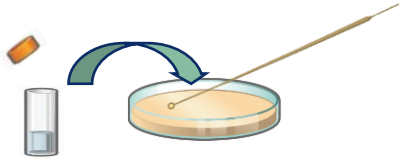
8. With a permanent marker, divide the bottom of the agar plate in half. Label each plate with today's **date** and the **code** for your screw-capped tube.

   Each person should label **one half** of the plate with **their initials.**



Code:
GR = green
YE = yellow
OR = orange

9. Using the sterile inoculating loop, **GENTLY** transfer the mixture from the tube onto the agar.
   You can make a squiggle or some other design, if you like.



10. The plates will be incubated at 31°C overnight to allow the bacteria to grow.

Supported in part by NSF/ATE grants: DUE #9454555; 9752027

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Transformation Results

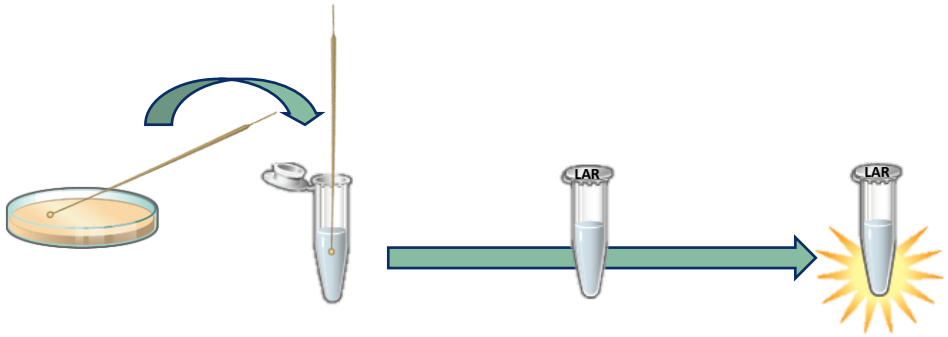
1. Each table should have:

   a) A 1.5ml microcentrifuge tube marked **LAR**, which contains 1mM luciferin.
   b) A plate of grown *E. coli* bacteria.

2. With the inoculating loop that was previously used, each student should sweep some bacteria from the plate and **CAREFULLY** add it to the LAR tube.

   Try to get the clump of bacteria to release from the loop into the LAR liquid by quickly stirring the loop within the tube.

3. Continue to harvest the bacteria until the tube is very cloudy with bacteria.
   The more bacteria, the brighter the light should be.



4. After each student has added some bacteria, vigorously shake the tube to break up the clumps of cells.

5. View the tube in a dark place to see the bioluminescence.

Note: *The disposable pipettes, inoculating loops, gloves, bacterial tubes, and plates used in this experiment should be disposed of appropriately in biohazardous waste.*

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