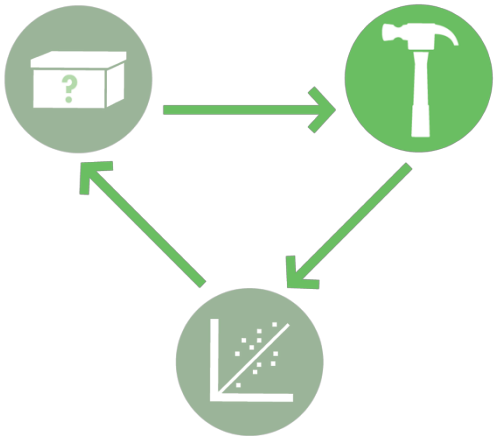
STUDENT MANUAL

BioBuilder

Synthetic Biology for Students

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



BioBuilder

What A Colorful World

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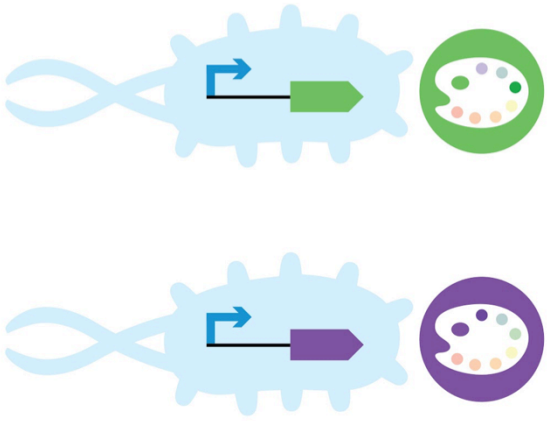
ABOUT WHAT A COLORFUL WORLD

Synthetic biologists often use lab strains of E. coli because they are well understood, easy to grow and generally safe to work with, but it's important to realize that using E. coli as a host cell to run our designed genetic programs is a choice. This BioBuilder activity is a reminder that, just as you carefully your design for a genetic program, you also need to carefully choose the host cell, or "chassis," that will run it.

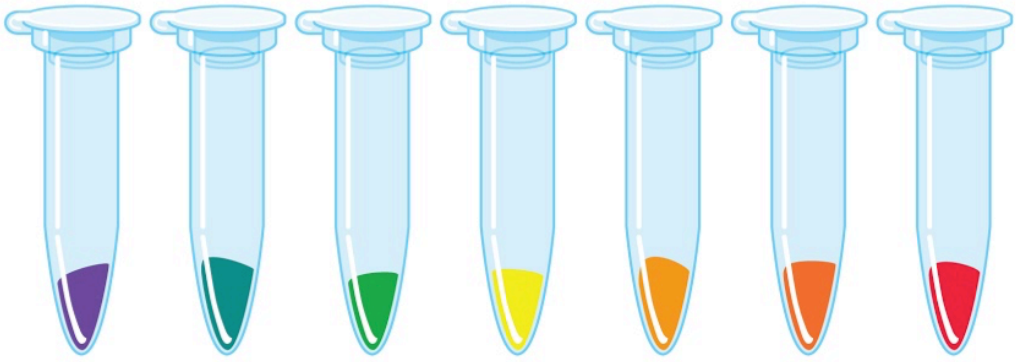


In this lab, you will build several color-generating systems to explore how the chassis affects the output of a designed genetic program. Because the colors are visible to the naked eye, you can easily decide whether the color outputs are different between the chassis.

The DNA programs that generate purple or green pigments have already been written and assembled for you, but you will complete the final building step by inserting the DNA that encodes the programs into a few different bacterial chassis.

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?

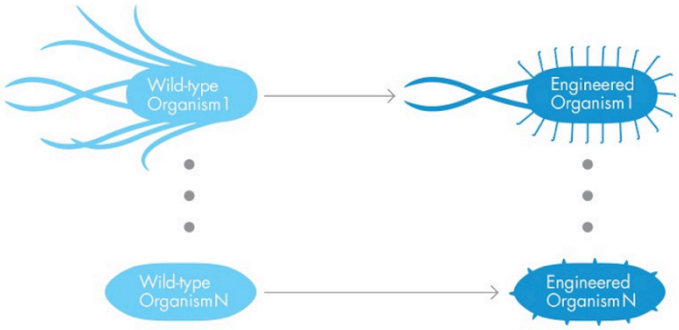
The DNA programs come from a 2009 International Genetically Engineered Machines - iGEM project called, "E. chromi," in which students from the University of Cambridge designed and engineered E. coli to produce a spectrum of pigments.



INTRODUCTION TO CHASSIS DESIGN

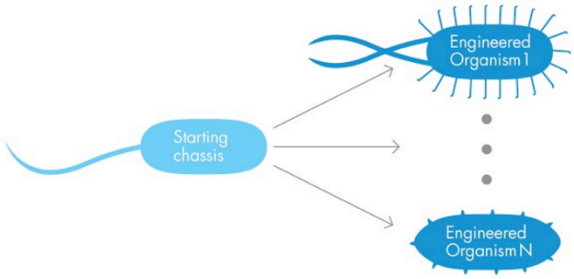
Just as a car manufacturer must take into account the entire car when designing an engine, synthetic biologists must consider the entire system they are building, including the cellular environment itself. And in the same way that there is a huge variety of cars on the road, cells vary dramatically in their size, shape, organelles, and basic metabolic functions. Consequently, choosing the best host cell, or chassis, for any engineered genetic program is an important step in the design process.

Nearest Neighbor vs Standard Chassis



In this approach, a chassis is identified from nature based on its ability to do something similar to the task a synthetic biologist has in mind for a new system.

For example, the bacterium Ralstonia eutropha naturally converts carbon dioxide into energy-storing polymers. This makes the chassis attractive to engineers who want to generate energy-storing molecules like biofuels.

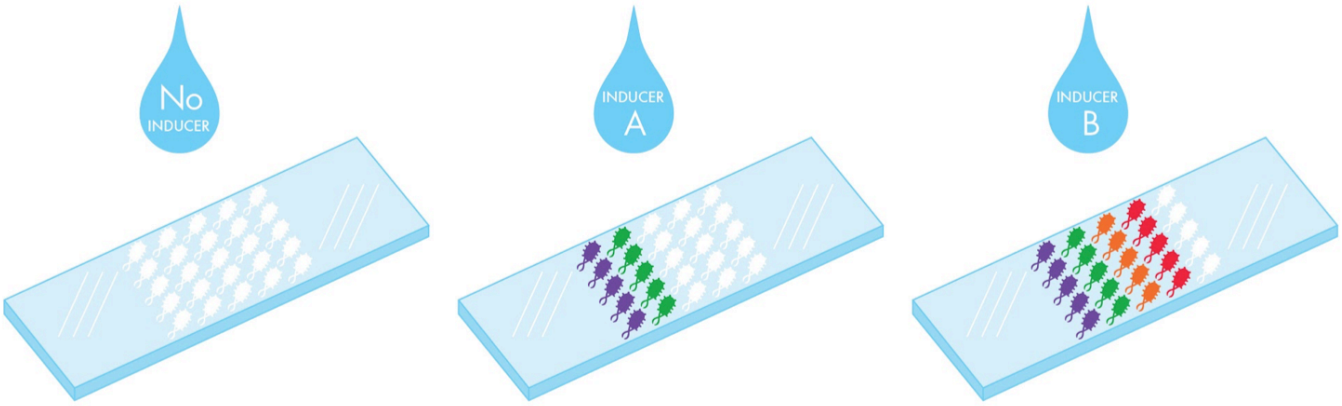


In this approach, a more generic chassis is chosen because it either has a minimal number of natural components or it is well understood and highly engineerable.

*Some research groups are using engineered E. coli to make biofuels.. The researchers see E. coli as an attractive platform for **many** purposes, including the conversion of carbon dioxide and electrical energy into isooctane, even though this reaction is unlike anything else these cells normally do.*

ENGINEERING A LIVING BIOSENSOR

In 2009, the University of Cambridge iGEM team designed, specified, and built a set of biosensors that the team called "E. chromi." The system was designed to detect heavy metals such as arsenic and mercury. These metals pose significant health risks and contaminate many waterways around the world. The iGEM team wanted their engineered biosensor to report both the identity and the concentration of several metals in a water sample. The presence of metals would trigger one or more color outputs. Because the colors were pigment-based, the readout of their system was visible to the naked eye, alleviating the need for specialized equipment and thus making it easy to use.



Pigment Production

Given the wide variety of natural colors found in the living world (orange carrots, purple flowers and so on), the iGEM team has plenty of sources for their color generating devices. Whenever possible, they focused on genetic pathways that could be modified to produce more than one color. Carotenoids, for example, are a family of pigments that are structurally related and so can be modified to appear yellow, red or orange.

PIGMENT FAMILY	COLOR(S) PRODUCED	BACTERIAL SOURCE
Violacein	Green and purple	*Chromobacterium violacein*
Carotenoids	Red and orange	*Pantoea ananatis*
Melanin	Brown	*Rhizobium etli*

Your experiment will focus on the color-generating devices in the Violacein pigment family. These genetic devices can change the color of cells so they appear purple or green.

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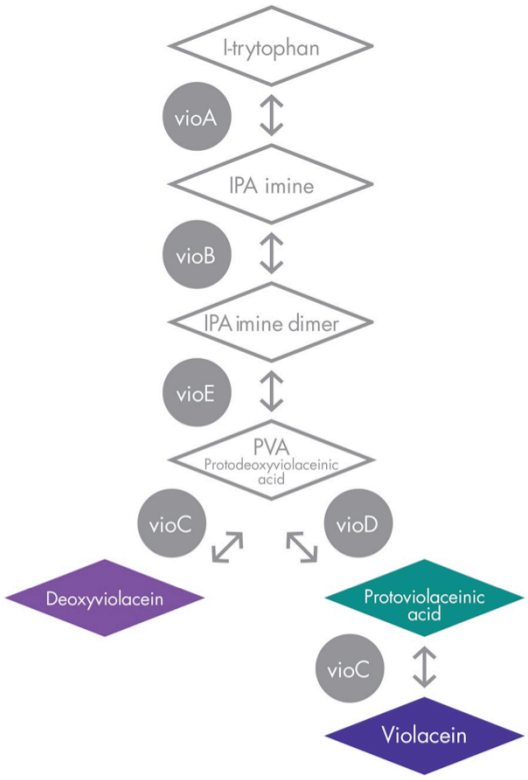
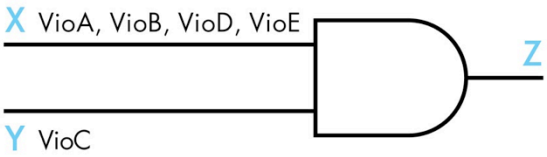
CHASSIS SELECTION CHALLENGE

When the violacein family of pigments are produced, the cells can appear purple or green, depending on a relatively small genetic change. Normally the genetic pathway uses five enzymes (VioA, VioB, VioC, VioD and VioE) converts an abundant and natural compound, tryptophan, to produce violacein, a purple pigment. When the VioC gene is removed, however, the pathway branch is blocked and the final conversion to violacein can't occur. In this case, the system's endpoint becomes proto-violacein, which is a green pigment.





INPUT X	INPUT Y	OUTPUT Z
0	0	0
1	0	Green
0	1	0
1	1	Purple

A logic gate and truth table can be used to summarize system's behavior. Both show that VioA, VioB, VioD and VioE are necessary for generating the green pigment and that the addition of the VioC gene gives rise to the purple pigment output.

Emergent Behavior

In building their system, the iGEM team members presumed that all the color-generating devices would predictably generate a visible palette of colors. To the team's surprise and chagrin, there were idiosyncrasies for each pigment-generating device. Some strains expressing the color-generating devices were colorful and healthy while others less stable and more sick. To examine the impact of the chassis on the behavior of the genetic system, the team experimented with a number of bacterial strains. You will continue this investigation to identify what factors make a chassis the "best" one for running each device. What makes identical genetic programs behave differently, even in two closely related cellular chassis?

In this lab, you will compare two color-generating devices in two different chassis to investigate the reliability of color outputs.

BACTERIAL TRANSFORMATION

The Colorful World experiment will test for reliable production of purple and green pigment generators. Each genetic program will be introduced into two closely related E. coli strains so the number of transformants and their colors can be compared.

The strains of E. coli you'll work with are routinely used in laboratories around the world to study the behavior of bacterial cells and to perform molecular biology techniques. Both have acquired mutations and can survive in only a narrow set of environments. They have all but lost the ability to thrive outside laboratory growth conditions. As such, they offer the beginnings of a standard chassis for synthetic biology. The genetic differences between the two strains are known, though, and are listed here.

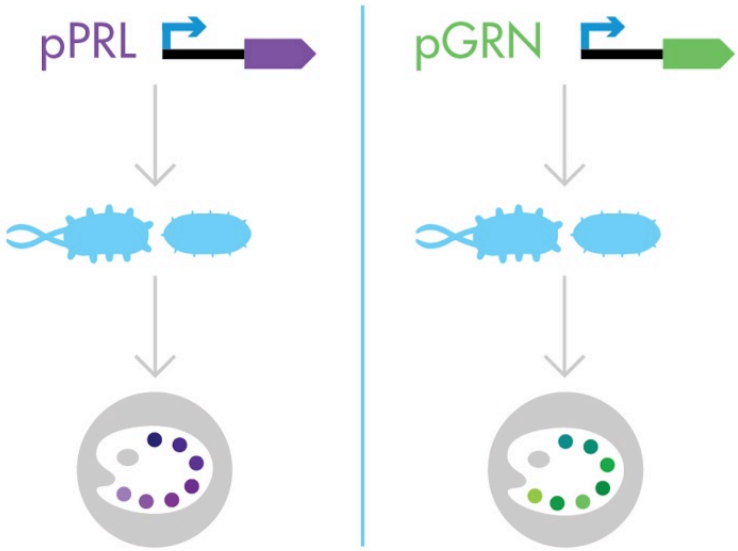
4-1 *Strain 4-1 = E. coli K12 (NEB catalog# ER2738) = F'proA+B+ lacIq Δ(lacZ) M15zzf::Tn10(TetR)/ fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5*

4-2 *Strain 4-2 = E. coli BL21 (NEB catalog# C2523) =: fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10-- TetS)2 [dcm] R(zgb-210::Tn10--TetS) endA1 Δ(mcrC-mrr)114::IS10*

You will compare the performance of the purple and green color-generators in the E. coli K12 and the E. coli B-type chassis

Experimental Method

The process of introducing new DNA into bacterial cells is called transformation. The E. coli strains you'll study do not naturally take up new DNA in from the environment. To prepare the cells for this experiment, they must be treated with a $CaCl_2$ salt solution that makes the cells porous. New DNA can be introduced through the pores when the cells are in this "competent" state.

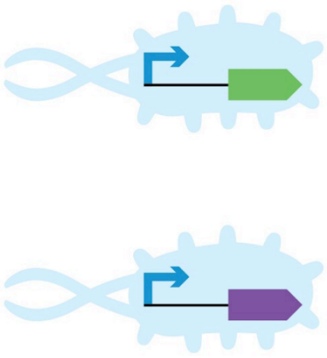


The DNA that encodes the color-generating devices also encodes the ampicillin resistance gene. Because the media contains ampicillin, only the cells having the color-generating device should grow. After one night of growth, each cell that survived the transformation and antibiotic exposure will have grown into a colony of cells visible to the naked eye. The ampicillin is used to select for the cells that have been transformed. Cells that have not taken in the DNA will not grow on the ampicillin-containing media.

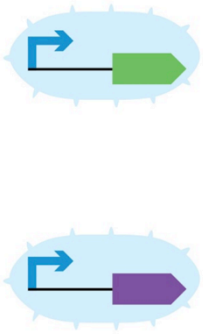
BACTERIAL TRANSFORMATION Continued

The Colorful World protocol includes instructions for transforming the purple and the green color generators into two bacterial strains, 4-1 (an E. coli K12 strain) and 4-2 (an E. coli B-type strain). Successfully transformed cells will be selected for using media that contains ampicillin. After inserting the devices (top vs bottom in the figure below) into the different chassis (left versus right in the figure below), you will investigate the behavior of the systems you have built. You will count the colonies to determine your transformation efficiency, and you will note the color, shape, and size of the colonies to determine whether the strain chosen as the chassis affects the system's output.

4-1



4-2



CONTROLS

Though they are not explicitly described in the lab protocol, several control reactions are easy to include in this experiment. Including controls is a big part of good experimental design. Controls make it possible to isolate variables and troubleshoot problems encountered while doing experiments.

As a NEGATIVE CONTROL FOR GROWTH on ampicillin, you can use any leftover competent cells from step 6 in the protocol that follows. These leftover cells will not have been exposed to plasmid DNA but they can be otherwise treated identically to the experimental samples. No colonies should grow when these control reactions are plated on media that contains ampicillin. If growth IS observed, then perhaps the media's antibiotic has degraded or a reagent (e.g. the media or the the cells themselves) have been contaminated.

A POSITIVE CONTROL FOR GROWTH is not included with the instructions but can be run by plating any of the leftover transformation mixes on media that contains no antibiotics. Cells should grow as a lawn on this non-selective media. If NO growth is observed, then perhaps the cells did not survive the transformation treatment (e.g. perhaps the water bath was boiling rather than 42°) or the antibiotic in the selection media is not the correct one (e.g. tetracycline rather than ampicillin)

PRE-LAB QUESTIONS

Briefly explain the goal of synthetic biology.

Why might an engineer want to use bacteria instead of mechanical or electronic equipment to detect and report on environmental changes?

When talking about cars, the chassis is the framework that houses the engine. When talking about synthetic biology, the chassis is the _____ that runs the _____.

An engineer who wants to design a biofuel could choose a chassis that already makes something like a biofuel. This engineer is choosing a _____ chassis rather than a standard chassis.

The 2009 Cambridge (UK) iGEM team put a color-generating device in E. coli bacteria as a way to sense a toxin. The device modified an operon isolated from Chromobacterium violacein, a bacterium. Describe how this operon can be modified to generate either purple or green pigment.

True or False: the color-generating devices produced the same amount of color in all bacterial chassis the iGEM team tested?

What is the term used to describe cases when unexpected results arise from combining components?

What is meant when we say bacteria are "competent"?

How are bacteria being made competent in this lab?

Identify the bacterial chassis that are going to be used in this experiment.

How will we select for cells that have been transformed with plasmid DNA?

WHAT A COLORFUL WORLD

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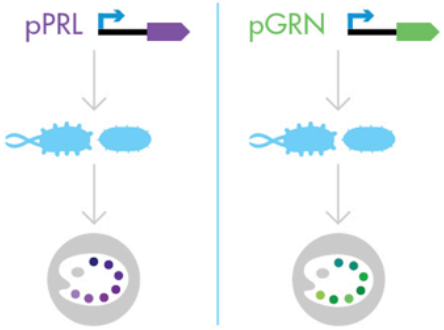
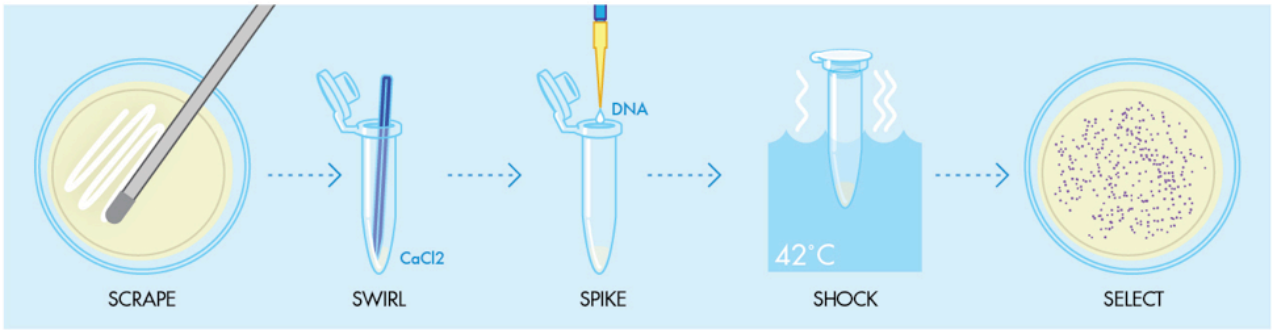
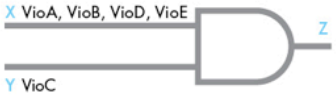
BIG PICTURE



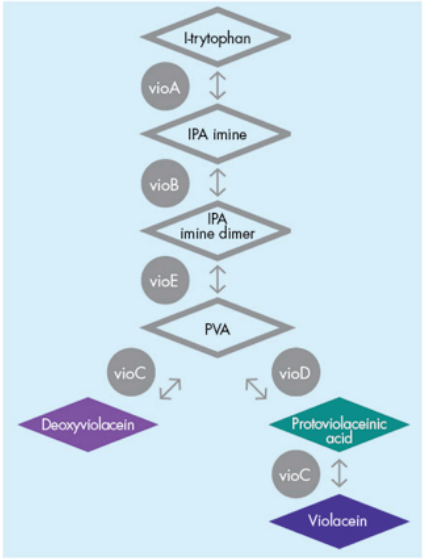
THE DETAILS



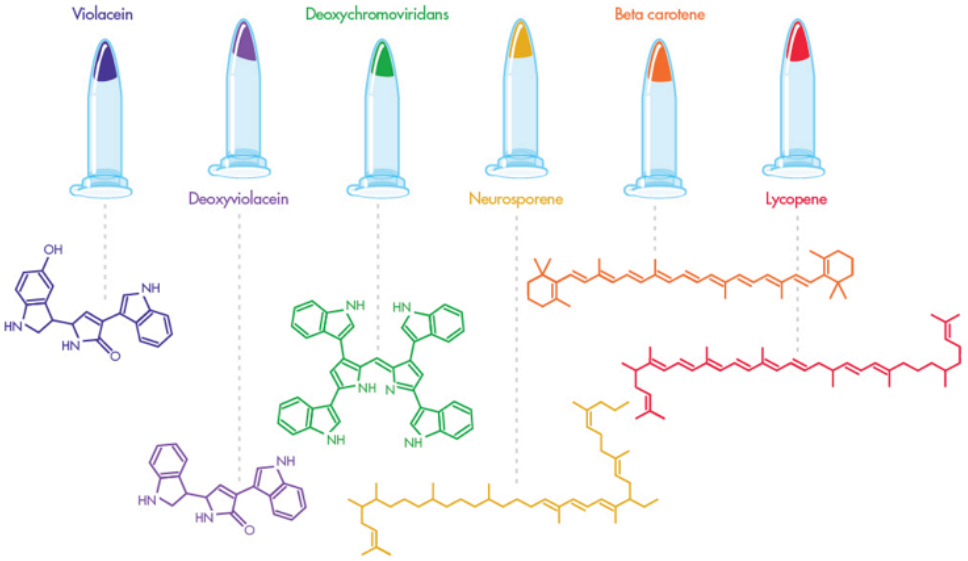
UNDER THE HOOD



INPUT X	INPUT Y	OUTPUT
0	0	0
1	0	Green
0	1	0
1	1	Purple



NATURE'S PALETTE



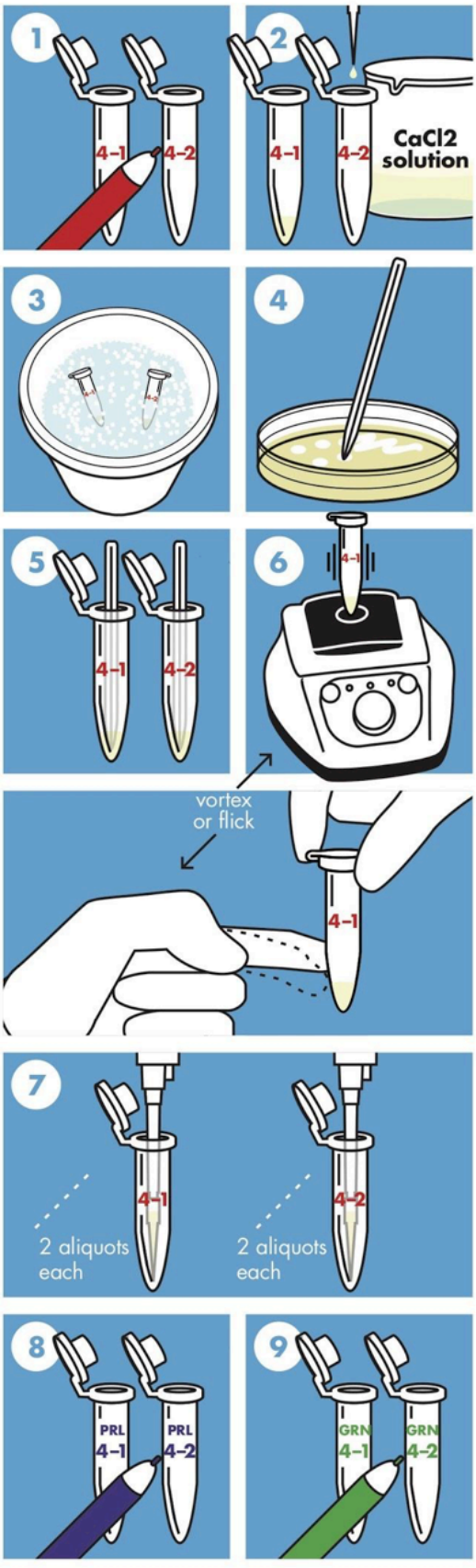
QUICK GUIDE: WHAT A COLORFUL WORLD

IN ADVANCE

Patch **4–1** and **4–2** bacteria on LB petri dishes**

DAY OF LAB

1. Label 2 small microfuge tubes either "**4–1**" or "**4–2**."
2. Pipet 200 µl of CaCl2 solution into each microfuge tube.
3. Place the tubes on crushed ice.
4. Using a sterile pipet tip, toothpick or inoculating loop, scrape a patch of cells off the **4–1** or **4–2** petri dish**. Avoid scraping up the agar.
5. Swirl the cells in the appropriate tube with cold CaCl2 then repeat for the other patch of bacteria.
6. Gently vortex the cells to resuspend them. If no vortex is available, gently flick and invert the microfuge tubes, then return them to your icebucket.
7. Retrieve 2 aliquots of each plasmid for a total of 4 samples (2x **pPRL**, 2x **pGRN**).
8. Label one of the **pPRL** tubes "**4–1**" and label the other **pPRL** tube "**4–2**."
9. Label one of the **pGRN** tubes "**4–1**" and label the other **pGRN** tube "**4–2**."



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10. Flick the tube with the competent "**4–1**" strain and then pipet 100 µl of the bacteria into the tube labeled "**pPRL**, **4–1**." and an additional 100 µl into the tube labeled "**pGRN**, **4–1**."

11. Flick the tube with the competent "**4–2**" strain and then pipet 100 µl into the tube labeled "**pPRL**, **4–2**" and an additional 100 µl into the tube labeled "**pGRN**, **4–2**"

12. Incubate the tubes on ice for ~5 minutes.

13. Heat shock the transformation reactions at 42°C for 90 seconds exactly.

14. Move the tubes to a rack at room temperature and add 0.5 ml LB to each. Close the caps, and invert the tubes to mix the contents.

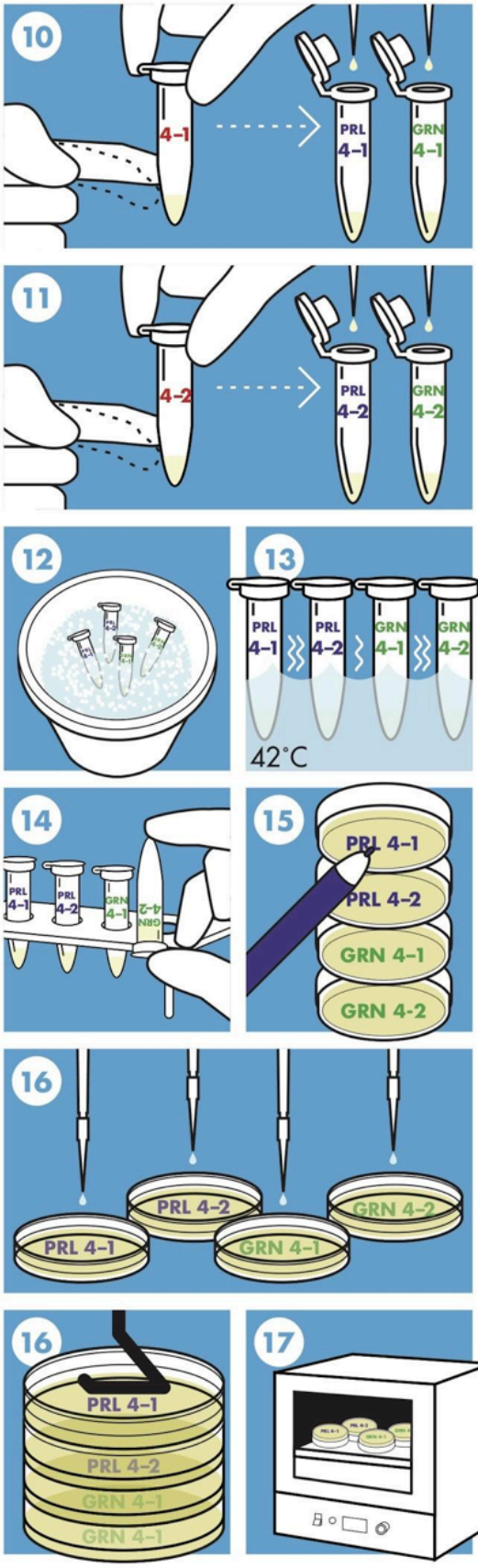
15. Label the media-side of the LB + amp petri dishes to indicate the strain you've used ("**4–1**" or "**4–2**") and the DNA you've transformed them with ("**pPRL**," "**pGRN**")

16. Pipet 250 µl of each sample onto the media of the appropriate petri dish. Spread the sample evenly across the dish with a sterile spreader.** Discard spreader and remainder of transformation mix in 10% bleach solution.

17. Incubate petri dishes, media side up, overnight at 37°C.

After the petri dishes have incubated overnight, count the colonies in each dish.

** VIDEO OF PROCEDURE AVAILABLE ONLINE



POST-LAB QUESTIONS

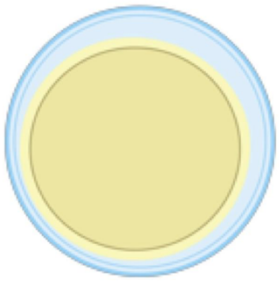
What is expected in each of the following cases? You can sketch your prediction below.
a. Growing E. coli that has undergone transformation with pPRL or pGRN on an LB+Amp petri dish

b. Growing E. coli that has undergone transformation with pPRL or pGRN on an LB agar petri dish

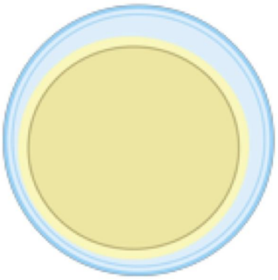
c. Growing E. coli that has NOT undergone transformation on an LB + Amp petri dish.

d. Growing E. coli that has NOT undergone transformation on an LB agar petri dish without Ampicillin

LB + Amp petri dish

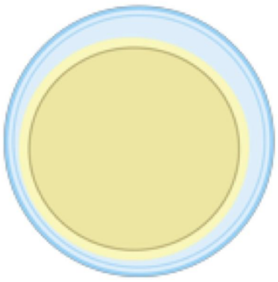


a. + pPRL (or pGRN)

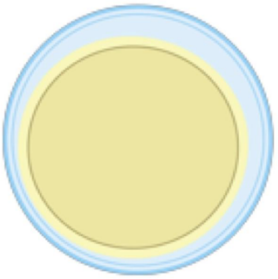


c. No DNA

LB agar petri dish



b. + pPRL (or pGRN)



d. No DNA

What were some potential problems that may have affected the outcome of this experiment? List at least 2 problems.

What is synthetic biology and what are some examples of what you can do with this field?

Ideally, the interpretation of these results should encourage more experimentation, provide ideas for improved designs, and build excitement to explore and do more.

STUDENT MANUAL

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Do you have an idea for improving and extending the units? Please email us: **info@biobuilder.org**