BioBuilder

Synthetic Biology for Teachers

introduction | acknowledgments | manual

This teacher's booklet will minimize the barrier for trying the units we offer. Let us know what you need and how it goes.

You have an idea for improving and extending the units?
Please email us

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

Using synthetic biology to teach engineering.

The engineering approach taught here focuses on two important principles: abstraction, and standardization, and relies on numerous enabling technologies such as DNA synthesis.
These principles and technologies provide biology teachers with a means to extend the teaching of molecular genetic 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

This curriculum has been developed in conjunction with the BioBuilder.org website, which provides educational animations for students and teachers to explore the underpinnings of synthetic biology. Single page animations frame the topics and lab activities. All the material is modular and can be taught completely, in any order, or piecemeal, as individual exercises to supplement an existing program.

Biology teachers can use these available materials to conduct engineering challenges with students. Students gain first-hand experience with the engineering paradigm:

Since the engineering activities are performed in the context of living systems, the students will have to understand the underlying science. Through synthetic biology, students can learn these concepts within an authentic context of engineering challenges. These tools of synthetic biology provide biology students with a means to be more than technicians; they can be engineers. 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.

What a Colorful World	The iTune Device	Picture This
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.	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.	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	Golden Bread
Compares two alternative genetic designs. Both programs should make the cells smell like ripe bananas as the cells grow.	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 statistical analysis of data.

What a Colorful World Lab

Exploring the role of the cellular chassis

context | check lists | lab protocol

In this lab, bacterial transformation techniques will be used to introduce some plasmid DNA into two cell types. The engineering concept of chassis is explored by comparing the function of identical genetic programs in two different strains of *E. coli*. Sterile technique and solid-state microbial culture are the biotechnology skills emphasized.

If you're inclined to think like a scientist

you're excited by getting into the lab, finding that something new and unexpected has happened to the system you're working on. This emergent behavior might make you wonder, "What explains the results I observe?"

If you're inclined to think like an engineer

this behavior might make you think, "ugh" and then get to work defining the best way to optimize a system's performance with hopes of avoiding unexpected behaviors like these.

Both approaches have merit, and together the scientific and engineering approaches should lead to more reliable designs in the future and should minimize the number of surprising behaviors we see as we build new living systems.

In this lab, 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.

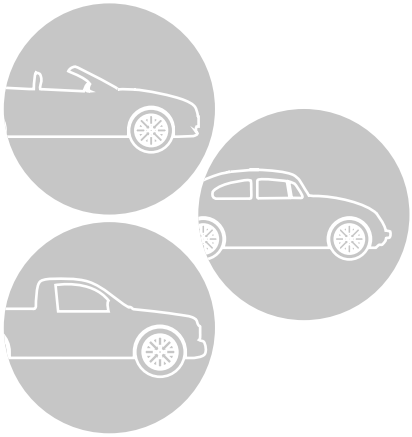
The DNA programs come from a 2009 International Genetically Engineered Machines - iGEM project called, "E. chromi," in which students from the University of Cambridge designed and engineered *E. coli* to produce a spectrum of pigments.

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

Before detailing the experiment, though, we will provide a general discussion of chassis design and decisions, and we'll walk through the E. chromi project to provide some context for your investigation.

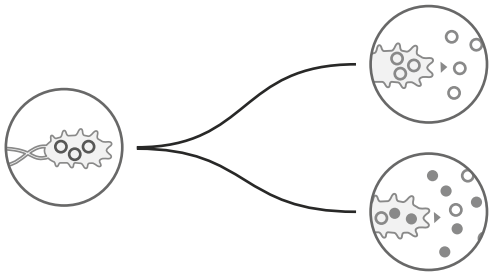
Introduction to chassis

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.



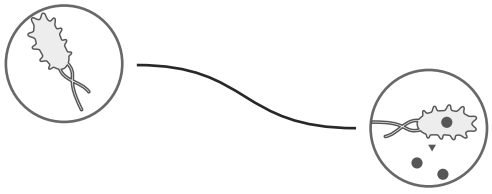
How to engineer it?

1 - NEAREST NEIGHBOR, the “nearest wild-type organism” 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.



The work of some biofuel researchers illustrates well this approach. Because the bacterium *Ralstonia eutropha* naturally converts carbon dioxide into energy-storing polymers, engineers felt it was an attractive chassis to generate biofuels instead of the polymers they normally make.

2 - STANDARD CHASSIS, “blank canvas” approach
A more generic chassis is chosen that either has a minimal number of natural components or is well understood and highly engineerable. It is essentially a blank canvas that is theoretically an ideal chassis for a variety of engineered systems with different desired outputs.



For example, some research groups are using engineered *E. coli* to make biofuels. *E. coli* have no existing talents to recommend them for biofuel production. However, they are the closest organism that the field has to a standard bacterial chassis. Thus, the researchers see *E. coli* as an attractive platform for many purposes, including the conversion of carbon dioxide and electrical energy into isooctane.

A little bit more about chassis

NEAREST NEIGHBOR

STANDARD CHASSIS

+

When only minor changes are needed in an existing organism to obtain the desired outcome, it might be relatively easy to use the nearest wild-type organism approach.

The standard-chassis approach is more scalable than the nearest wild-type organism approach because it provides a generic starting point for designers.

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An existing organism can be unexpectedly complicated because all organisms have their own metabolic pathways and needs, any of which might interfere in surprising ways with the desired output.

The hope for a “blank canvas” cell that could be reliably programmed with any and every genetic circuit is still futuristic. Cells cannot yet be thought of as a virtual machine for biology.

For now, a compromise is being struck between the nearest wild-type and the standard-chassis approaches. In this compromise, a small menu of chassis serves as a starting point for many biodesign goals. One or two reliable standard chassis may be available for bacterial applications and a few others for mammalian work. This hybrid approach to the engineering of cellular chassis is perhaps more likely to yield accessible tools for synthetic biologists in the near term.

Safety, how to engineer it?

Researchers must consider their personal safety in working with synthetic living systems, the safety of their lab environment, and any harm that might occur if their engineered organisms enter the wider world.

Existing guidelines and regulations largely protect the personal safety of the researchers and the lab communities where they work. These regulations came into existence in the 1970s with the advent of recombinant DNA technologies.

One radical example of “safety by design” is the synthetic biology research to engineer orthogonal DNA that is decoded differently from naturally occurring DNA. Because DNA can transfer between organisms and such transfer is relatively common, there is concern about the potential consequences if an engineered organism were to take up DNA from a naturally occurring organism, and vice versa. However, if engineered organisms were expressing orthogonal DNA that can’t be decoded properly outside the original host, these possible dangers are minimized.

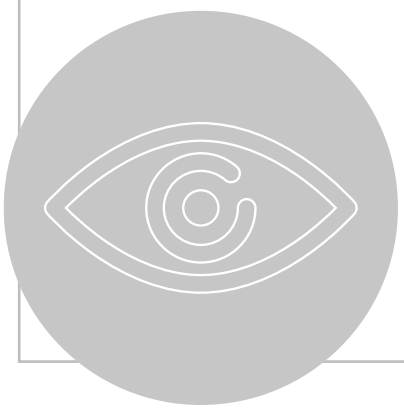
Any chassis that uses the altered genetic code couldn’t implement a new—and potentially dangerous—genetic function that it acquired from the outside environment, and the genes from the engineered organisms couldn’t be implemented if they were to be transferred to naturally occurring organisms.

The iGEM project

In 2009, the University of Cambridge team designed, specified, and built a set of biosensors that the team called E. chromi. Given the wide variety of natural colors to be found in the living world (orange carrots, purple flowers, and so on), the team decided to use color as the system's output.

PIGMENT FAMILY	COLOR PRODUCED	BACTERIAL SOURCE
Violacein	green purple	*Chromobacterium violacein*
Carotenoids	red orange	*Pantoea ananatis*
Melanin	brown	*Rhizobium etli*

The team also decided to make sensors for particular metal compounds, including arsenic, mercury, and other heavy metals that are persistent contamination concerns in some countries.



These environmental contaminant inputs would trigger pigment-based outputs visible to the naked eye for easy, immediate deployment.

About their device

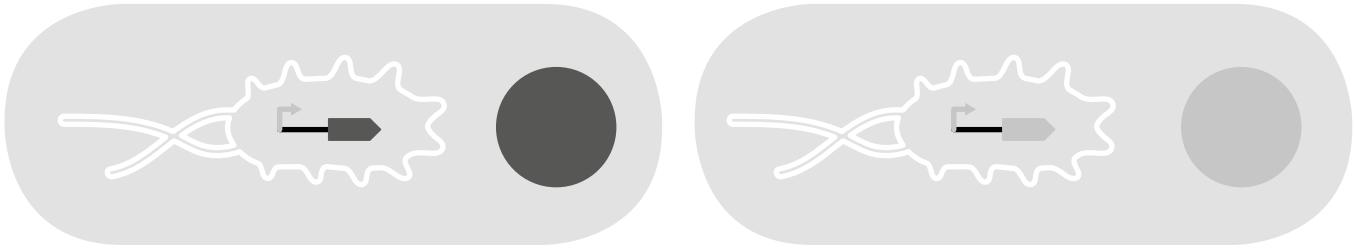
To deploy the system's devices, the iGEM team also had to consider the distinct mechanisms used to produce the violacein and the carotenoid pigments. The carotenoid devices use a pathway that produces a red pigment first and then uses a downstream enzyme to convert some fraction of the red pigment to an orange one. By contrast, the violacein devices rely on a branched metabolic pathway to produce both green and purple colors.

Parts and devices

The BioBuilder Colorful World activity uses components from the violacein system that can produce either green or purple pigment, depending on a relatively small genetic change.

Normally the input to this pigment-producing system is the amino acid tryptophan. Through the action of five enzymes VioA, VioB, VioC, VioD, and VioE, a purple pigment called violacein is produced.

When the VioC gene is removed, however, the pathway branch is blocked and the final transformation to violacein can't occur, so the system's endpoint instead becomes protoviolaceinic acid, a pigment with a dark green color.



EXPERIENCE OVERVIEW

Design choices

In designing their system, iGEM team members presumed that the color-generating devices would predictably generate a visible palette of colors when exposed to a given concentration of metal contaminants. The team realized, however, that there were idiosyncrasies for each pigment-generating device. To address these idiosyncrasies, the team experimented with a number of bacterial strains to find the “best” one for running each device.
In this lab, you will pick up this aspect of the iGEM team’s project. You will compare two color-generating devices in two different chassis to investigate whether reliable color output is attainable.

Experimental Question

Do identical genetic programs behave differently, even when expressed in two closely related cellular chassis?

HOW?

This experiment tests for reliable, reproducible production of purple and green pigment generators by transforming them into *E. coli* K12 and B-type chassis.

1 - Methods

Inserting the DNA encoding the color-generating devices. The steps involved in this process include patching the strains to grow the cells you'll need, and then treating the cells with a salt solution so that they become competent to take in DNA.

Then you will mix your competent *E. coli* strains with the DNA encoding the color-generating devices, and you'll expose the cells to a high temperature for a brief period of time. This heat shock helps the cells take up DNA from the environment.

In the final experimental step some fresh media is added to the transformation mixes to help the cells recover, and then the transformed cells are plated onto Petri dishes filled with agar-solidified media that also contains the antibiotic ampicillin.

2 - Selection & control

Selected growth
The DNA that encodes the color-generating devices also encodes the ampicillin resistance gene. Thanks to the media, which contains ampicillin, only the cells having the color-generating device should grow.

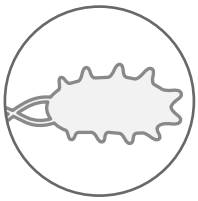
Controls
The procedure specifies a control sample in which cells are not exposed to plasmid DNA but are plated on media that contains ampicillin. No colonies should grow in this case but if growth is observed, perhaps the media's antibiotic has degraded or the cells have been contaminated. A positive control for growth is not included with the instructions but can be run by plating any of the transformation mixes on media that contains no antibiotics.

3 - Results

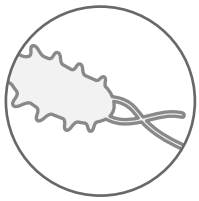
After one night of growth, each cell that survived the transformation and antibiotic exposure will have had time to grow into a colony of cells visible to the naked eye.

The data collected will be based on visually inspecting the plates. Students will count the colonies to determine the transformation efficiency, and will note the color, shape, and size of the colonies on the different plates to determine whether the strain chosen as the chassis affects the system's output.

This lab requires two strains of *E. coli*



NEB *E. coli* K12 ER2738: F'proA+B+ lacIq Δ(lacZ) M15 zzf::Tn10(TetR)/ fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5



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

This lab requires two plasmids

Plasmid
pPRL
Registry plasmid #
BBa_K274002
Plasmid description
pUC18 plasmid backbone, AmpR

Plasmid
pGRN
Registry plasmid #
BBa_K274004
Plasmid description
pSB1A2 plasmid backbone, AmpR

Engineering safety

E. coli K12 and B strains are routinely used to study the behavior of bacterial cells and to perform molecular biology techniques. They have acquired mutations and can survive in only a narrow set of environments. Indeed, 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, at least regarding safety concerns.

SUGGESTED WORKFLOW

Day 1	Introduce Synthetic Biology

PRE-LAB Q&A

Day 2	Introduce lab, have students prepare materials

PREPARE MATERIALS
PREPARE BACTERIA

Day 3	Students perform transformation Incubate plates overnight

LAB PROTOCOL - Part 1
LAB PROTOCOL - Part 2

Day 4	Students observe and discuss results

DATA COLLECTION
KEY TEACHING POINTS
POST-LAB Q&A

PRE-LAB Q&A

Pre-lab and Post-lab questions are provided in the student manual. The answer key is provided below.

1. Briefly explain the field of synthetic biology.

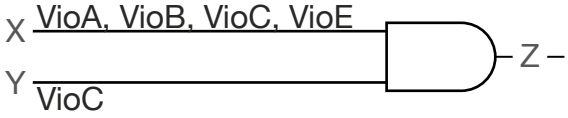
Synthetic biology involves construction of novel living machines in order to solve problems and improve people's lives.

2. Briefly explain why bacteria are often used as "sensors" instead of using mechanical or electronic equipment?

The bacteria may be cheaper and easier to distribute than equipment and materials that are manufactured with traditional methods. They are also exquisitely sensitive to changes in the environment.

3. The 2009 Cambridge (UK) iGEM team put a color-generating device in *E. coli* bacteria as a way to sense a toxin. The device was an operon isolated from Chromobacterium-violacein, a bacterium. Describe how this color generator, an operon in this case, works.

The violacein operon consists of five genes that 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.



inputs		output	
X	Y	Z	
0	0	0	
1	0		green
0	1	0	
1	1		purple

4. Why transform *E. coli* with the color producing generators originally found in the *C. violacein bacterium*? In other words, why not do the experiments in *C. violacein*?
Synthetic biologists like to use *E. coli* because it is well understood and easy and safe if proper strains are used to work with.

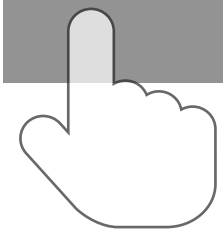
5. To a synthetic biologist, what is a chassis?
The chassis is the cell in which the genetic device is placed. It must provide an environment that allows the genetic device to function.

6. To a biologist, what is transformation?
Transformation is the altering of an organism's genome by making the cell's membrane permeable and then transferring genes into the cell.

7. What is meant when we say bacteria are "competent"?
Competency is the ability of the cells to take up plasmids containing genetic material during transformation.

8. What strains of *E. coli* will be used?
The two chassis we will use are strain 4-1, which is a K12 strain, and 4-2, which is a B-type strain.

9. What plasmids will be used?
The two plasmids we will use are pPRL and pGRN.



Online you can find a rubric and score sheet for a lab report assignment: http://biobuilder.org/teacher/colorful-world-teachers/

WHAT I HAVE

Checklist of the kit content

For a class of 24 students - 6 groups of 4

Item	Amount
Stab vial with strain 4-1	1
Stab vial with strain 4-2	1
pPRL plasmid DNA, 1µg/µl	60 µl
pGRN plasmid DNA, 1µg/µl	60 µl
Vials of Luria Broth	6 vials, 5 ml/vial
Vial of 50 mM $CaCl_2$	3 vials, 10 ml/vial
1.5 ml microcentrifuge tubes	2 packages, 30/pkg
10 µl inoculating loops	1 package, 30/pkg
Spreaders	4 packages, 10/pkg
LB + 50 µg/ml Amp petri dishes	4 sleeves, 10/sleeve
LB petri dishes	2 sleeves, 10/sleeve
Teacher + Student Instructions	downloadable

Something missing?
Contact us: info@biobuilder.org

In order to be ready to carry out the transformation on day 3, you need to prepare some material in advance and to replate the colonies as patches with some of the provided elements of your kit. Each patch will provide sufficient bacteria for one lab group, and up to six patches will fit comfortably on one plate.

These steps can be performed by students on day 2.

2 days in advance

PREPARE MATERIALS

! max 1 month in advance

2 days in advance

CELLS FROM STABS

! max 7 days in advance

1 day in advance

PATCH THE COLONIES

! max 7 days in advance

2 days in advance

PREPARE MATERIALS

1 **Aliquot $CaCl_2$, prepare Luria Broth**

The CaCl2 has arrived 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 50 mM CaCl2 solution since each group will need only 400 µl of it. If you aliquot the solution, we recommend giving the students a 500µL 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.

! max 1 month in advance

2 days in advance

CELLS FROM STABS

1 Using 3 sterile inoculating loops or toothpicks transfer from one stab to an LB, not LB+amp, petri dish.

Watch the video - https://youtu.be/KU1XgordEY8

2 Repeat so both 4-1 and 4-2 are growing.

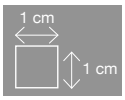
3 Place petri dishes in the incubator at 37°C overnight, or at room temperature but it may take 2 days.

37°C

1 day in advance

PATCH THE COLONIES

1 Using a sterile inoculating loop or toothpick, transfer some bacteria from one of the petri dishes to an LB (not LB+Amp) 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

Watch the video - http://youtu.be/qI_t868exWs

2 Repeat for each strain you will need for the transformation lab, six patches can fit comfortably on one LB Petri dish.

3 Place petri dishes in the incubator at 37°C overnight, or they can be grown at room temperature but it may take 2 days.

! max 7 days in advance

What a Colorful World Lab
DAY 3 - PREPARATION

In order to be ready to transform the cells, here are 2 checklists of the equipments and accessories that have to be ready for day 3.

Some have been prepared previously or on day 2 with students.

Equipment checklist

The accessories for the class

Item	Amount	
42° heat block or water bath	1	
Thermometer	1	
Decontamination solution	1	
Vortex, optional	1	
37°C incubator, optional	1	

Workstation checklist

What the students will need

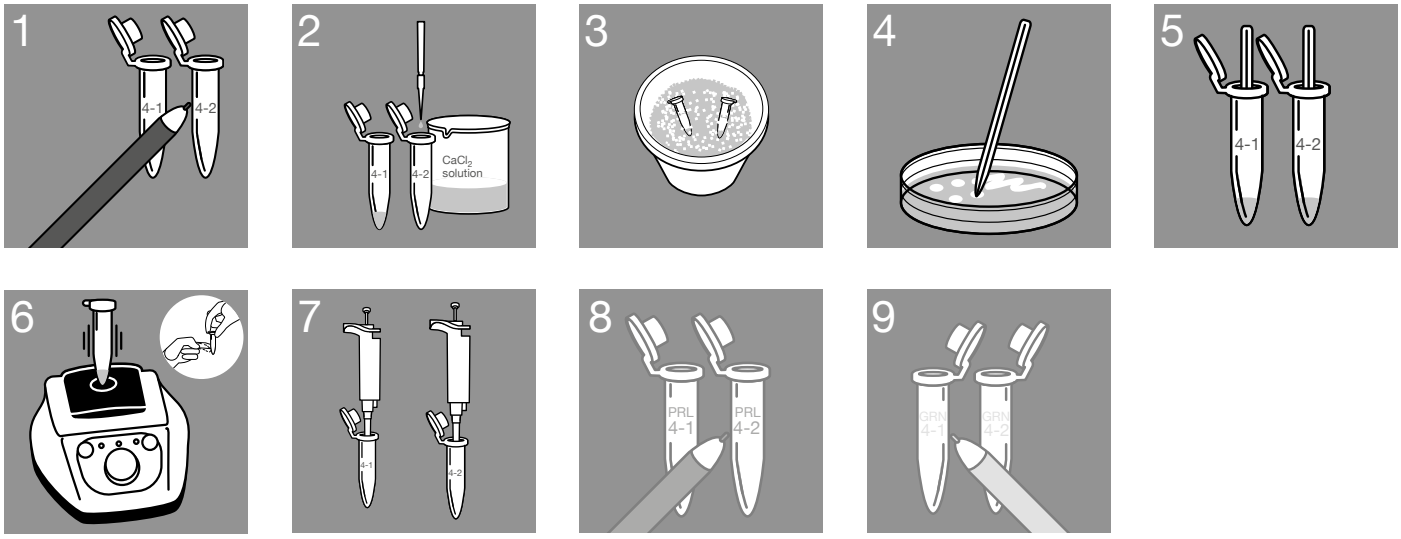
For each group

Item	Amount per group	✓
Bacterial strain 4-1	1 (patch)	
Bacterial strain 4-2	1 (patch)	
pGRN plasmid	2 (5 µl in each tube)	
pPRL plasmid	2 (5 µl in each tube)	
3.0 ml Luria Broth	1	
0.5 ml $CaCl_2$	1	
LB + amp agar plate	6	
Microcentrifuge tubes	6	
Rack for microcentrifuge tubes	1	
Micropipets and tips to measure 5 µl	1	
Micropipets and tips to measure 100 µl	1	
Micropipets and tips to measure 500 µl	1	
Sterile dowels or inoculation loops	2	
Sterile spreaders	6	
Container for contaminated waste	1	
Ice and ice bucket	1	
Timer	1	
Sharpie	1	

LAB PROTOCOL

Part 1:
Preparing strain 4-1 and 4-2 for transformation

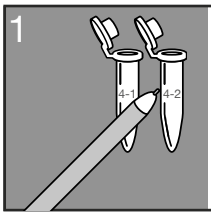
Neither of these E. coli strains will take up DNA from the environment until they are treated with a salt solution that makes their outer membrane slightly porous. The cells treated as directed here will become “competent” for transformation. They will be ready to bring DNA that’s external to the cell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



Teachers

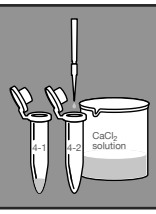
You should emphasize that this procedure can be tough on the cells. The students should be gentle and work quickly. It is essential that the tubes be kept on ice, preferably crushed.

1



Label two small microfuge tubes
“4-1” and **“4-2.”**

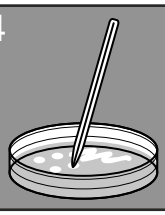
2



Pipet 200 µl of $CaCl_2$ transformation solution into each tube,
and then place the tubes on ice.

3

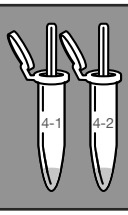
4



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 into its appropriate tube of cold $CaCl_2$.
A small bit of agar can get transferred without consequence to your experiment.

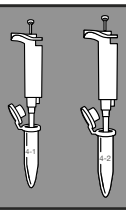
6

If you have a vortex, you can resuspend the cells by vortexing gently.
If no vortex is available, gently flick and invert the tube.

It’s okay for some clumps of cells to remain in this solution.

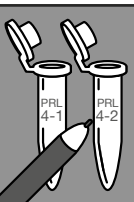
Keep these competent cells on ice while you prepare the DNA for transformation.

7



Retrieve two aliquots in microfuge tubes of each plasmid for a total of four samples:
2x pPRL, purple-generating device plasmid,
2x pGRN green-generating device plasmid.
Each aliquot has 5 µl of DNA in it. The DNA is at a concentration of 1 µg/µl.
You will need these values when you calculate the transformation efficiency at the end of this experiment.

8



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.

9

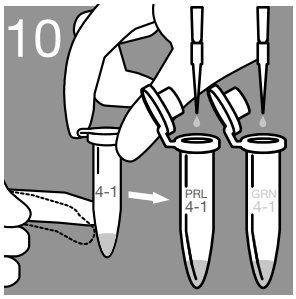
Label one of the **pGRN** tubes **“4-1.”**
Label the other **pGRN** tube **“4-2.”**

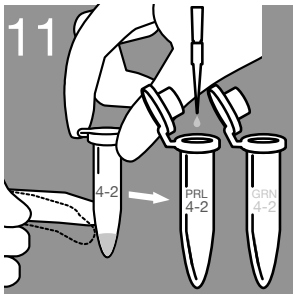
Ensure that the labels are readable.
Place the tubes in the ice bucket.

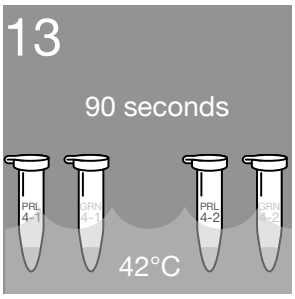
LAB PROTOCOL

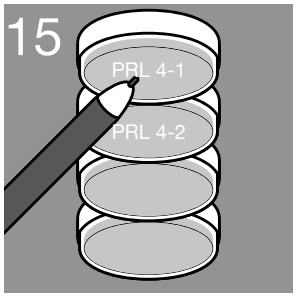
Part 2:
Transforming strains 4-1 and 4-2 with plasmid pPRL and plasmid pGRN

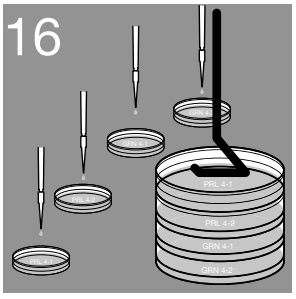
The cells you've prepared will be enough to complete a total of 4 transformations. You will transform the purple-color generator into each strain, and also the green-color generator into each strain. If you choose to, you can also use the last bit of competent cells as negative controls for the transformation.

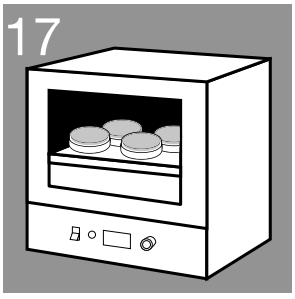










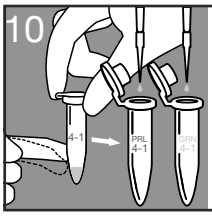


Watch the video - http://youtu.be/ayvElUIc0pg

Teachers

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

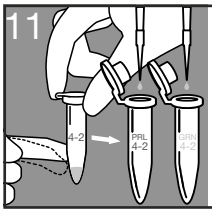
10



Flick the tube with the competent **4-1 strain.**
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.”**

Flick to mix the tubes and return them to the ice.
Save the remaining small volume of the 4-1 strain on ice.

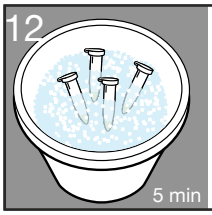
11



Flick the tube with the competent **4-2 strain**.
Then pipet 100 µl into the tube labeled **“pPRL, 4-2”** and an additional 100 µl into the tube labeled **“pGRN, 4-2.”**

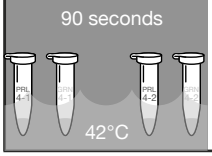
Flick to mix and store them, as well as the remaining volume of competent cells, on ice.

12



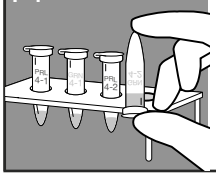
Let the DNA and the cells sit on ice for at least five minutes.

13



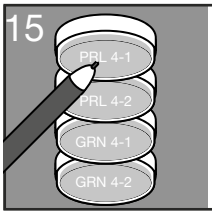
Heat shock all of your DNA/cell samples by placing the tubes at 42°C for 90 seconds exactly - use a timer.

14



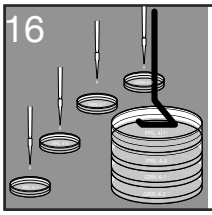
Move the tubes to a rack at room temperature and add 0.5 ml LB to each.
Close the caps, and invert the tubes to mix the contents.

15



While your DNA and cells are incubating, you can label the bottoms - media side of the six 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”.**

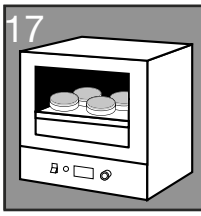
16



Pipet 200–250 µl of the transformation mixes onto the surface of LB+ampicillin agar petri dishes.

Immediately spread using a sterilized spreader or sterile beads.

17



Incubate the petri dishes with the agar side up at 37°C overnight not more than 24 hours.

The plates will be stored upside down to prevent condensation from dripping onto the bacteria.

After the petri dishes have incubated, count the colonies and observe their color.

DATA COLLECTION

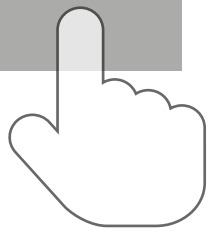
To achieve high transformation efficiency and clear color differences, it is important that students be precise when conducting the transformation protocol. For instance, a water bath in excess of 42°C or leaving the cells in the bath for more than 90 seconds may damage the cells and adversely affect 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 few days old since the transformation efficiency drops as the cells age.

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. We generally observe transformation efficiencies around $1x10^3$ 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.

If you find a great way to teach this or a follow-up experiment that other teachers could benefit from, please consider telling us about it by email: info@biobuilder.org



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 = $1x10^3$ colonies/microgram of DNA = transformation efficiency

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					

UNDERSTANDING THE DATA

1. 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.

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. 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.

4. 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.

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

NOTES

POST-LAB Q&A

1. What is expected to grow in each of the following cases

a. A LB agar plate with Ampicillin, growing E. coli that has undergone transformation with pPRL or pGRN.
We should see purple or green colonies, depending on the plasmid.

b. A LB agar plate without Ampicillin, growing E. coli that has undergone transformation with pPRL or pGRN.
We should see a lawn of untransformed cells due to lack of ampicillin in the media. Though some 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. A LB agar plate with Ampicillin, growing E. coli that has NOT undergone transformation.
There should be no bacteria because the ampicillin will kill the untransformed bacteria.

d. A LB agar plate without Ampicillin, growing E. coli that has NOT undergone transformation.
We should see a lawn of untransformed cells due to lack of ampicillin in the media.

2. What were some potential problems that may have affected the outcome of our 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.

3. If we had a plasmid that had a gene that turned bacteria green and a gene that broke down the antibiotic gentimycin, would we expect to get green bacteria on:

a. an LB plate?
b. an LB + ampicillin plate?
c. an LB + gentimycin plate?

a. We expect that the bacteria could grow as a lawn on the LB plate as there is no antibiotic present, but the cells would not be green since so few are likely to have the plasmid.

b. No bacteria could not grow on the ampicillin plate as they do not express a gene for ampicillin resistance.

c. The transformed cells could grow green on the gentimycin plate as colonies as they do express the gene for gentimycin resistance.

4. 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.

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Eau that Smell

Golden Bread

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