BioBuilder

Synthetic Biology for Students

introduction | acknowledgments | manual

WELCOME

We're glad you're here! You've just found a place where the most amazing technology is under construction, and it is biology.

Imagine all that's possible if we could intelligently apply what we know about the living world to solve its problems. What would you build from biology if you could build anything? Specialized cells that travel in your body to diagnose or treat a disease? Yeast that bake biofuel instead of bread? How about plants that grow into tree houses large enough for people to live in? Or maybe you'd want to purify contaminated drinking water by sprinkling an algae sponge on the surface?

Today, these ideas are more fiction than science. That can change, but before it does, we first have to get much better in the engineering of living systems. Other engineering disciplines have a lot to teach us. Nature has a lot to teach us. With the BioBuilding activities you'll find here, you can start learning.

The world is filled with examples of robust, self-assembling, self-correcting systems.

- You, for example -

What is synthetic biology?

Synthetic biology relies on all the facts from those thick biology textbooks and some of the tried and true principles of engineering. It puts them together to make and model useful living systems. Best case scenario for synthetic biology: we make novel systems that work reliably and address important world problems. Worst case scenario: the systems we build fail the first time... and the second and third... possibly failing in surprising or dangerous ways.

So at this early stage in synthetic biology both the successes and the failures have a lot to teach us. And though we still have a long way to go before it's easy to genetically program cells to perform particular tasks, you'll learn a lot by trying. And if you share your successes and failures with our BioBuilding community, you'll advance everyone's understanding— and in this way advance this new field.

A little bit more about

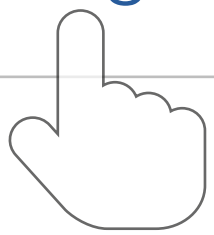
BioBuilder.org

These challenges have been developed in conjunction with the BioBuilder.org website.

BioBuilder.org provides animations to explore the underpinnings of synthetic biology, with links to the activities you find here. Feel free to look around. All the content is modular and so can be looked at in any order and at any time.

Once you've tried the BioBuilder activities, please share your data with the community. You'll be able to see how your findings compare to others. You can help us all learn how to better build with biology. Plus we'd love to hear how your construction projects are going.

Share your science
on
- www.biobuilder.org -



Acknowledgments

The lab you are about to enter was developed with materials from the University of Cambridge, UK, 2009 iGEM team, as well as guidance and technical insights from Drew Endy and his BIOE.44 class at Stanford University.

What a Colorful World

Exploring the role of the cellular chassis

background | protocols | analysis

Now is the time for you to become a real scientist and perform the very cutting-edge science that the best labs in the world are using. You will explore some of the most important questions that they are facing. Which cell will best meet the requirements for synthetic biology? Can we anticipate which cell will do the job we are trying to do? What are the difference between cells, and how do we control all that?

In other words, let's do some organism engineering.

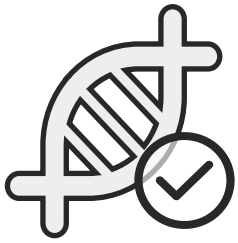
Student manual

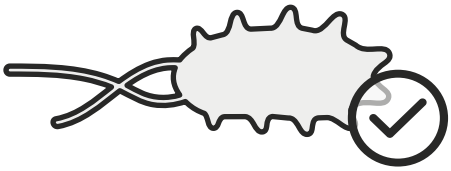
What a Colorful World
INTRODUCTION

Biodesign principles

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 for our genetic programs is a choice.

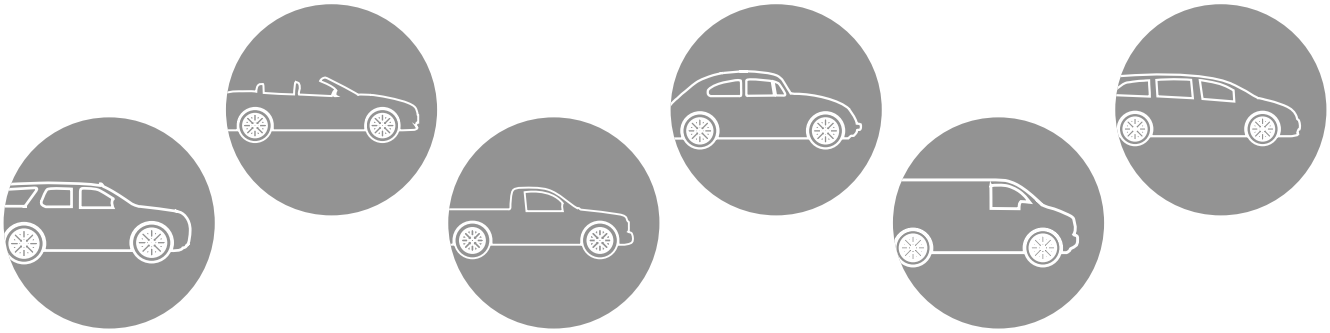
This BioBuilder activity is a reminder that, just as you carefully design a genetic program, you also need to carefully choose the host cell, or “chassis,” that will run it.





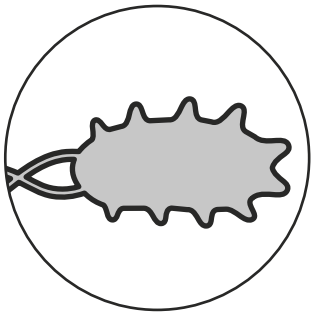
Let’s speak about cars

Cars are a useful analogy for our experiment because they represent a highly engineered system of interconnected parts. Many car parts perform similar functions in different chassis, but they must be tailored to the size and function of the car they’ll run in. For instance, we might be able to move a radio from a Ford truck into a Porsche 911, but we probably can’t move the drive train or the engine.

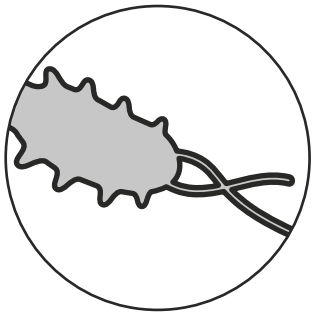


Choosing a Cellular Chassis

One trick that researchers use is to focus on strains they are familiar with, like *E. coli* and other domesticated cells, as chassis for experiments. In the case of *E. coli*, most of the strains that are used in research labs are one of two kinds. One strain is known as K-12 and the other B. Both strains are known to be safe and have been effectively used for genetic experiments for almost 100 years.



Originally isolated around 1922 from a diphtheria patient and characterized at Stanford University.



Originally isolated for phage research in Paris around 1918.

But even small genetic differences in these strains can trip up researchers, who often find that a genetic program works great in one chassis but will run differently in another. A key goal of synthetic biology is the reliable programming of cells, so fixing this lack of interoperability is a very active area of current research.

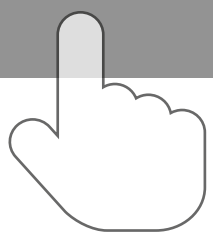
One approach to managing the complexity of the cellular chassis is to build new genetic programs in cells that can already do some of what the researcher wants. This approach uses a “nearest neighbor” for organism engineering.

Another approach to engineering a cellular chassis is to build a standard chassis for all organism engineering projects. It is essentially a blank canvas that is theoretically an ideal chassis for a variety of engineered systems with different desired outputs.

What a Colorful World
INTRODUCTION

In 2009, a Cambridge (UK) iGEM team designed a bacterial system to sense chemicals and turn colors. They called their system E. chromi.

Discover more about their project
http://2009.igem.org/Team:Cambridge

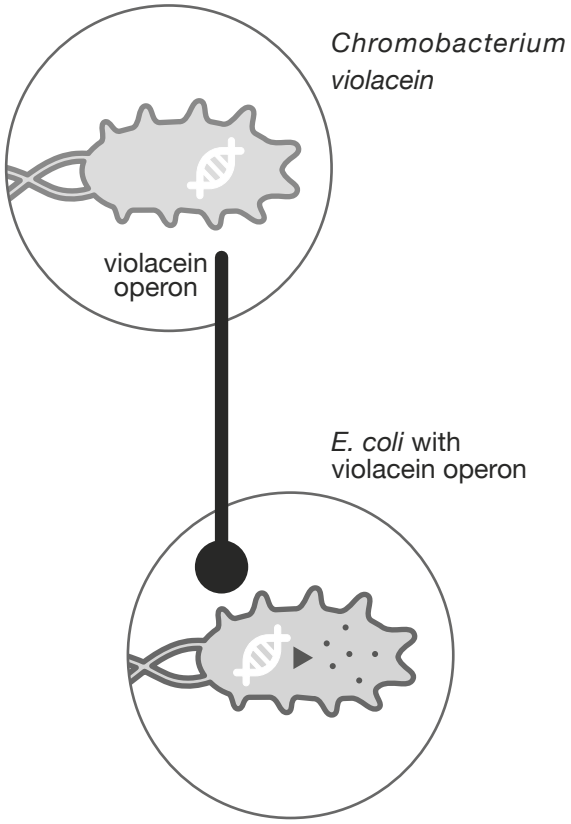


A bit of context

One potential use of engineered bacteria is as an indicator of toxic substances, such as arsenic or lead. Indeed, bacteria are very sensitive to the toxin levels, so if you find a way to see when they react to such chemicals, they would be a very good sensor.

One pigment they used is Violacein, a pigment produced by a handful of genes originally found in a different bacterial strain, *Chromobacterium violacein*. These genes were re-engineered and combined to produce either purple or green colors in *E. coli*.

To make the purple color, the team transferred to *E. coli* the entire violacein operon, which encodes five enzymes to metabolize L-tyrosine into a purple pigment. To vary the color, the team removed the third gene in the operon sequence, which makes the cell metabolize the L-tyrosine into a green pigment. These pigments are easily visible to the naked eye so could be used to make a biosensor that turns color in response to toxins.



What a Colorful World
INTRODUCTION

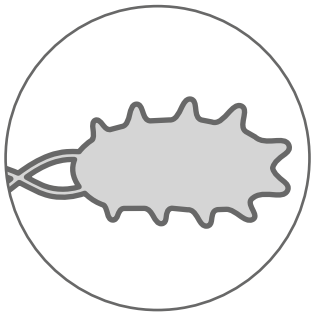
A few questions

Imagine that a group of engineers is manufacturing an arsenic sensor in *E. coli*. This group would like the intensity of purple color to vary as a function of arsenic level.

And now think about a second group of engineers who are also doing this but they use a different strain of *E. coli*. How sure can we be that the pigment will be expressed to the same extent in a different chassis? Thinking back to our analogy with car chassis: would an engineer put a V-8 engine from a Lexus into a Mercedes chassis? Would the engine behave the same? Would the car?

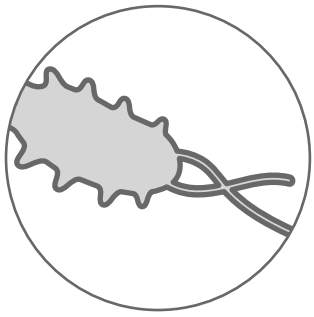
About your experiment

In this lab you will transform bacteria from two different strains of *E. coli*, in other words, two different chassis.



CHASSIS 1

Strain 4-1 is a
E. coli **K-12 strain**



CHASSIS 2

Strain 4-2 is a
E. coli **B-type strain**

Into each strain you will insert plasmids containing violacein-pigment devices. One plasmid, pPRL, has the purple version of this device while the other plasmid, pGRN, has the dark green version.

Purple device

Plasmid pPRL

Green device

Plasmid pGRN

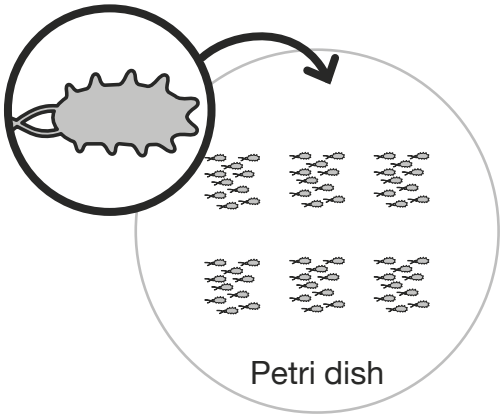
Both plasmids also code for resistance to an antibiotic, allowing cells containing the plasmid to grow on ampicillin-containing media. This will help you a lot.

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? Will the transformation efficiency be the same?

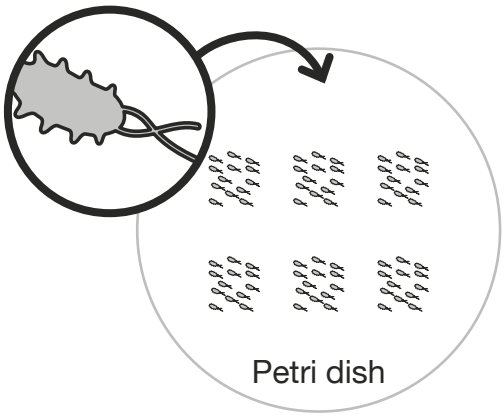
What you will do

Grow two strains

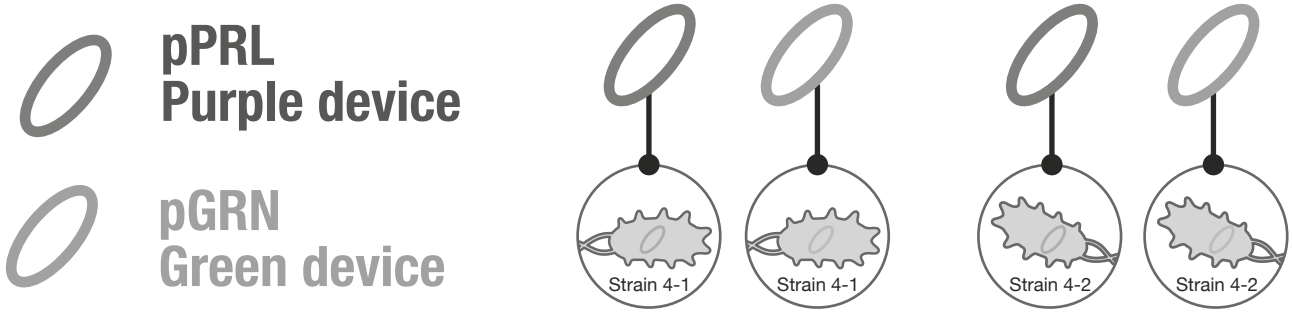
E. coli K-12



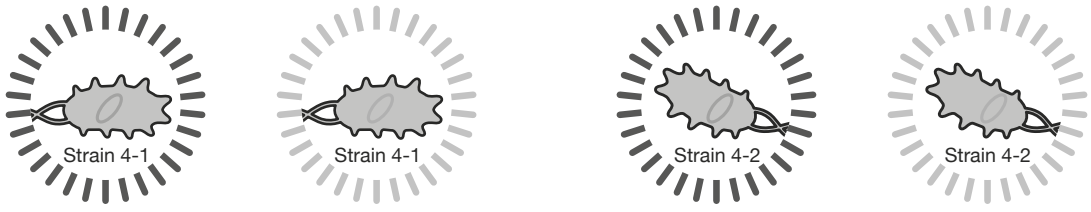
E. coli B



Insert plasmids into them



Analyze results



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

Define and properly use synthetic biology terms: chassis, minimal cell, synthetic cell, sensor, color generator.

Define and properly use molecular genetics terms: operon, gene expression, bacterial transformation.

Explain the role of the cellular chassis in synthetic biology and engineering.

Conduct and interpret the results of a bacterial transformation.

And that's awesome!

Pre Lab Questions to What a Colorful World Lab

To get ready to perform the experiment, let's see what you know about synthetic biology and this lab.

1. Briefly explain the field of synthetic biology.

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

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.

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*?

What a Colorful World
PRE-LAB Q&A

5. To a synthetic biologist, what is a chassis?

6. To a biologist, what is transformation?

7. What is meant when we say bacteria are “competent”?

8. What strains of *E.coli* will be used?

The two chassis we will use are _____ and _____.

9. What plasmids will be used?

The two plasmids we will use are _____ and _____.

NOTES

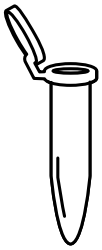
WHAT A COLORFUL WORLD - LAB PROTOCOL

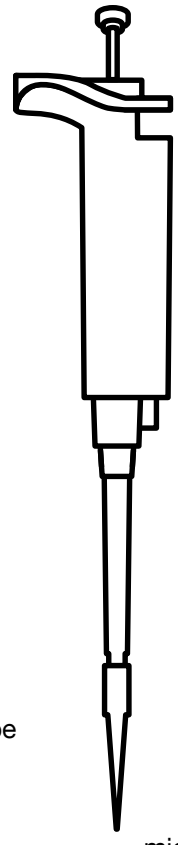
PART 1
Preparing strain 4-1 and 4-2 for transformation

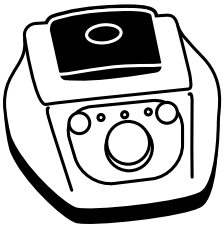
Neither of the two *E. coli* strains will take up DNA from the environment until they are treated with a salt solution that makes their outer membrane slightly porous. The cells will become "competent" for transformation. They will be ready to bring DNA that's external to the cell into the cytoplasm where the DNA code can be expressed.

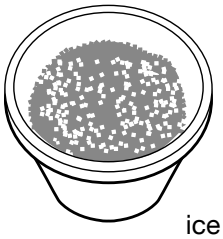
The cells will also become fragile. Keep the cells cold and don't pipet them roughly once you have swirled them into the $CaCl_2$ transformation solution.

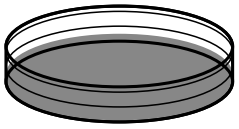

sterile pipet tip


microfuge tube


micropipet


vortex

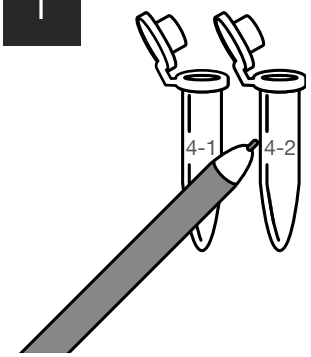

ice bucket


Petri dish

What a Colorful World Lab
PROTOCOL AT A GLANCE

PART 1

1



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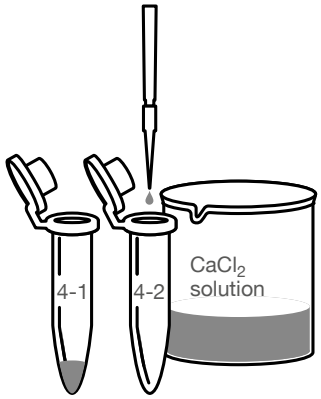

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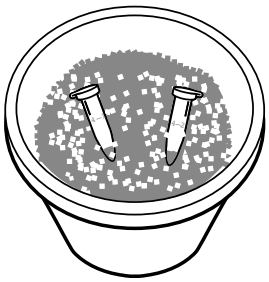

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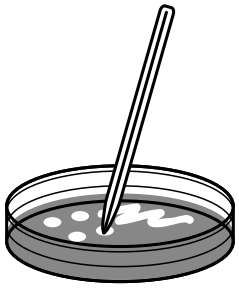

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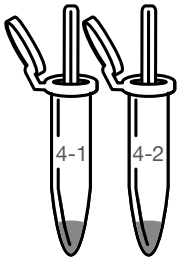

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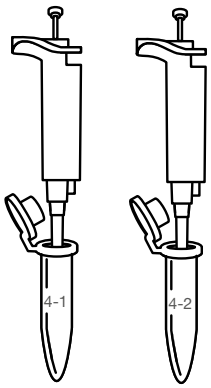

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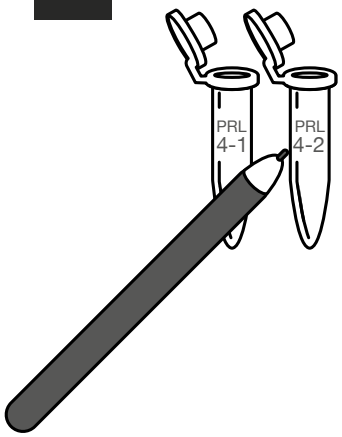

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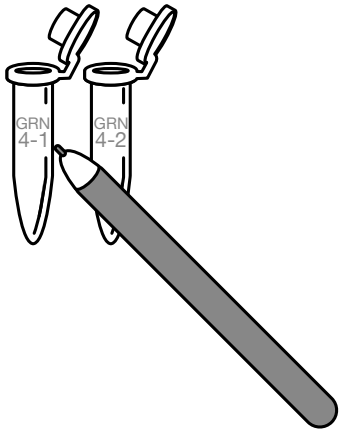
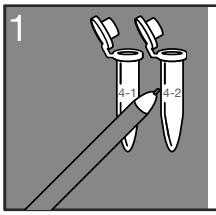


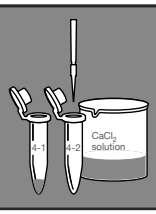
You should be gentle and work quickly.
It is essential that the tubes be kept on ice.

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.

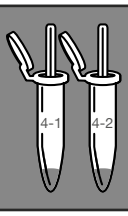
3

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.

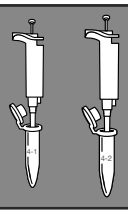
6

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.

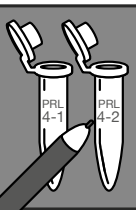
Keep these competent cells on ice while you prepare the DNA for transformation.

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.

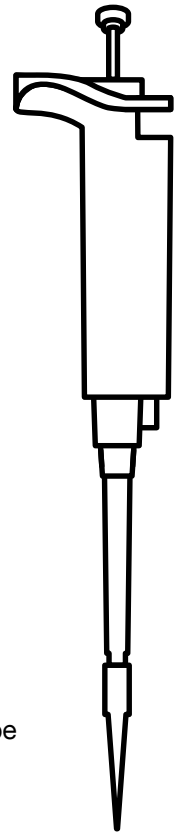
WHAT A COLORFUL WORLD - LAB PROTOCOL

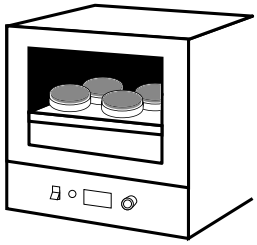
PART 2

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

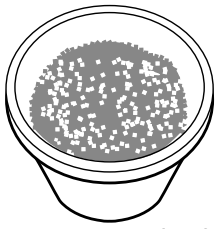
The cells you've prepared will be enough to complete a total of 4 transformations. You will transform the purple-color generator into each strain, and also the green-color generator into each strain. You will also use the last bit of competent cells as negative controls for the transformation.

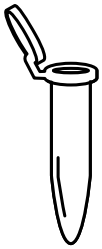

sterile pipet tip

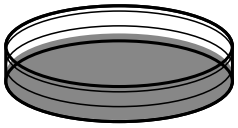

micropipet


incubator


sterilized spreader


ice bucket


microfuge tube

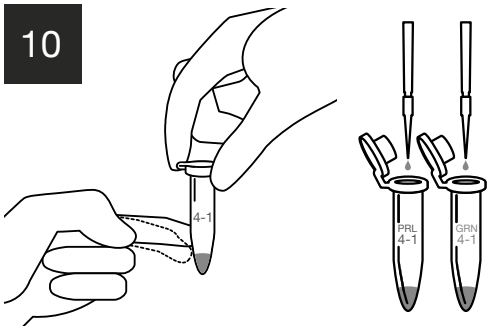

Petri dish

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

What a Colorful World Lab
PROTOCOL AT A GLANCE

PART 2

10



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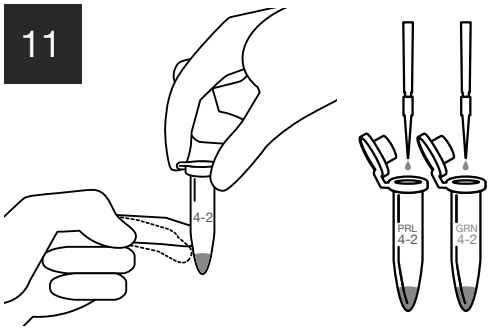

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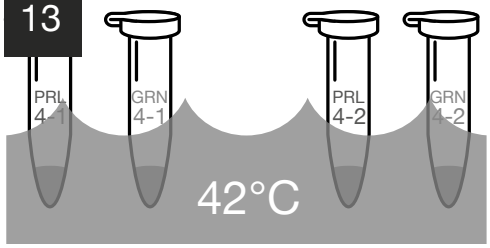

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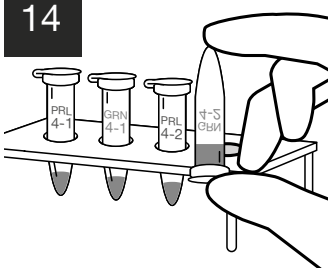

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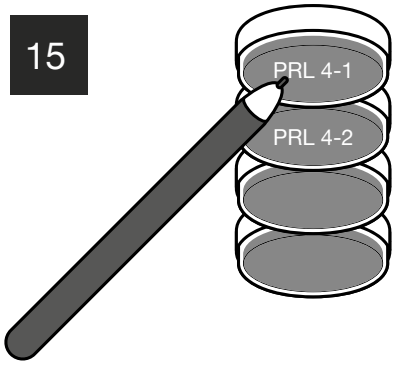

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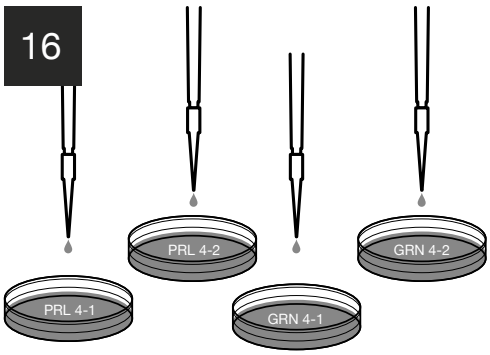

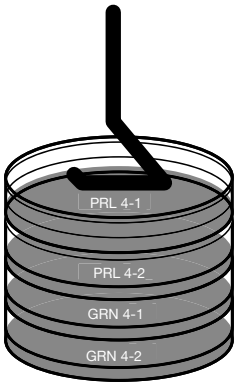

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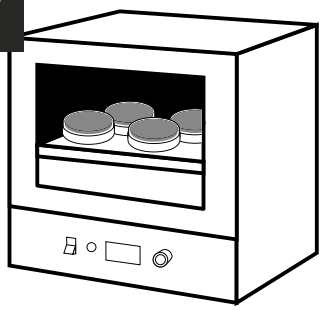
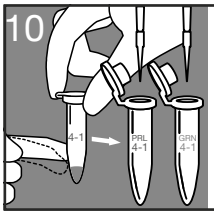


Watch the video - http://youtu.be/ayvElUIc0pg

It is important that each tube and plate is correctly labeled.

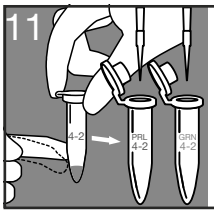
10



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.

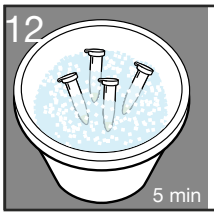
11



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.”**

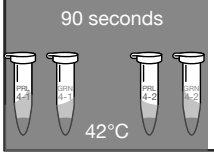
Flick to mix and store them, as well as the remaining volume of competent cells, on ice.

12



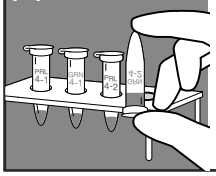
Let the DNA and the cells sit on ice for at least five minutes.

13



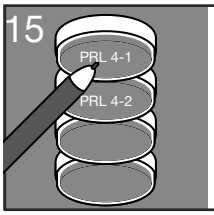
Heat shock all of your DNA/cell samples by placing the tubes at 42°C for 90 seconds exactly - use a timer.

14



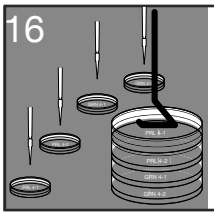
Move the tubes to a rack at room temperature and add 0.5 ml LB to each.
Close the caps, and invert the tubes to mix the contents.

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”.**

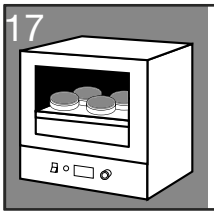
16



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 record their color.

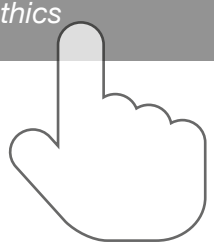
THE RESULTS

Collect and interpret

In your lab notebook, you will need to construct a data table as shown below.

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					

Also be sure to share your data with the BioBuilder community at
http://www.biobuilder-submitdata.org/users/login *password = natbioethics*



1 Count the number of colonies growing on each Petri dish

You can feel most confident in your results if there are between 20 and 200 colonies on the petri dish. Fewer than 20 and your value is affected by errors in pipeting that make large percentage differences in the outcome. Greater than 200 colonies and they become hard to count reliably. If the petri dish has many colonies growing on it, try to divide the dish into pie sections (1/4th or 1/8ths or even 1/16ths of the area), and then count a representative area. Finally, multiply the number you get for the section to get your total number of colonies. You'll still have some counting error, but perhaps less.

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.

Based on the number of colonies you find on each petri dish, calculate the transformation efficiency for each. Transformation efficiency is a measure for how well the cells incorporated the DNA. The units for transformation efficiency are "colonies per microgram of DNA." Each transformation used 200 nanograms (=0.2 micrograms) of DNA and you plated only 1/2 the transformation mixes on the petri dishes.

2 Data and Analyis

Based on these observations, do the DNA programs seems to be behaving identically in both strains for *E. coli*?

For example, does the **pPRL** plasmid give the same number of transformants and the same color in both strains?

What about the **pGRN** plasmid?

If you see differences, how can you explain them?

How could you test your explanations?

CALCULATION & LAB REPORT

CALCULATION

A simple way to know the transformation efficiency

Transformation efficiency	=	**number of colonies**	÷	**micrograms of DNA used**

Data

	100 colonies on a petri dish **but** 1/2 of the transformation mix plated	0.2 micrograms of DNA used

Calculation

	100 x 2 = 200 colonies if all were plated	0.2 micrograms of DNA used

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

What a Colorful World Lab
REPORTING YOUR DATA

LAB REPORT

Document and understand what you did and think about next steps.

1. Provide a brief introduction describing the field of synthetic biology.

2. What is a color generator? How does this color generator work? How might a color generator be useful?

3. Briefly describe the purpose of the lab. What are we trying to do here? Presume that a reader of your lab report has not read the assignment.

4. What is the role of the chassis?

5. How does chassis effect the expression of a genetic system?

6. How might synthetic engineers modify the relation between a chassis and an engineered genetic system to reduce the chassis effect on the system?

7. Why is it important to engineer a minimal or synthetic cell?

8. What are the advantages/concerns of engineering a minimal cell?

9. How might we test for the differences in the chassis that may be affecting a genetic system?

You may find helpful information at
http://en.wikipedia.org/wiki/Genetic_screen
http://en.wikipedia.org/wiki/DNA_m

METHODS

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

RESULTS

Present the data tables in clear format.

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

DISCUSSION

Draw a conclusion: Do the color generators produce the same results in different chassis? Justify your answer.

Analyze the data: Be sure to discuss how each part of the experiment and results adds to your conclusion.

Are we sure that the transformation worked? What do the controls that lacked plasmid tell us?

Discuss errors and other reasons for data variability.

Use your results to explain why it is important for synthetic biologists to fully characterize the chassis used in an engineered system.

Post Lab Questions to What a Colorful World Lab

Let's wrap this up and see what you know now.

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.

b. A LB agar plate without Ampicillin, growing E. coli that has undergone transformation.

c. A LB agar plate with Ampicillin, growing E. coli that has NOT undergone transformation.

d. A LB agar plate without Ampicillin, growing E. coli that has NOT undergone transformation.

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

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?

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

5. What is one thing you learned from this lab and one thing that you are still confused about? Did you like the lab?

MORE SYNTHETHIC BIOLOGY ON

BioBuilder.org

What a Colorful World

The iTune Device

Picture This

Eau that Smell

Golden Bread

Design of this manual by
Norman Wozniak - www. normanwozniak.com