Purpose

- To transform bacteria with a plasmid containing a gene of interest in order to produce a specific protein.

Big Idea

Transformation is the process by which exogenous, or foreign, DNA is taken into a cell. The plasmids used in transformation can be designed by researchers to confer different traits onto a cell. This simple yet powerful technique is often used:
- To make many copies of DNA (“DNA cloning”)
- To make large amounts of specific proteins such as insulin.
- To genetically modify a bacterium or cell

In this lab, we will be transforming Escherichia coli (E.coli) so that the bacteria will express a particular gene of interest located on the pFLO plasmid. This gene codes for a fluorescent protein found in marine coral. To help identify and isolate transformed cells, the plasmid also carries the gene for ampicillin resistance.

Station Inventory

- 50mM CaCl2 (cold)
- 1 container with ice
- disinfectant (10% bleach or 70% alcohol)
- 1 floating tube rack
- 1 LB agar / amp
- 1 LB agar plate
- micropipets and tips (P20, P50-200, P1000)
- 1 microtube rack
- 2 microtubes
- pFLO plasmid (0.01 ug/ul)
- 1 permanent marker
- sterile distilled water
- sterile Luria Broth (LB)
- 2 sterile plate spreaders
- sterile toothpicks
- 2 sterile transfer pipets
- waste container
Procedure
Make notes and observations in your lab notebook as you work through the procedure.

Part 1: Prepare Tubes
Purpose: Sterilize your workspace to reduce the chance of contamination. Set up your control and pFLO transformation.

1. Disinfect your work space by cleaning with 10% bleach or 70% alcohol. Wash your hands before doing any work.

2. Obtain 2 sterile 1.5 ml microtubes containing 250 µl of cold calcium chloride solution (CaCl₂).
   a. One microtube will be for the control. Label this tube “C”.
   b. The other microtube will be for pFLO transformation. Label this tube “pFLO”.
   c. Label both tubes with your team name and date.
   d. Place both tubes on ice.

Part 2: Add Bacteria to Tubes
Purpose: Each colony on the stock plate originates from a single “mother” cell. Selecting a single colony ensures that all the bacteria you’ll be growing and transforming are genetically alike.

3. Carefully remove a sterile toothpick from the container.

4. Using the toothpick, gently scrape up a large, single bacterial colony from the stock plate.

5. Insert the toothpick into the control tube and vigorously tap or twirl the toothpick against the side of the tube.
   a. Check that there is a clump of cells in the solution.
   b. Use a sterile transfer pipet to suspend the cells by pipetting repeatedly. Check that there are no clumps visible.
   c. Return the tube to ice and discard the toothpick and transfer pipet.

6. Using a new toothpick, transfer another colony to the pFLO tube just as you did for the control.
   a. Check that there is a clump of cells in the solution.
   b. Use a sterile transfer pipet to suspend the cells by pipetting repeatedly. Check that there are no clumps visible.
   c. Return the pFLO tube to ice and discard the toothpick and transfer pipet.

Part 3: Add Plasmid to Bacteria
Purpose: Transform bacteria with the pFLO plasmid to express antibiotic resistance and the gene of interest.

7. Using a 2-20 µl micropipet and a new tip, add 10 µl pFLO plasmid to only the pFLO tube.
   a. Once added, mix by closing the tube and tapping or flicking the tube with your fingers.
   b. Return the tube to ice.
8. Using a new tip, add 10 µl of sterile water to the control tube.
   a. Once added, mix by closing the tube and tapping or flicking the tube with your fingers.
   b. Return the tube to ice.
9. **Wait 15 minutes.** This gives time for the pFLO plasmid to settle onto the surfaces of the bacteria. While you’re waiting, think ahead about the next two steps. The timing of these steps is important to the success of the procedure. **During this time answer the questions on the Data & Analysis sheet.**

### Part 4: Heat shock

*Purpose:* The CaCl₂ and heat shock alter the cell membrane of the bacteria so that foreign DNA can pass through more easily. **These cells are called “competent”. This is an essential step for successful transformation.**

10. When the 15-minute waiting period is over, take the ice container with the pFLO and control tubes to the 42°C water bath (check the temperature).
11. Transfer the tubes into a floating tube rack.
12. It is essential that cells are given a sharp and distinct heat shock.
   a. Place the rack with the tubes in the water bath for **exactly 90 seconds.**
   b. Return the tubes to ice immediately after the water bath. Wait at least **2 minutes.**
13. Using a P1000 micropipet and sterile tip, add 250 µl of sterile Luria Broth (LB) to each tube. Tap each tube with your finger to mix its contents.

### Part 5: Plating Cells

*Purpose:* To spread individual cells evenly over the surface of the plate resulting in visible colonies after incubation.

14. Label plates:
   a. Obtain 1 LB plate and 1 LB/amp plate.
   b. Handle the plates carefully so that they remain sterile while you label them.
   c. Turn over the plate and draw a line down the middle of each plate with a permanent marker. Label one side “pFLO” and another side “C” for control.
   d. Then, write your team name, class period, and date on the bottom of both plates (see Figure 3).
15. In this lab, we are using a technique in which you will plate your control and pFLO bacteria on the same plate (see Figure 4). By drawing a line on the back of the plate and growing your experiment and control on the different halves you can compare results and minimize any variables. Be careful not to mix the control and pFLO bacteria when plating. **Do not cross the center line on the plates!**
16. Plate the “C” or control side of both plates.
   a. Set a P50 (or P100/200) micropipet to 50 µl.
   b. Suspend the bacteria in tube C by pipetting up and down.
   c. Carefully drip 50 µl on the “C-side” of both your plates.
   d. Using a cell spreader, evenly spread the cell suspension on the “C-side” of both plates. Do not cross the middle line.

17. Plate the “pFLO” side of both plates.
   a. Using a new tip, suspend the bacteria in tube pFLO by pipetting up and down.
   b. Carefully drip 50 µl on the “pFLO-side” of both your plates. Make sure that you do not cross the middle line.
   c. Using a new cell spreader, evenly spread the cell suspension on the “pFLO-side” of both plates. Do not cross the middle line.
   d. Do not dispose of the cell spreaders! Place the cell spreaders in a container of disinfectant.

18. Allow 5 minutes for the plates’ surfaces to absorb the cell suspensions.

19. Place your plates upside-down and incubate at 37°C overnight.

20. Make predictions about what you expect to happen on each side of each plate.

Clean Up
- Dispose of all waste materials, tips, and tubes in the trash.
- Disinfect your work surfaces and micropipets.
- Wash your hands.