

BACTERIAL TRANSFORMATION LESSON PLAN

Primary Learning Outcomes: Understanding the process of bacterial genetic engineering through plasmid insertion.

High School Georgia Performance Standards

- SCSh2. Students will use standard safety practices for all classroom laboratory and field investigations.
 - SCSh3. Students will identify and investigate problems scientifically.
 - SCSh4. Students use tools and instruments for observing, measuring, and manipulating scientific equipment and materials.
 - SCSh6. Students will communicate scientific investigations and information clearly.
 - SCSh8. Students will understand important features of the process of scientific inquiry.
 - SB1. Students will analyze the nature of the relationships between structures and functions in living cells.
 - SB3. Students will derive the relationship between single-celled and multi-celled organisms and the increasing complexity of systems.
 - SB2. Students will analyze how biological traits are passed on to successive generations.
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Middle School Georgia Performance Standards

- S7CS2.* Students will use standard safety practices for all classroom laboratory and field investigations.
 - S7CS4.* Students use tools and instruments for observing, measuring, and manipulating scientific equipment and materials.
 - S7CS6.* Students will communicate scientific investigations and information clearly.
 - S7CS9.* Students will understand important features of the process of scientific inquiry.
 - S7L2.* Students will describe the structure and function of cells, tissues, organs, and organ systems.
 - S7L3.* Students will recognize how biological traits are passed on to successive generations.
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Materials:

<i>Kit provides:</i>	<i>Teacher provides:</i>
Microcentrifuge tubes	Ice
Biohazard bag	Microwave
Large-range micropipettors	Permanent markers
Tips for large-range micropipettors	Cotton swabs
Water bath, with rack for small tubes	Toothpicks
Small-range micropipettors	Box large enough to hold 13X9 disposable metal cooking pan
Tips for small-range micropipettors	14 Beakers for disposables and ice
Disposable Petri dishes (2 100 x 15 mm and sleeve of 60 x 15 mm)	
Racks for microcentrifuge tubes	
Plate of nontransformed <i>E. coli</i> cells and plate of GFP-transformed <i>E. coli</i>	
Premixed LB agar (250 ml)	
1 vial (25 mg) of ampicillin (AMP)	
GFP plasmid (80µl)	
7 Glass vials of 50 mM calcium chloride solution	
Oven mitts	
14 Disposable "bulb" pipettes	
37°C Incubator (heating pad, aluminum pan & wire rack)	
UV light	

Materials needed for a class of 28 students (7 groups of 4 students each):

- 14 microcentrifuge tubes
- 7 beakers of ice
- 7 fine-tipped permanent markers
- 7 large-range micropipettors
- Large-range micropipette tips

7 small-range micropipettors
Small-range micropipette tips
7 microcentrifuge tubes, prefilled with 500 μ l 50mM calcium chloride solution
Freshly streaked plate of *E. coli* cells
14 LB+Amp agar plates (prep directions follow)
7 small microcentrifuge tubes, prefilled with 10 μ l GFP plasmid each
42°C water bath, with small rack to hold microcentrifuge tubes
7 microcentrifuge tube racks
7 beakers for disposables
14 "bulb" pipettes
Heating pad
Oven mitts
Water bath

Duration of activity:

Prep time: Pouring plates, approximately 30 - 45 minutes
Plating *E. coli* and pre-filling tubes, approx. 30 minutes
Class time: Day 1, 1 class period of approximately 55 minutes
Day 2, approx. 20 - 30 minutes for observation and discussion

Advance preparation:

- You will need to prepare one large LB agar only plate for each class you plan to perform the lab with. These plates will contain the bacteria used for transformation. You will also need to prepare enough small LB+ AMP agar plates for the students to use. The LB agar is provided and should be microwaved to liquefy. Loosen cap on the bottle and heat on defrost or 50% power setting for a minute or two at a time. Repeatedly remove and swirl. This should take approximately 8 minutes. The bottle will be very hot! Use protective MITTS. When the agar is liquid, allow to cool (use a water bath for this). Dissolve the AMP in 250 μ l deionized water. When medium has cooled to about 50° C (the bottle will be cool enough to touch), the vial of AMP can be added, the media swirled to mix, and the plates poured. Prior to adding the AMP you should pour your LB agar plates.

- If you chose to pour the LB+ AMP agar plates prior to the day of the lab you must store them in the refrigerator and take out to warm before use.
- The day prior to the student experiment, plate *E. coli* bacteria onto a LB agar plate (*with no antibiotic added!*) and allow to grow overnight at 37°C. To do this place the heating pad (on medium heat) in an aluminum baking pan with the wire rack resting on the edges of the pan. Take a box and place this over the heating apparatus. Place a thermometer into the side of the box to monitor temperature. **IMPORTANT:** Bacterial cells are most receptive to transformation at approximately 24 hours.

Additional tips:

- Fill waterbath with distilled water and set at 42° C before class begins.
- **Students need much practice in micropipetting.** Allow for practice time before or during activity.
- The teacher should demonstrate procedure steps throughout.
- Bacteria will express the GFP trait best if grown at 37° C.
- Bacterial colonies will be larger if allowed to grow for more than one day.
- When observing colonies, first discuss why colonies are expected in the + plate but not in the - plate. Then, make the room as dark as possible. It may take a minute or two for students' eyes to adjust to the dark to see their "glow-in-the-dark" bacteria.
- The items contaminated by bacteria during the experiment (toothpicks, cotton swabs, etc.) should be placed in the orange biohazard bag, sealed, and returned to NESPAL for proper disposal.

- **UV lights should only be handled by the teacher.** Please take the batteries out of the lights after use. If the UV lights are not working a black lightbulb can substitute for them.

MAKING FLUORESCENT BACTERIA

The **GFP** gene is normally found in Jellyfish. In this experiment you will add this jellyfish gene to *E. coli* bacteria cells. Adding this new gene to *E. coli* gives them the ability to **fluoresce** in the dark! When the bacteria are **transformed**, they receive the jellyfish gene and express the jellyfish trait. This technique is called **genetic engineering** and is used to move genes from one organism to another.

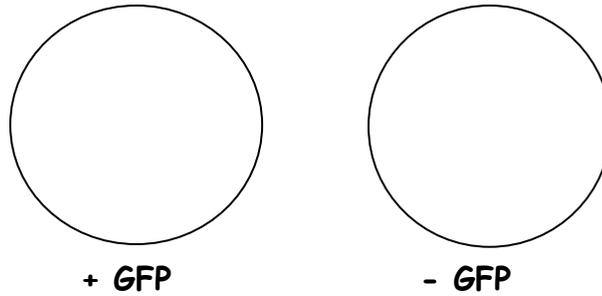
PROCEDURE

1. Obtain 2 (1.5 ml) microcentrifuge tubes.
2. Use a permanent marker to label one 1.5 ml microcentrifuge tube "+" and the other "-".
3. Use a large range (100 - 1000 μ l) micropipettor to add 250 μ l of calcium chloride (CaCl_2) solution to each tube.
4. Place the tubes in a beaker of ice.
5. Follow these steps to transfer *E. coli* cells from a petri plate to both tubes:
 - Gently scrape up 2 to 3 colonies with a tooth pick. Be careful not to dig at or take up any agar.
 - Carefully place the tooth pick into the calcium chloride solution and shake off the cells. You should see the small clump of cells on the bottom of the tube.
 - Use a new tooth pick to repeat the procedure for the other tube.
6. Using the plastic bulb pipettes, mix the cells in the tube by squeezing the bulb. Lower the tip into the mixture, and squeeze the bulb to move the liquid gently up and down several times. Use a new pipette for each tube. Your solution should look slightly cloudy. If it does not, scrape off more bacterial colonies.

7. Return the tubes to ice after mixing. Be sure that the solution in each tube is cloudy.
8. The teacher will use a small-range (2 - 20 μ l) micropipettor to add 10 μ l of GFP plasmid to the "+" tube only. Add it directly to the mixture.
9. Return the "+" tube to ice until the next step.
10. Heat shock the cells:
 - Carry the beaker of ice, with your tubes in it, to a water bath. Remove both tubes from the ice and immediately place them in a 42° C water bath for 90 seconds.
 - Immediately return the tubes to ice for 1 minute.
11. Place both tubes in the rack on your table to rest at room temperature.
12. Label 2 petri plates (on the bottom) -GFP and +GFP. Put your initials on each plate. (Write around the edges so that it will not interfere with viewing your bacterial cultures.)
13. Use a large-range micropipettor to transfer the bacterial cells onto the plates:
 - Transfer 250 μ l of the bacteria in the "-" tube onto the -GFP plate. (Lift the lid just enough to squirt the bacteria onto the plate---This is called "clamshelling".)
 - Transfer 250 μ l of the bacteria in the "+" tube onto the +GFP plate in the same manner as the - plate.
14. Spread the cells over the surface of the agar:
 - Clamshell the lid of one plate and carefully use the cotton swab to spread the liquid evenly on the agar.
 - Repeat the process with the other plate and a fresh cotton swab.
15. Allow the plates to sit upright until all the liquid soaks into the agar.

16. Once the liquid has soaked in, store the plates upside down in the heating-pad incubator and allow bacteria to grow at least overnight.

Sketch your predictions below:



Conclusion and Analysis

1. Did the results of the experiment match your predictions? Why or why not?

2. Why was there no growth on the - GFP plate?