**Colorful World\_Workflow**

**Advance preparation**

*TEACHERS: You will be receiving two strains of bacteria to transform. These strains will consist of colonies of each strain on a petri dish. To obtain sufficient amounts of bacteria for a class to conduct transformation, you will need to replate these colonies as patches. Each patch will provide sufficient bacteria for 1 lab group and up to 6 patches will fit comfortably on one plate. This process can be done by the student or the teacher* ***up to a week prior to the transformation.*** *You may wish to conduct this procedure yourself. However, if time allows, the students will enjoy learning these microbiological techniques.*

1. Using a sterile inoculating loop or toothpick, transfer a bacterial colony from one of the petri dishes to a new LB agar petri dish, drawing a 1 cm x 1 cm square of each strain. Each square you draw this way will yield enough cells to transform with 2 plasmids.
2. Repeat for each strain you will need for the transformation lab.
3. Place petri dishes in the incubator at 37°C overnight.

*TEACHERS: On the day that the students transform the cells, you will need to complete the transformation buffer by adding 50 ml of bottled spring water to the 0.73 g CaCl2 to make a 0.1M solution. If the CaCl2 has arrived in solution already then you only need to pre-chill the tube in the fridge or on ice.*

*You could also choose to aliquot the 0.1M CaCl2 solution since each group will need only 0.4 ml of it. We recommend giving the students a 500μL aliquot to account for pipet variations. In all cases, you’ll want to chill the CaCl2 in the fridge or on ice.*

**Annotated Laboratory Procedure***TEACHERS: Part 1 of this procedure can easily be done in one lab period and the petri dishes allowed to incubate overnight. Part 2 can be completed the next day. Since precision is required for optimal transformation, you may want to have the students complete a practice session with blank plates and using water instead of buffer and DNA. This will allow the students to practice the timing and pipeting techniques that are required.*

**Part 1: Preparing Strain 4-1 and 4-2 for transformation**

Neither of these E. coli strains will take up DNA from the environment until they are treated with a salt solution that makes their outer membrane slightly porous. The cells will become "competent" for transformation (i.e. ready to bring DNA that's external to the cell into the cytoplasm where the DNA code can be expressed). The cells will also become fragile. Keep the cells cold and don't pipet them roughly once you have swirled them into the CaCl2 transformation solution.

*TEACHERS: You should emphasize that this procedure can be tough on the cells. The students should be gentle and work quickly.**TEACHERS: It is essential that the tubes be kept on ice, preferably crushed.*

1. In advance of lab today, a small patch of each strain was grown for you on an LB agar petri dish. [Strain 4-1](http://www.neb.com/nebecomm/products/productE4104.asp) is a K-12 type of E. coli. [Strain 4-2](http://www.neb.com/nebecomm/products/faqproductC2523.asp#1132) is a B-type strain.
2. Label 2 small eppendorf tubes either "4-1" or "4-2".
3. Pipet 200 ul of CaCl2 transformation solution into each eppendorf and then place the tubes on ice.
4. Use a sterile wooden dowel or inoculating loop to scrape up one entire patch of cells (NOT including the agar that they're growing on!) labeled "4-1," and then swirl the cells into its tube of cold CaCl2. A small bit of agar can get transferred without consequence to your experiment, but remember you're trying to move the cells to the CaCl2, not the media they're growing on. If you have a vortex, you can resuspend the cells by vortexing for one minute. If no vortex is available, gently flick and invert the eppendorf tube.
5. Repeat, using a different sterile wooden dowel to scrape up the patch of cells labeled "4-2." Vortex briefly if possible. It's OK for some clumps of cells to remain in this solution.
6. Keep these competent cells on ice while you prepare the DNA for transformation.

**Part 2: Transforming Strains 4-1 and 4-2 with pPRL and pGRN**

The cells you've prepared will be enough to complete a total of 6 transformations. You will transform the purple-color generator into each strain, and also the green-color generator into each strain. You will also use the last bit of competent cells as negative controls for the transformation.

*TEACHERS: It is important that the students correctly label each tube and plate.*

1. Retrieve 2 aliquots (in eppendorf tubes) of each plasmid for a total of 4 samples (2x [pPRL,](http://openwetware.org/images/9/97/PGRN%28pSB1A2_BBa_K274004%29.ape) 2x [pGRN](http://openwetware.org/images/9/97/PGRN%28pSB1A2_BBa_K274004%29.ape)). Each aliquot has 5 ul of DNA in it. The DNA is at a concentration of 0.04 ug/ul. You will need these values when you calculate the transformation efficiency at the end of this experiment.
2. Label one of the pPRL tubes "4-1." Label the other pPRL tube "4-2." Be sure that the labels are readable. Place the tubes in the ice bucket.
3. Label one of the pGRN tubes "4-1." Label the other pGRN tube "4-2." Be sure that the labels are readable. Place the tubes in the ice bucket.
4. Flick the tube with the competent 4-1 strain and then pipet 100 ul of the bacteria into the tube labeled "pPRL, 4-1" and an additional 100 ul into the tube labeled "pGRN, 4-1." Flick to mix the tubes and return them to the ice. Save the remaining small volume of the 4-1 strain on ice.
5. Flick the tube with the competent 4-2 strain and then pipet 100 ul into the tube labeled "pPRL, 4-2" and an additional 100 ul into the tube labeled "pGRN, 4-2." Flick to mix and store them, as well as the remaining volume of competent cells, on ice.
6. Let the DNA and the cells sit on ice for 5 minutes. Use a timer to count-down the time.
7. While your DNA and cells are incubating, you can label the bottoms (not the tops) of the 6 petri dishes you'll need. The label should indicate the strain you've used ("4-1" or "4-2") and the DNA you've transformed them with ("pPRL," "pGRN," or "no DNA control").
8. Heat shock all of your DNA/cell samples by placing the tubes at 42° for 90 seconds exactly (use a timer). This step helps drive the DNA into the cells and closes the porous bacterial membranes of the bacteria.
9. At the end of the 90 seconds, move the tubes to a rack at room temperature.
10. Add 0.5 ml of room temperature LB to the tubes. Close the caps, and invert the tubes to mix the contents.
11. Using a sterilized spreader or sterile beads, spread 200-250 ul of the transformation mixes onto the surface of LB+ampicillin agar petri dishes.
12. Cover the plate and set aside for a minute. Then, turn the plate over. The plates will be stored upside down to prevent condensation from dripping onto the bacteria.
13. If desired the remaining volumes of transformation mixes can be plated on LB plates without ampicillin to show the effect of antibiotic selection on the outcome.
14. Incubate the petri dishes with the agar side up at 37° overnight, not more than 24 hours.

*TEACHERS:* [*This video*](http://youtu.be/VN28WysDzXc) *illustrates the use of a sterilized spreader as well as sterile beads. Packaged sterile spreaders could also be used and do not have to be sterilized with alcohol before spreading the cells, but do have to be changed between samples.*