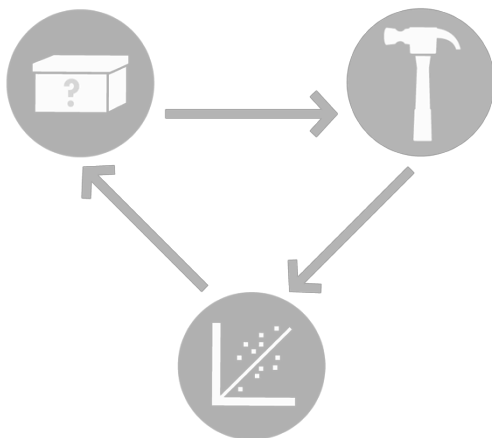# BioBuilder

Synthetic Biology for Teachers

▶ ## *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
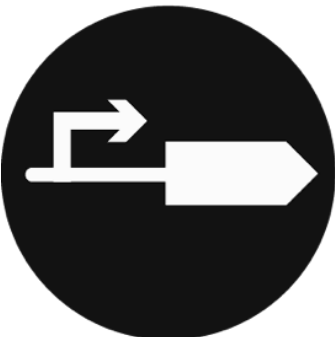
This teacher's booklet is meant to help support you and your students with the BioBuilder units. Let us know what you need and how it goes. Email us: **info@biobuilder.org**

BioBuilder

What A Colorful World

Table of Contents

About Synthetic Biology

For the last decade, teachers have introduced genetic engineering techniques to students. It is becoming commonplace for students in Biology and AP Biology courses to conduct a standard set of "experiments" using gel electrophoresis and bacterial transformation techniques. Students who perform these experiments learn several basic techniques, but that is where the laboratory experience ends. There is little room for student inquiry or creativity. The students are more technicians than scientists.

A solution to this limitation comes not from biology but a relatively new field, Synthetic Biology. Synthetic biologists apply engineering principles and extend genetic engineering techniques to construct synthetic living systems. The synthetic biology approach familiarizes teachers and students with molecular biology, genetic engineering and microbiology methods in an engineering setting. The students learn designing, building or testing designs of engineered biological systems. In addition, this approach provides science teachers with a means of fulfilling state and national teaching standards that are hard to address in most biology classes.

Using synthetic biology to teach engineering

Standardization

Abstraction

Synthesis

$$A + A + C + T + T...$$
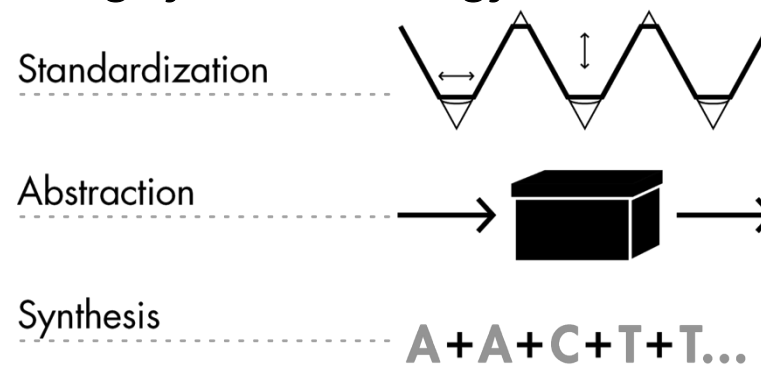
BioBuilder's engineering approach focuses on two important principles: abstraction and standardization, and relies on enabling technologies such as DNA synthesis. These principles and technologies extend the teaching of molecular techniques into real world, authentic applications. In the way that physics teachers can have students create functioning circuits and computer teachers can have students create 3-D animations, biology teachers can have students safely design, construct and analyze engineered biological systems.

The BioBuilder Curriculum

BioBuilder provides educational materials for students and teachers to explore the underpinnings of synthetic biology. All the material is modular and can be taught completely, in any order, or piecemeal, as individual exercises to supplement an existing program. BioBuilder's curriculum includes both classroom lessons and laboratory activities. Biodesign and Bioethics lessons can be carried out in any sized classroom and with many age groups. The laboratory investigations provide standard protocols as well as modifications to meet local situations and needs.
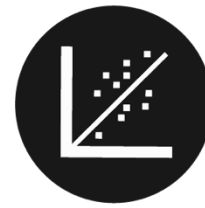
Biology teachers can use our materials to lead engineering challenges with students. Students gain first-hand experience with the engineering paradigm:

DESIGN   BUILD   TEST

Students are motivated to understand the underlying science within an authentic context of engineering challenges. BioBuilder students become more than technicians; they become engineers.

What A Colorful World

Examines the role of the cellular chassis in system performance. Students transform different strains of *E. coli* with DNA that turns the cells several bright colors. Students then observe how different the color intensity can be from strain to strain, despite being encoded by the same DNA sequence.

iTUNE Device

Examines the role of parts, such as promoters and ribosome binding sites, in predicting the output of a genetic device. The students measure β-galactosidase enzymatic activity as the device's output, thereby looking through the lens of molecular genetics to predict and then evaluate a device's behavior.

Picture This

Three activities to explore the role of modeling in circuit design. These activities include a downloadable program to computationally vary the parameters of a genetic circuit, an exercise to mimic a genetic circuit with electronic parts, and an opportunity to send a stencil that will be turned into a bacterial photograph.

Eau That Smell

Compares two alternative genetic designs. Both programs should make the cells smell like ripe bananas as the cells grow.

Golden Bread

Explores the science, engineering and bioethics of a yeast that's genetically modified to make a vitamin-enriched food. Lab activities include PCR, yeast transformation, codon shuffling and quantitative analysis of data

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.
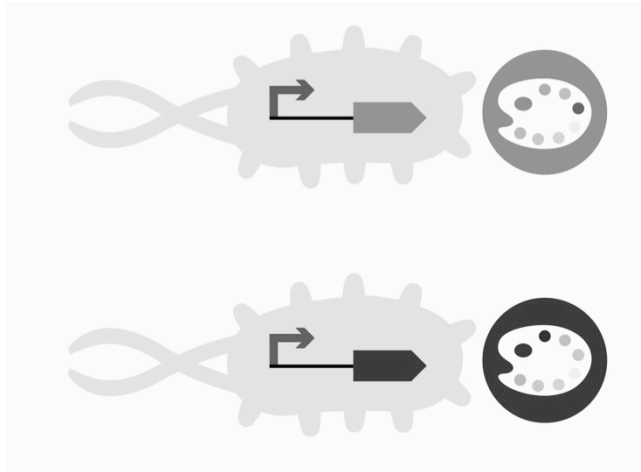

In this lab, you will build several color-generating systems to explore how the chassis affects the output of a designed genetic program. Because the colors are visible to the naked eye, you can easily decide whether the color outputs are different between the chassis.

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.
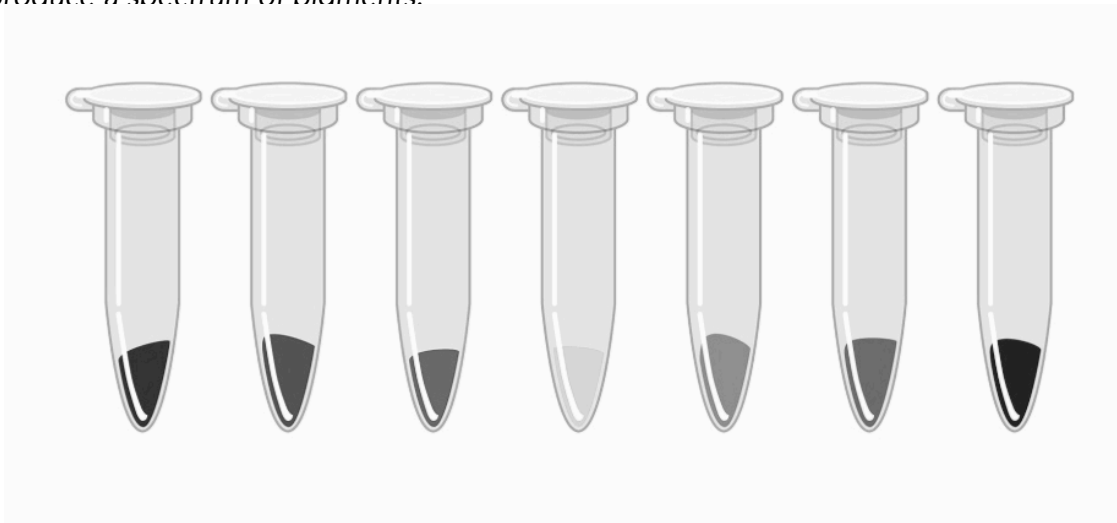
INTRODUCTION TO CHASSIS DESIGN

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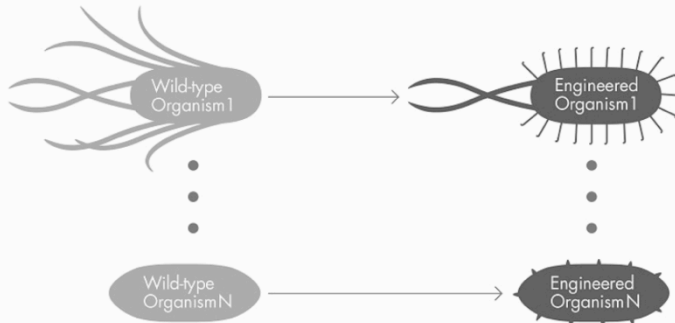
In this approach, a chassis is identified from nature based on its ability to do something similar to the task a synthetic biologist has in mind for a new system.

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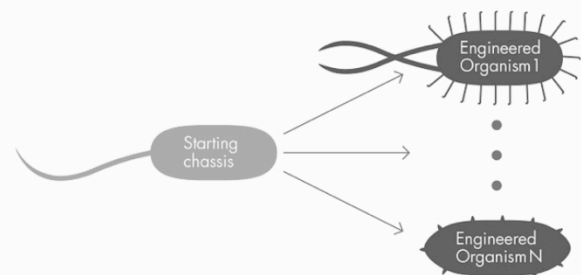
In this approach, a more generic chassis is chosen because it either has a minimal number of natural components or it is well understood and highly engineerable.

Some research groups are using engineered *E. coli* to make biofuels.. The researchers see *E. coli* as an attractive platform for <u>many</u> 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.
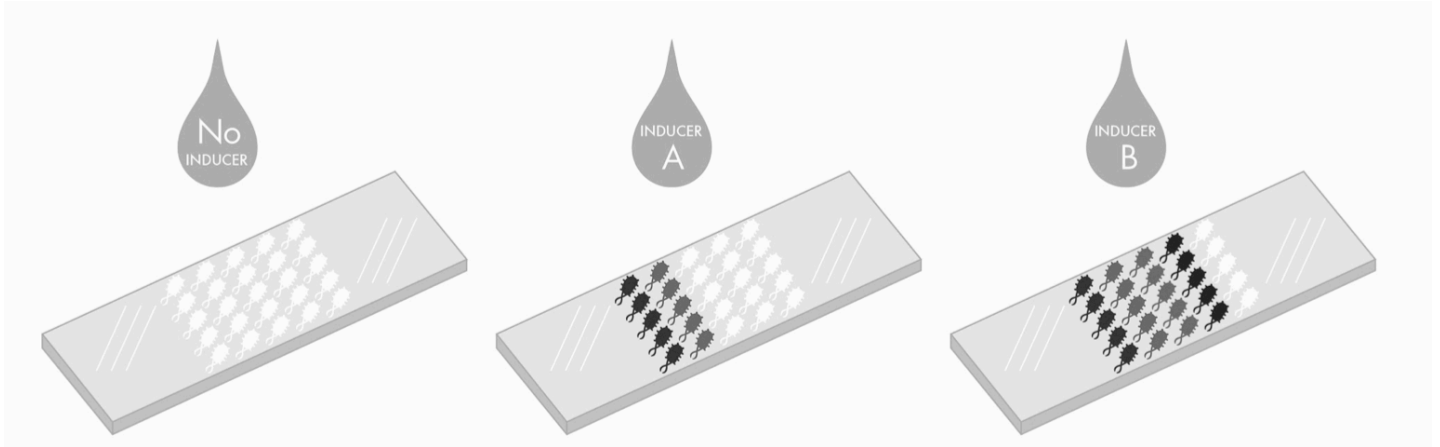
Pigment Production

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.*

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.

| X | VioA, VioB, VioD, VioE |
| Y | VioC |

INPUT X	INPUT Y	OUTPUT Z
0	0	0
1	0	Green
0	1	0
1	1	Purple
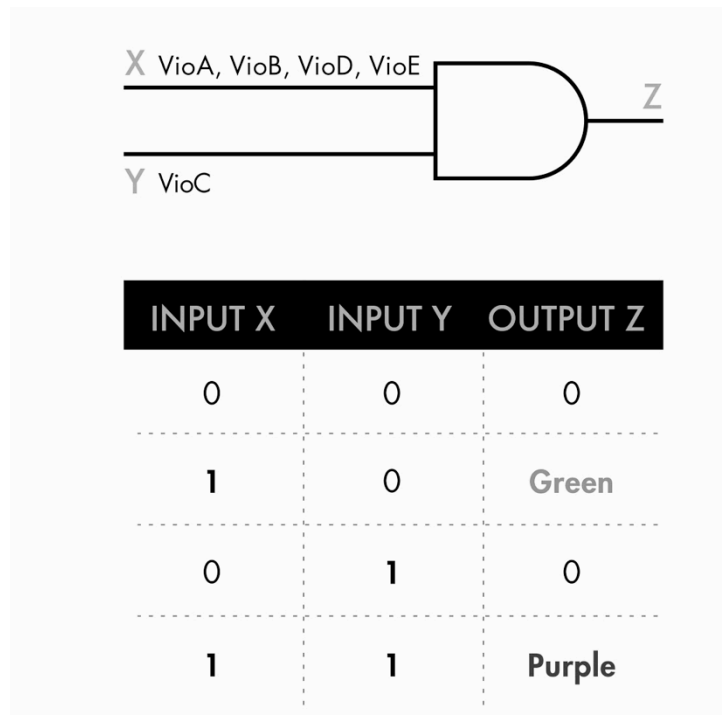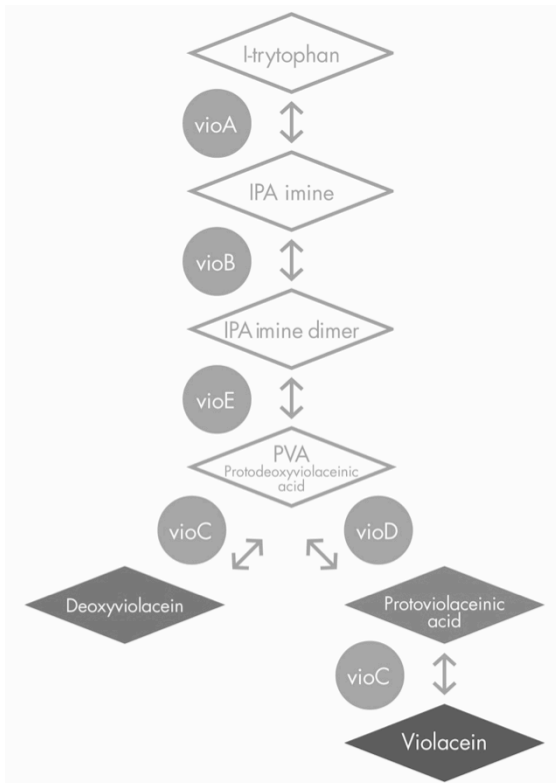
A logic gate and truth table can be used to summarize system's behavior. Both show that VioA, VioB, VioD and VioE are necessary for generating the green pigment and that the addition of the VioC gene gives rise to the purple pigment output.

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.
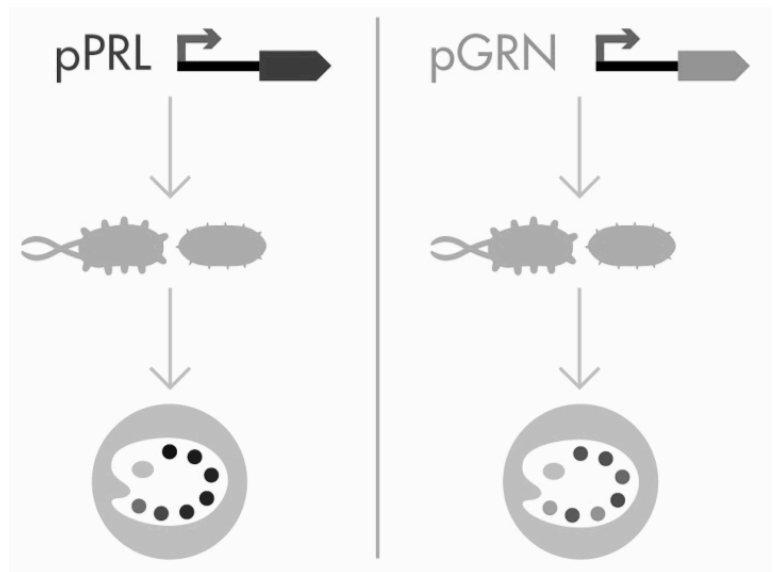

The DNA that encodes the color-generating devices also encodes the ampicillin resistance gene. Because the media contains ampicillin, only the cells having the color-generating device should grow. After one night of growth, each cell that survived the transformation and antibiotic exposure will have 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
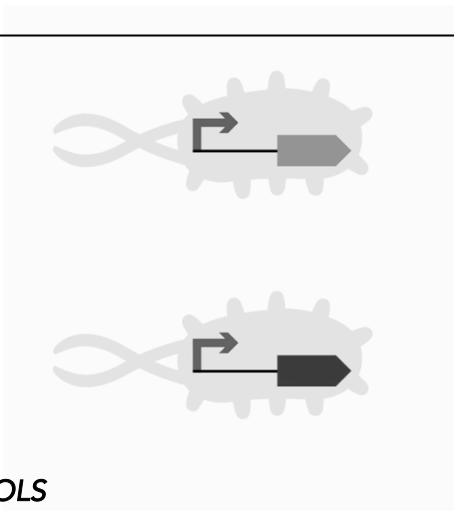
4-2
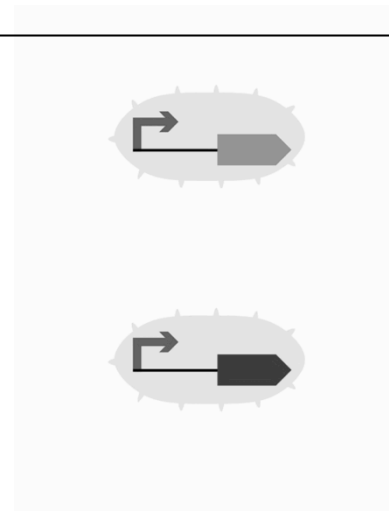
CONTROLS

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.
Synthetic biology involves construction of novel living machines in order to solve problems and improve people's lives.

Why might an engineer want to use bacteria instead of mechanical or electronic equipment to detect and report on environmental changes?
Bacteria may be cheaper to produce and easier to distribute than more traditional sensing materials and equipment. Bacteria also make more copies of themselves, while mechanical or electronic equipment cannot. Bacteria are also exquisitely sensitive to changes in the environment.

When talking about cars, the chassis is the framework that houses the engine. When talking about synthetic biology, the chassis is the __ cell ___ that runs the __genetic (DNA) program___.

An engineer who wants to design a biofuel could choose a chassis that already makes something like a biofuel. This engineer is choosing a ____nearest neighbor_____ 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.
The violacein operon consists of five genes (VioA, VioB, VioC, VioD, VioE). Expression of all five genes will convert tryptophan through a series of steps into violacein, a purple pigment. However, removal of VioC will block the last step in the pathway, causing the cell to stop once it has made proto-violacein, a green pigment.

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

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

What is meant when we say bacteria are "competent"?
Competent cells are ready to be transformed because they can accept plasmid DNA from the environment

How are bacteria being made competent in this lab?
The cells are treated with $CaCl_2$

Identify the bacterial chassis that are going to be used in this experiment.
The two chassis that will be compared are strains 4-1 which is a K12 strain and 4-2 which is a B-type strain

How will we select for cells that have been transformed with plasmid DNA?
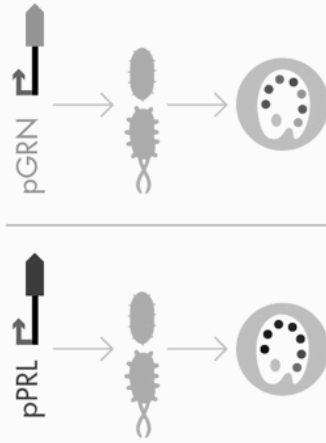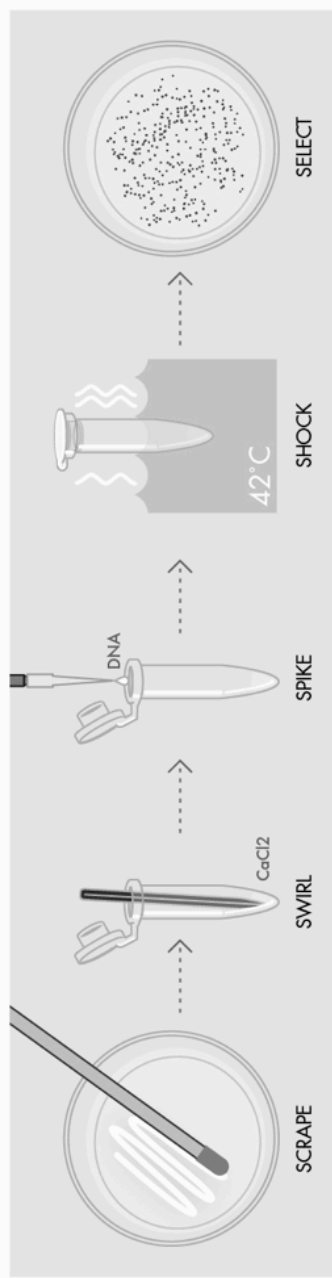*Transformed cells will be able to grow as colonies on media with ampicillin*

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BIG PICTURE

pPRL
X VioA, VioB, VioD, VioE

pGRN

THE DETAILS

SCRAPE → SWIRL → SPIKE → SHOCK → SELECT

CaCl2

DNA

42°C

NATURE'S PALETTE

Violacein

Deoxyviolacein

Deoxychromoviridans

Neurosporene

Beta carotene

Lycopene

UNDER THE HOOD

X VioA, VioB, VioD, VioE

Y VioC

Z

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

Ltryptophan → vioA → IPA imine → vioB → IPA imine dimer → vioE → PVA

vioC
vioD → Protoviolaceinic acid → vioC → Violacein

vioC → Deoxyviolacein
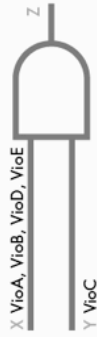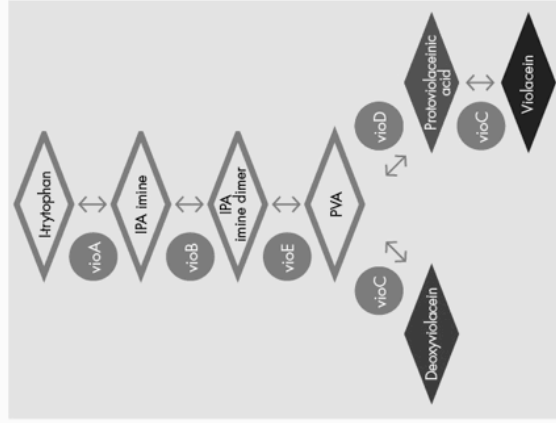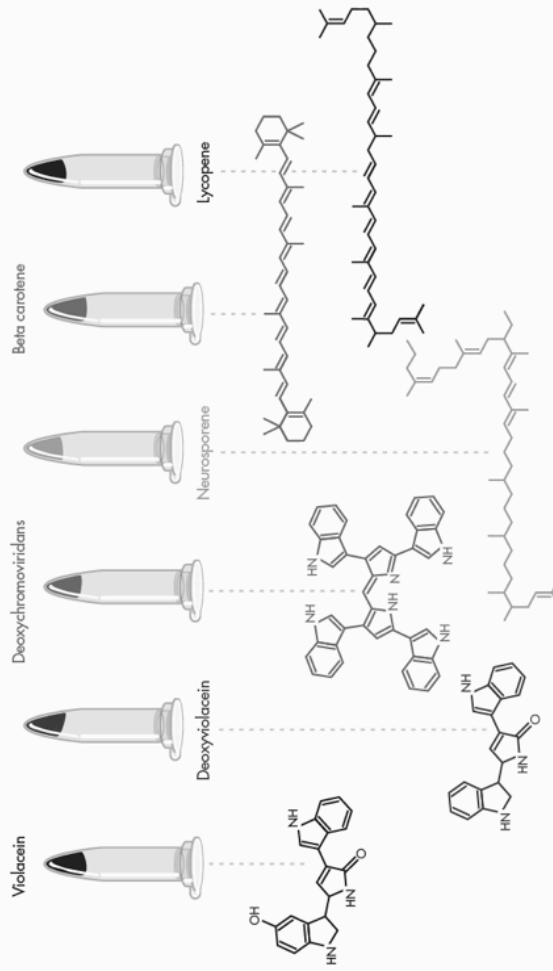
CHECKLIST FOR KIT CONTENTS

☐ Eppendorf Tubes (2 packages, 30/pkg)
☐ Disposable spreaders (4 packages, 10/pkg)
☐ Sterile inoculating loops (1 package, 30/pkg)
☐ Bacterial Strains 4-1 and 4-2 (sent as stabs)
☐ pPRL and pGRN Plasmid DNA (60 ul of 1 ug/ul)

☐ Vials of Luria Broth (6 vials, 5 ml/vial)
☐ Vials of 50mM CaCl2 (3 vials, 10 ml/vial)
☐ LB + 50 ug/ml Amp petri dishes (4 sleeves, 10/sleeve)
☐ LB petri dishes (2 sleeves, 10/sleeve)

Unpacking your kit

- Store the Luria Broth and CaCl2 on the shelf until the day of the experiment
- Store the LB and LB+Amp petri dishes in the fridge (4°C)
- Store the plasmid DNA in the fridge (4°C) or freezer (-20°) if you have one
- The stabs can be kept at room temperature or in fridge

Up to two weeks in advance of lab

▶ *Streak the bacteria from the stab: Using a sterile loop, touch the bacteria that will arrive growing in the stab or slant vial, picking up a small but noticeable amount of cells. Touch the loop to an area on an LB (NOT LB+AMP) petri dish you have prepared and patch the cells on the media as shown in the video on the BioBulder.org website. Repeat so one patch of 4-1 and one patch of 4-2 is growing for each group of students. Place the petri dishes in the incubator at 37°, or at room temperature but it may take 2 days.*

▶ *The CaCl2 will arrive in solution so you only need to pre-chill the tube in the fridge or on ice on the day of lab. You may choose to aliquot the 50mM CaCl2 solution since each group will need only 400 ul of it. If you aliquot the solution, we recommend giving the students a 500 ul aliquot to account for pipet variations. In all cases, though, you'll want to chill the CaCl2 in the fridge or on ice before the students use any.*

▶ *Students will need at least 3 ml of LB per team. The LB has arrived in 3 ml aliquots so you can distribute the LB, one tube per team. This should minimize the potential for contamination.*

Pre-lab preparation

Each lab group will need

- A patch of 4-1 and 4-2 cells. Each patch will yield enough cells to transform two plasmids. Several groups can work from one LB plate that has several patches streaked out, or each group can work with a plate of their own
- An aliquot of each plasmid: 5 ul of pPRL and 5 ul of pGRN for each group in eppendorf tubes

- An aliquot of CaCl2
- An ice bucket with ice
- An aliquot of LB media
- Four LB+Amp petri dishes
- Two eppendorf tubes Two loops or toothpicks
- Four sterile spreaders

The lab will need

- Microfuge
- Biohazardous waste disposal
- 42° heat block or water bath
- 37° incubator, though petri dishes can be incubated at room temperature for one day longer

- Vortex, optional
- Timer
- Micropipets and tips
- Sharpies
- Latex gloves

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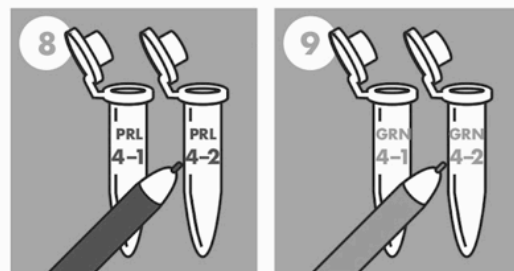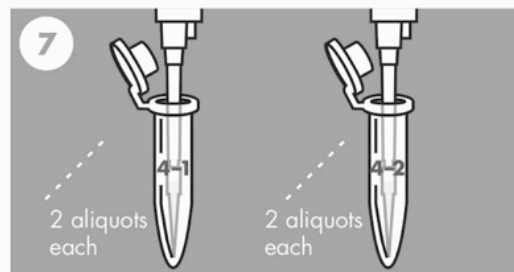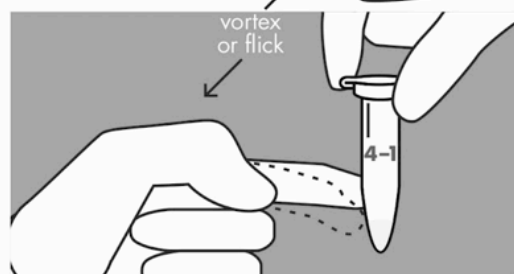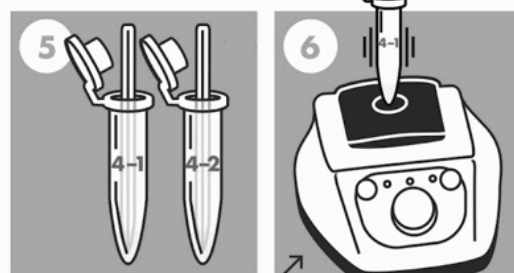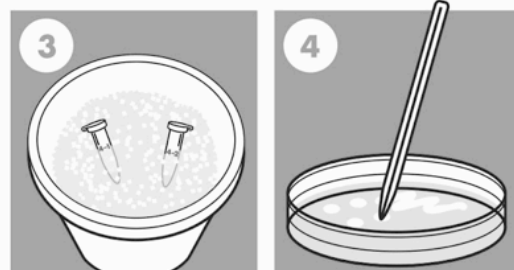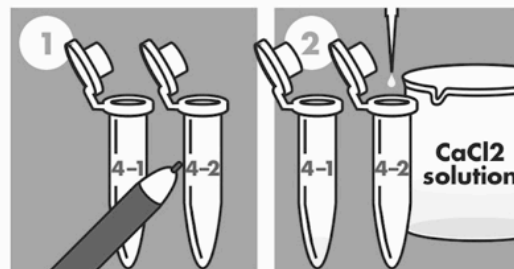
10. Flick the tube with the competent "**4–1**" strain and then pipet 100 µl of the bacteria into the tube labeled "**pPRL**, **4–1**." and an additional 100 µl into the tube labeled "**pGRN**, **4–1**."

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
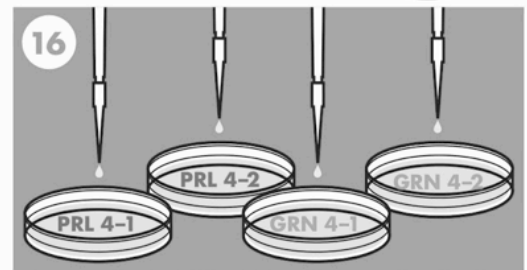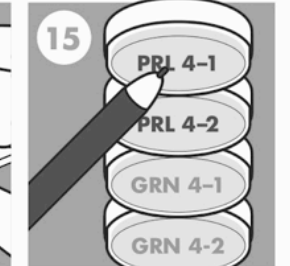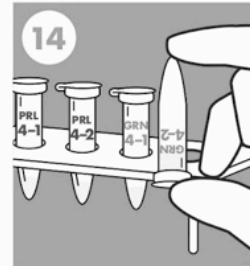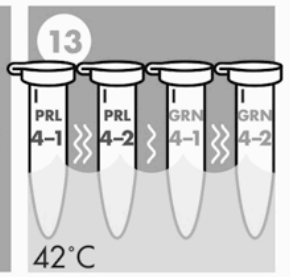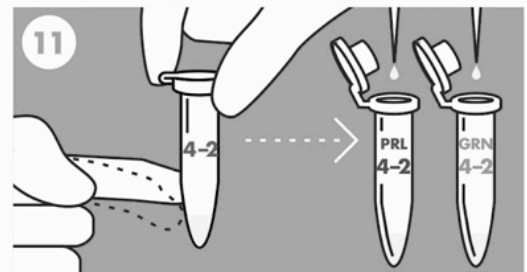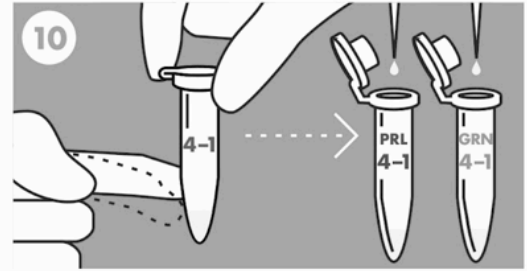
INTERPRETING THE RESULTS

Using the protocol presented here, 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.

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.

4-1

4-2
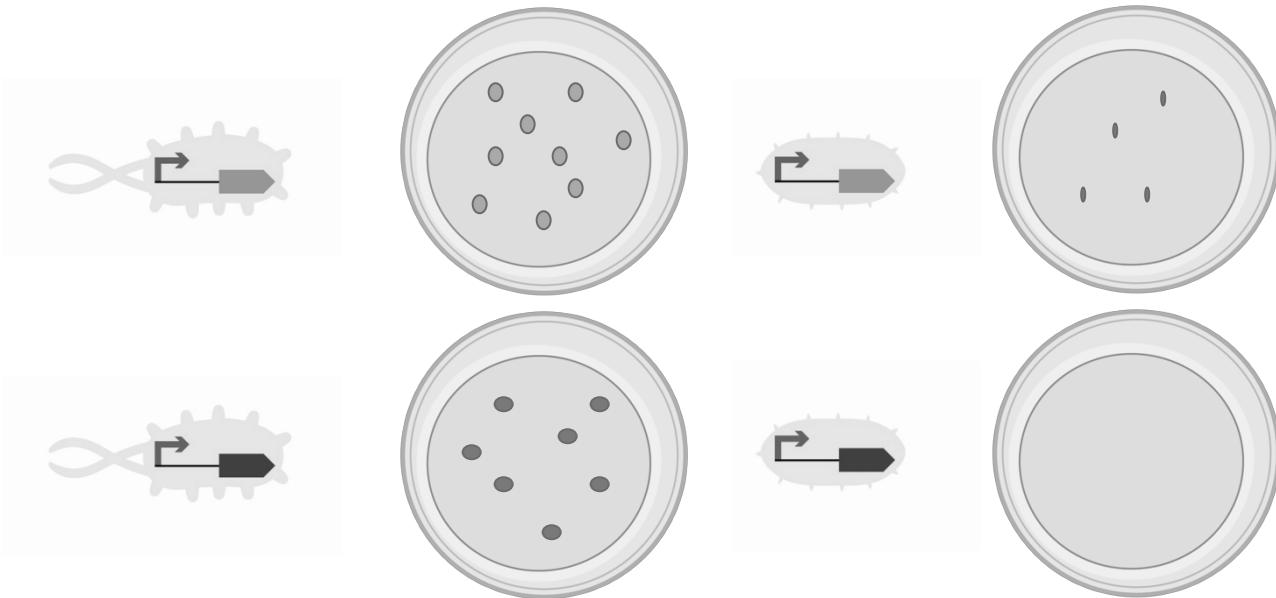
Transformation Efficiency

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 $CaCl_2$. 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 will realize they can't all have made that "mistake."

We generally observe transformation efficiencies around 200 colonies/microgram of DNA for strain 4-1. However, variations on the protocol, such as incubation of the petri dishes at room temperature, may produce different results.

SAMPLE CALCULATION

- • 500 colonies seen on an LB+Amp petri dish
- • 5 micrograms of DNA used for the transformation
- • 1/2 of the transformation mix plated

500 x 2 = 1000 colonies if entire transformation mix had been plated
1000 colonies/5 micrograms of DNA = 200 colonies/microgram of DNA = transformation efficiency

TIPS AND POST-LAB QUESTIONS

TEACHING TIPS

- *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 $CaCl_2$ should not be more than a week or two old since the transformation efficiency drops as the cells age.*
- *pGRN does not behave the same in two strains. The explanation may relate to the "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.*
- *pPRL is not well tolerated in the 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.*

POST-LAB QUESTIONS

What is expected in each of the following cases:

a. Growing E. coli that has undergone transformation with pPRL or pGRN on an LB+Amp petri dish

> *We expect to see purple or green colonies, depending on the plasmid.*

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

> *We expect to see a lawn of untransformed cells due to lack of ampicillin in the media. Though some cells may have received the purple or green color-generating plasmid, these transformants will not be detectable since the vast majority of cells will not have been transformed.*

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

> *There should be no growth because the ampicillin will kill the untransformed bacteria.*

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

> *We should see a lawn of untransformed cells due to lack of ampicillin in the media.*

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

Answers will vary but may include problems with sterile techniques, liquid measurement errors, temperature variations, health of bacteria, stability of the plasmids etc.

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

Synthetic biologists construct novel cellular machines that function to solve problems and improve lives. Examples of synthetic biology include bacteria that can sense heavy metals in the environment and improve yields in biomanufacturing of medicines and other compounds.

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

BioBuilder

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