Lab 4: What a Colorful World

Simplifying assumptions about "the cell" are brought into question when different strains are transformed with DNA that makes them grow in colorful ways.

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Objectives

By the conclusion of this laboratory investigation, the student will be able to:

- Define and properly use synthetic biology terms: chassis, system, device, minimal cell, sensor, color generator.
- Define and properly use molecular genetics terms: operon, gene expression, bacterial transformation.
- Explain the role of chassis in synthetic biology and engineering.
- Conduct and interpret the results of a bacterial transformation.

Introduction

One potential use of engineered bacteria is as indicator of toxic substances. Bacterial sensing systems have been designed for arsenic and lead. Bacteria are cheap and easy to produce and store. This reduces the need for expensive and technologically complex chemical tests. The bacteria are also much more sensitive to the toxin levels. However, there is one potential drawback. The bacteria respond to the toxin metabolically. This means we may be able to detect a change in pH or other indicator of metabolism. This requires further equipment such a pH indicator. Sensors have been linked by synthetic biologists to other forms of output such as the green fluorescent protein. However, this also requires further equipment such as a fluorescent light. This reduces the practicality in impoverished areas of the world, the very areas most at risk for arsenic or lead contamination.

The 2009 Cambridge iGEM team took up the challenge to design an indicator that could be used without additional technology. They designed color generator devices that could be linked to sensors. *E. coli* are naturally colorless, but other bacteria make pigments and so do appear colored. The iGEM team designed “e chromi,” engineered *E. coli* capable of producing colors through the synthesis of pigments. One pigment they used is Violacein, a pigment produced by a handful of genes originally found in *Chromobacterium violacein*. These genes were re-engineered and combined to produce purple and green in *E. coli*. The violacein operon consists of

five genes which metabolize L-tyrosine. Expression of all five genes will produce a purple pigment. However, removal of the third gene in the sequence will cause the cell to metabolize the L-tyrosine into a green pigment. These pigments are easily visible to the naked eye. This device could be linked to a biosensor for a toxin and the bacteria will turn color in response to the toxin concentration.

It's reasonable to wonder: Why didn't the team just use the *Chromobacterium*? Synthetic biologists like to use *E. coli* because it is well understood and easy and safe (if proper strains are used) to work with. But it's important to realize that this was a choice! Synthetic biologists refer to the host cell as the chassis, and just as you'd carefully design a genetic program to encode, you'd also need to carefully choose the chassis that will run it. For an engineered genetic system to function in a chassis, the chassis must supply the cell with energy, materials for protein synthesis and materials those proteins will use when they function. The chassis will take care of all the material needs to meet the engineer's specifications. The better the chassis is understood, and the better it can provide materials for the engineered system, the better the results. By primarily using one chassis, synthetic biologists are managing complexity. A standard chassis allows engineers from many labs across the world to compare results.

Note how we also manage complexity in our everyday life. When we buy bananas or bell peppers, we simply call them bananas or bell peppers. In actuality, many varieties get mixed together in the store. But is it really important that we are aware of this when we shop? As long as the taste is similar, does it matter what variety of peppers you use? Cars, however, are a different story. A car is a highly engineered system of interconnected parts. While many of these parts are similar, they must be tailored to the size and function of the car. So, while the chassis of a truck, a GTO muscle car and a Toyota hybrid are different, so are many of the internal parts that make up the engine and the drive train. We might be able to move a radio from a truck chassis to a sports car chassis, but not much else. The car manufacturers are comfortable with this complexity and it has little effect on the user of the car.
What about your computer? You can think of your computer and its operating system as a chassis, making Macs and PCs different chassis (though in computer lingo they are known as platforms). There was a time in the past when word processing files written on one platform could not be viewed or edited on the other. But interoperability was clearly needed and so the computer companies have agreed on certain standards. Through re-engineering of the programs and the chassis/platforms, users no longer get lost in the complexity. Thankfully, files written on one platform can be viewed and edited on the other.

Synthetic biologist George Church is working to further remove the complexity from engineered systems by creating what are known as minimal cells. The idea is to design a cell that contains just the minimum genome to maintain its existence. These

cells will only be able to survive on special media and all of their metabolic functions will be well characterized. Another example of research into this idea was published by Craig Venter in May of 2010. His lab replaced the genome of a bacterial cell with a fully synthesized genome and were able to produce bacteria that expressed the synthetic genome. As appealling as these chassis are for synthetic biology, the work has a way to go before they can be in general use.

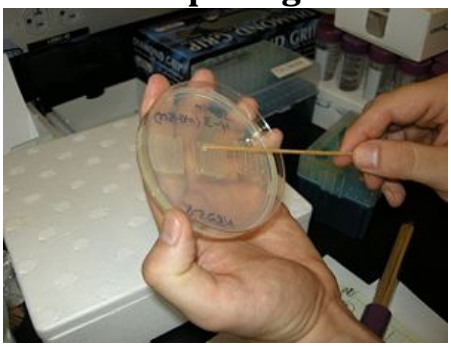
So, until minimal cells or synthetic cells are a viable option, researchers continue to use E coli and other domesticated cells as chassis for experiments. Mostly, the strains of E. coli that are used in research labs are one of two kinds. One strain is known as K-12 and the other B. Both strains are known to be safe and have been effectively used for genetic experiments for almost 100 years. The differences between these strains seem to be minor. Most are related to metabolism and none would seem likely to affect the color generator system. You can read about the interesting history of these strains here.

So now imagine that a group of engineers is manufacturing an arsenic sensor in *E. coli*. This group would like the intensity of purple color to vary as a function of arsenic level. Now imagine that a second group of engineers are also doing this but they use a different strain of *E. coli*. How sure can we be that the pigment will be expressed the same in a different chassis? Thinking back to our analogy with car chassis: would an engineer put a V-8 engine from a Lexus into a Mercedes chassis? Would the engine behave the same? Would the car?

In this lab you will transform bacteria from two different strains of *E. coli*, in other words, two different chassis. Strain 4-1 is a K-12 strain, while strain 4-2 is a B-type strain. Into each strain you will insert plasmids containing violacein-pigment devices. One plasmid, pPRL, has the purple version of this device while the other plasmid, pGRN, has the dark green version. Otherwise, the plasmids are the same. Can we expect the devices to behave the same in each strain or will the chassis have an effect on the intensity of color produced?

Procedure

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 $CaCl_2$ salt solution.

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 $CaCl_2$ 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 $CaCl_2$. A small bit of agar can get transferred without consequence to your experiment, but remember you're trying to move the cells to the $CaCl_2$, not the media they're growing on. If you have a vortex, you can resuspend the cells by vortexing very briefly. If no vortex is available, gently flick and invert the eppendorf tube, then return it to your icebucket.
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.

1. Retrieve 2 aliquots 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 the procedure is here.
12. If desired the remaining volumes of transformation mixes can be plated on LB plates to show the effect of antibiotic selection on the outcome.
13. Incubate the petri dishes with the agar side up at 37° overnight, not more than 24 hours.

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

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

Lab Report

I. Introduction

- Provide a brief introduction describing the field of synthetic biology.
- What is a color generator? How does this color generator work? How might a color generator be useful?
- Briefly describe the purpose of the lab. What are we trying to do here? Presume that a reader of your lab report has not read the assignment.
- What is the role of the chassis?
- How does chassis effect the expression of a genetic system?
- How might synthetic engineers modify the relation between a chassis and an engineered genetic system to reduce the chassis effect on the system?
- Why is it important to engineer a minimal or synthetic cell?
- What are the advantages/concerns of engineering a minimal cell?
- How might we test for the differences in the chassis that may be affecting a genetic system? You may find helpful information here and here.

II. Methods

- You do not have to rewrite the procedure.
- Explain why you did each step of the protocol.

III. Results

- Present the data tables in clear format.
- Present drawings of each slide.
- Describe the results: Describe the appearance of each plate. Are the colors different? Are the colonies different in number, size and/or shape? What was the transformation efficiency for each plate? Does it differ between the strains?

IV. Discussion

- Draw a conclusion: Do the color generators produce the same results in different chassis? Justify your answer.
- Analyze the data: Be sure to discuss how each part of the experiment and results adds to your conclusion.
- Are we sure that the transformation worked? What do the controls that lacked plasmid tell us?
- Discuss errors and other reasons for data variability.
- Use your results to explain why it is important for synthetic biologists to fully characterize the chassis used in an engineered system.