Lab 4: What a Colorful World

Teacher Considerations

This lab provides a streamlined and effective alternative to more common transformation protocols. The engineering concept of chassis gives this lab a context that takes it beyond a simple exploration of the technique. Using the protocol presented below, we generally observe strain 4-1 produces large, light green colonies and dark purple colonies. The 4-2 strain usually produces dark, small green colonies and no purple colonies. We generally observe transformation efficiencies around 1*10^3 colonies/microgram of DNA. However, variations on the protocol, such as incubation at room temperature, may produce different results. We encourage you to experiment and report your results back to us. BioBuilder's "share your data" page will allow you to compare your data to the data of other BioBuilders around the country. If you find a great way to teach this or a follow-up experiment that other teachers could benefit from, please consider posting it to the teacher's discussion page.

To achieve high transformation efficiency and clear color differences, it is important that students be precise when conducting the transformation protocol. For instance, a water bath in excess of 42°C or leaving the cells in the bath for more than 90 seconds may damage the cells and adversely affect the transformation efficiency. If time allows, it may be worth having the students practice the procedure using water samples the day before the actual lab. The patches that you prepare for the students to scrape into "TB+DMSO" should not be more than a few days old since the transformation efficiency drops as the cell age.

An introductory power point for this lab can be found here.

Needed Materials

Teacher Provides

- Petri dishes ("plates") containing LB (Luria Broth) agar medium (can be sent with kit as needed)
- Petri dishes ("plates") containing LB (Luria Broth) and ampicillin agar medium (can be sent with kit as needed)
- Luria Broth (can be sent with kit as needed)
- water bath
- alcohol or bunsen burners and spreading rods, or sterile beads to plate cells on petri dishes
- innoculating loops or sterile toothpicks
- Pipetmen and tips (P1000, P200, P20)
- Pipets (10 ml and 5 ml) and bulbs
- Timers or stopwatches
- Sharpies
- Nitrile or Latex gloves
- ice
- distilled water
- eppendorf tubes
- incubator @37^{o}C (cells can be grown at room temperature but grow more slowly)

Kit Provides

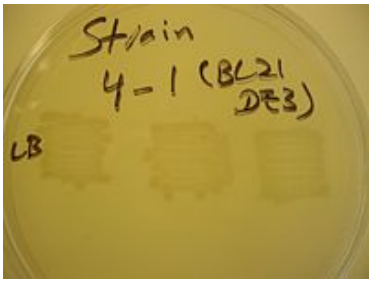
- Plate containing colonies of strains 4-1 and 4-2. Can be stored for one or two weeks at 4^{o}C until ready to create patches.
- pPRL and pGRN plasmids in 5µL aliquots. Store in freezer until ready for use.
- Transformation buffer. Store at room temperature until ready for use.
- DMSO. Store at room temperature until ready for use.
- sterile wooden dowels

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.

These instructions are not included on the student site but a video of this procedure is here.



LB agar petri dish showing 3 patches of bacterial strain "4-1." This petri dish would provide enough cells for 3 students (or group of students) to transform 2 plasmids

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. A video of this procedure is here.
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 will transform the cells, you will need to complete the transformation buffer by adding DMSO to a final concentration of 10%. Each group will need at least 0.4 ml of TB+DMSO. We recommend making 500µL to account for pipet variations. To make a TB+DMSO solution, add 50µL of DMSO to 450 µL of TB per each group, flick to mix and chill in fridge or on ice.

BioBuilding: Synthetic Biology for Teachers: What a Colorful World

Annotated Laboratory Procedure

TEACHERS: These are the same instructions as included on the student site but with special notes for the teacher.

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 "TB+DMSO" salt 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. A video of this procedure is here. Strain 4-1 is a K-12 type of E. coli. Strain 4-2 is a B-type strain.
2. Label 2 small eppendorf tubes either "4-1" or "4-2".
3. Pipet 200 ul of "TB+DMSO" transformation solution into each eppendorf and then place the tubes on ice.
4. Use a sterile wooden dowel 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 "TB+DMSO." A small bit of agar can get transferred without consequence to your experiment, but remember you're trying to move the cells to the "TB+DMSO," 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.

A video of this procedure is here.

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, 2x pGRN). 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 75 ul of the bacteria into the tube labeled "pPRL, 4-1" and an additional 75 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 75 ul into the tube labeled "pPRL, 4-2" and an additional 75 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 250 ul of the transformation mixes onto the surface of LB+ampicillin agar petri dishes. A video of this procedure is here.
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 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.

TEACHERS: Clean-up instructions. Provide containers at each work stations for student biological waste such as pipet tips, eppendorf tubes, spreaders, innoculating loops, and plates. Be sure to follow hazardous waste procedures as set forth by your school or municipality. Generally, it is safe to soak the material in each container with a 10% bleach solution for 2 hours. Materials can then be discarded into the regular trash. You can find more information about microbiology lab safety here

Next day

In your lab notebook, you will need to construct a data table as shown below. These may be provided. Also be sure to share your data with the BioBuilder community here.

Strain	Plasmid	Colony Number on LB (if used)	Colony Number on LB + Amp	Transformation Efficiency (colonies/microgram DNA)	Color/shape/size on LB (if used)	Color/shape/size on LB + Amp
4-1	no DNA					
	pPRL					
	pGRN					
4-2	no DNA					
	pPRL					
	pGRN					

1. Count the number of colonies growing on each petri dish.
    - Small white colonies that are growing around the perimeter of larger colored colonies are called "satellites." They should not be counted. They grow near the central colony only after the cells there have inactivated the ampicillin that's in the petri dish agar.
    - You can feel most confident in your results if there are between 20 and 200 colonies on the petri dish. Fewer than 20 and your value is affected by errors in pipeting that make large percentage differences in the outcome. Greater than 200 colonies and they become hard to count reliably. If the petri dish has many colonies growing on it, try to divide the dish into pie sections (1/4th or 1/8ths or even 1/16ths of the area), and then count a representative area. Finally, multiply the number you get for the section to get your total number of colonies. You'll still have some counting error, but perhaps less.
    - Based on the number of colonies you find on each petri dish, calculate the transformation efficiency for each. Transformation efficiency is a measure for how well the cells incorporated the DNA. The units for transformation efficiency are "colonies per microgram of DNA." Each transformation used 200 nanograms (=0.2 micrograms) of DNA and you plated only 1/2 the transformation mixes on the petri dishes.
2. Record the color of the colonies you see.
    - Based on these observations, do the DNA programs seems to be behaving identically in both strains for E. coli? For example, does the pPRL plasmid give the same number of transformants and the same color in both strains? What about the pGRN plasmid? If you see differences, how can you explain them? How could you test your explanations?

Calculations

Here is a sample calculation for transformation efficiency
Data:

- 100 colonies on a petri dish
- 0.2 micrograms of DNA used
- 1/2 of the transformation mix plated
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Calculation:

- 100 x 2 = 200 colonies if all were plated
- 200 colonies/0.2 micrograms of DNA = 1*10^3 colonies/microgram of DNA = transformation efficiency

Sample Data

Strain	Plasmid	Colony Number on LB (if used)	Colony Number on LB + Amp	Transformation Efficiency (colonies/microgram DNA)	Color/shape/size on LB (if used)	Color/shape/size on LB + Amp
4-1	no DNA	lawn	none	NA	white/cream-colored	NA
	pPRL	lawn	500	~10^3	white/cream-colored	Purple with satellites/round with crinkled edges/medium sized colonies
	pGRN	lawn	500	~10^3	white/cream-colored	Medium green with satellites, darker in the center/round with crinkled edges/medium sided
4-2	no DNA	lawn	none	NA	white/cream-colored	NA
	pPRL	lawn	none	0	white/cream-colored	NA
	pGRN	lawn	~100	10^2-10^3	white/cream-colored	Dark green with satellites/round /small

Key teaching points

1. ask the students to share their data and learn from others who have also run this experiment, using the BioBuilder "share your data" link that's here.
2. presence of ampicillin selection allows only cells with plasmids to grow (and the rare transformed cell from the non-selective plates cannot be seen)
3. pGRN does not behave the same in two strains. Explanation may be lon protease that is naturally missing in B-type strains such at strain 4-2. This may allow for greater concentration of protein to accumulate in 4-2 than 4-1.
4. pPRL is not tolerated in B-type strain and so no colonies are found when transformed into strain 4-2. Students are likely to think they've made a mistake but in fact, the lack of colonies may actually be due to higher expression level in this strain than in the K-type strain like 4-1. If these plates are allowed to incubate for longer, then white colonies may appear. These are cells that have either mutated so presumably no longer express this protein-generating device or may be cells that are growing because of

degradation of the ampicillin in the plates.

5. The transformation efficiency is generally higher into strain 4-1 than strain 4-2. This may be due to the clumpiness of 4-2 when it is resuspended in TB. The students can best see this difference by comparing the pGRN numbers into 4-1 and 4-2. Since the same DNA was used into both strains, the different # of colonies is a reflection of strain differences. This should also tip them off that the difference seen with pPRL is not just a "mistake"--and if you have several teams of students do this experiment, they can't all have made that "mistake."

Assessment

Lab Report Rubric
Download doc or pdf

Lab Report ScoreSheet
Download doc or pdf

Survey
To help us improve the labs, you can

1. send the students here where they can offer anonymous feedback.
2. "join a discussion" from the BioBuilder homepage
3. email us: "info AT biobuilder DOT org"

Thanks!

Variations to try

1. Can try the experiment using KanR versions of the plasmids rather than Amp. These have the advantage of giving rise to no satellite colonies. They have the disadvantage that they require a 30' outgrowth time at 37° after heat shock and before plating. If you would like to try the KanR versions of these color-generating plasmids, request DNA from strains NB442 and NB443.

Feedback
We're always looking to hear back from you if you've thought about this unit, tried it, or stumbled across it and want to know more. Please email us through BioBuilder, info AT biobuilder DOT org.