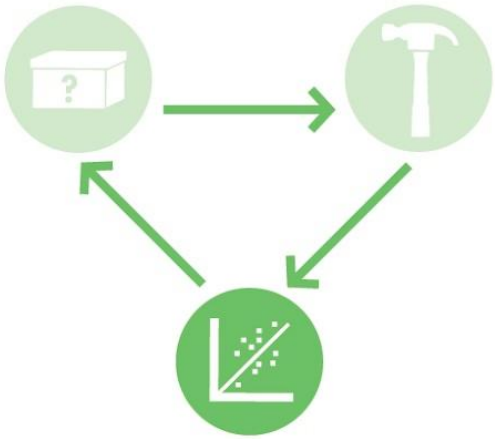
TEACHER MANUAL

BioBuilder

Synthetic Biology for Teachers

iTune Device

This activity focuses on predictable biodesign and the composition of genetic "parts," such as promoters and ribosome binding sites, to better understand how assembly influences the output of a genetic device.



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

iTunes Device

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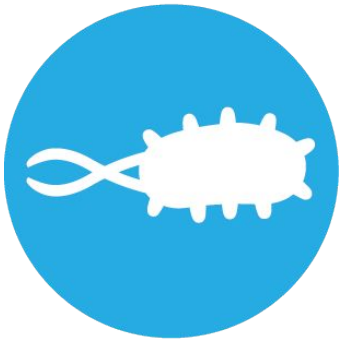
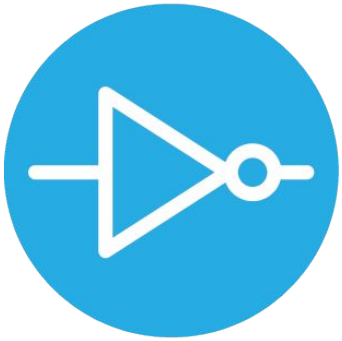
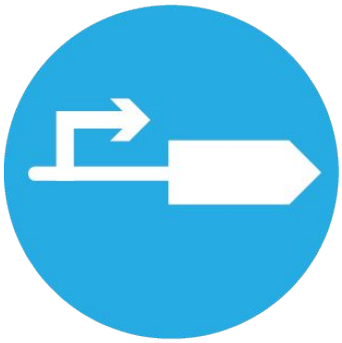




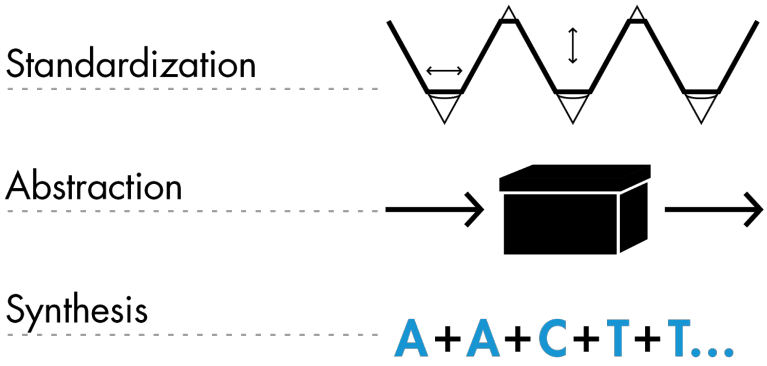


About Synthetic Biology

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 from 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



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:

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

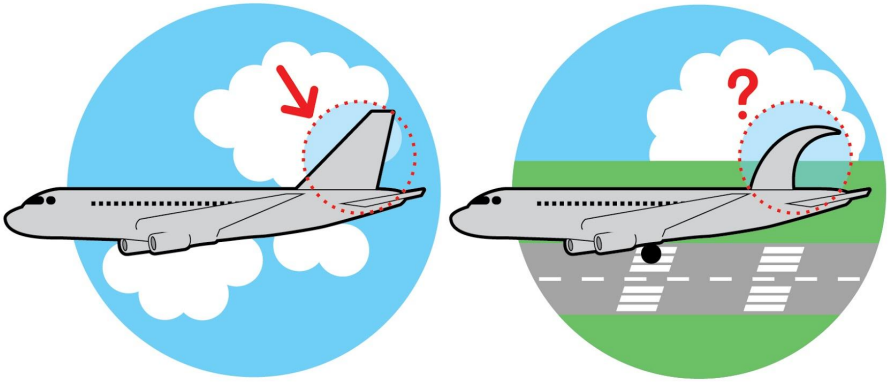
Compares two alternative genetic designs. Both programs should make the cells smell like ripe bananas as the cells grow.

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.

PREDICTABLE DESIGN

What would a company like Boeing or Airbus think if a new wing shape made the plane fly in unexpected ways? Designing and building in the face of such uncertainty would create huge expense and potentially put lives in danger.



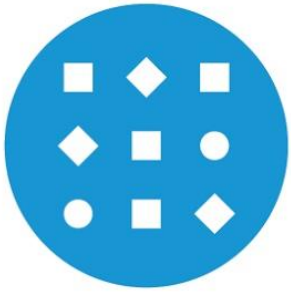
If an engineer modifies the standard tail of an airplane (left), then the novel design (right) must not introduce unintended behaviors that affect how the airplane can fly or land safely.

To predictably design new systems, engineers rely on modular components that can be functionally assembled in a variety of ways, customizing combinations according to an individual's needs. When the modular components are physically connected, the parts must function as expected. Significant differences between predicted and observed behavior are unacceptable.

At this point in the field of synthetic biology, biological engineers are still working toward such functional assembly of genetic parts. Researchers have characterized many cellular behaviors at the molecular level. In many cases it's possible to catalog the genetic elements necessary and sufficient to carry out a biological function. Yet, combining these genetic components in new ways often generates unexpected results.

To improve the functional assembly of biological parts, synthetic biologists have focused on the modularity, insulation, and measurement of genetic parts. These principles are more traditionally applied to the engineering of nonliving systems, but here they are tested in the context of an enzyme-producing genetic device. BioBuilder's iTune Device tests a variety of genetic circuits in cells, in order to compare their expected and their measured behaviors.

Modularity

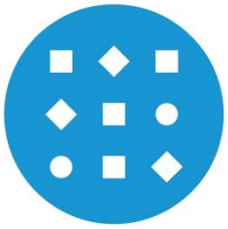


Insulation

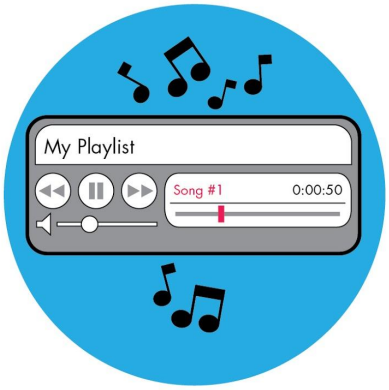
Measurement

Modularity, Insulation, Measurement

Modularity



Modularity refers to the idea that engineers can design and generate systems by combining functional units, or "modules." To understand the advantages gained from modularity, consider the way music distribution has changed. For much of the 20th century, the album was the standard unit for the music industry and its artists. Single songs were available but were significantly more costly, so even if people only liked a few of the songs on an album, they generally bought the album.

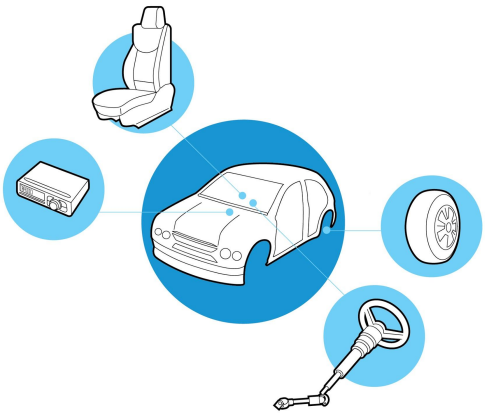


Once music was digitized, it became possible to download it. Suddenly, buying the physical album was no longer necessary, and individual songs from any digital album could easily be unbundled. The songs became independent modules that listeners could mix and match as desired and needed.

Modularity is sensible to apply to biology as well, because we can attribute discrete functions to particular snippets of DNA and protein domains. This notion of "genetic parts" is fundamental to synthetic biology.

Insulation

Insulation keeps modules from interacting from with one another in undesirable ways. This is a familiar feature of many engineered objects. For example, the operation of a car's stereo doesn't affect the operation of the driver's steering wheel. If it did, then twisting the knob on the radio might also turn the steering wheel -- presenting a real challenge for safe operation of the car.



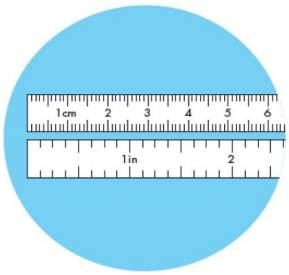
Unlike a car, the cell is a fluid environment. The molecules, proteins, and cellular structures are constantly mixing. How is it possible to insulate their behaviors when they encounter new partners and neighbors all the time?

One of the major challenges in synthetic biology is to rationally design living systems with component parts that do not interfere with one another or with the basic cellular machinery needed to maintain growth and life.

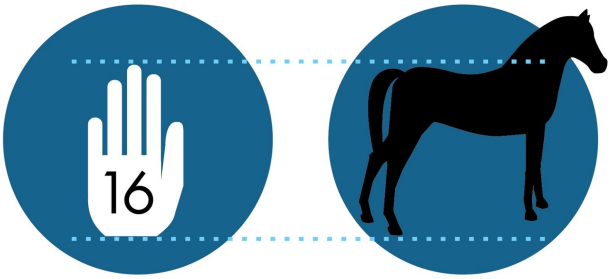
Modularity, Insulation, Measurement (con't)

Measurement

Whether it's the number of cards in a deck, the horsepower in a car engine or the score in a football game, measurements tell us about the state of the items, and their behaviors, relationships, or characteristics. Measurements allow us to compare items with one another, using an agreed upon unit for measuring them. Units might be taken for granted in something like sports, where teams get a standard number of points for each basket or goal. But some measurements are less clear and in some cases, like the English vs the Metric system, the units are not even agreed upon.



English v. metric



You might think it would be easy to measure the height of a horse, for example. From hoof to wither (~shoulder) could be 67 inches or maybe 1.7 meters. In fact, such a horse would be 16.3 hands high, since King Henry VIII standardized a hand (his hand) to be 4 inches and the units after the decimal to reflect additional inches.

The power of measurements

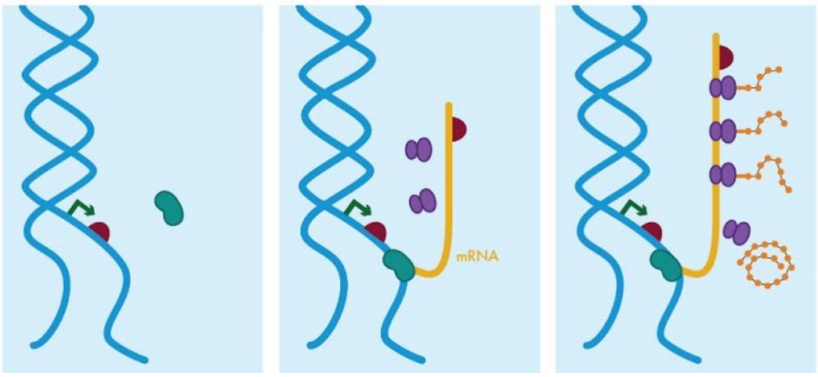
Scientists use measurement as a powerful tool for discovery. Mendel, for instance, uncovered the rules for inheritance by counting offspring with particular traits and looking for patterns. Engineers use measurement not only to describe systems but also to control, assemble, and improve the objects being measured. Some engineering measurements are described below.

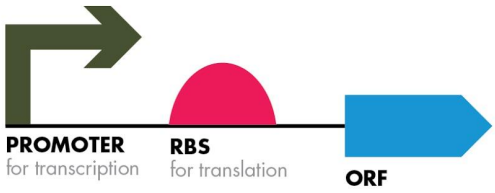
Measurement	Description	Utility
Static performance	Maps a range of controlled inputs to a part's measurable final output(s)	Helpful for ensuring one part's output will be sufficient to trigger the next part in a circuit
Dynamic performance	A part's output over time in response to a change in the input signal	Shows how a system will behave upon initial stimulation, which may differ from stabilized long-term behavior
Input compatibility	How a part responds to various inputs	Illustrates the part's flexibility for composition with various upstream parts/inputs
Reliability	Measured as Mean Time to Failure (MTF)	Used to determine how long the system can be expected to behave as originally specified
Consumption of materials or resources	Determines choice of power supply or resource pool	Affects chassis decisions among other things

By measuring the actual performance of synthetic living systems and comparing the measurements to what was predicted, synthetic biologists can assess their designs and move closer to correctly anticipating the success or failure of future designs.

Introduction to Gene Regulation

The mantra "DNA makes RNA makes protein" is shorthand for the knowledge that RNA sequences are transcribed from DNA sequences, and that those RNA sequences can be translated into the proteins that carry out many of the key jobs in a cell. Transcription and translation have been extensively studied so many of the core components that control these processes are known.

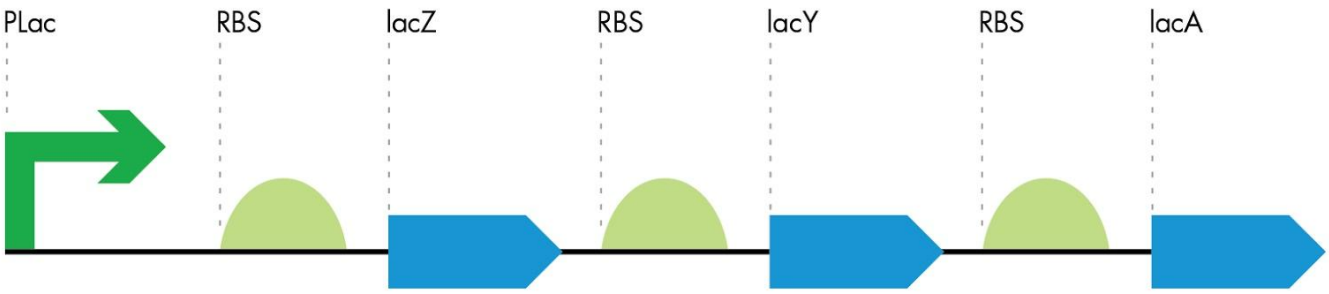




For example a promoter is the term for a DNA sequence where RNA polymerase binds and initiates transcription. Similarly, the sequence where translation initiates in bacteria is a site known as the ribosome binding site (RBS), defined as the DNA sequence that allows ribosomes to begin protein synthesis from the RNA copy. Additionally, an open reading frame, abbreviated "ORF," represents a DNA sequence that encodes a protein. It is often illustrated as an arrow or a box. The direction of the arrows indicate the direction on the DNA strands in which the promoter or ORF are read.

The lac operon

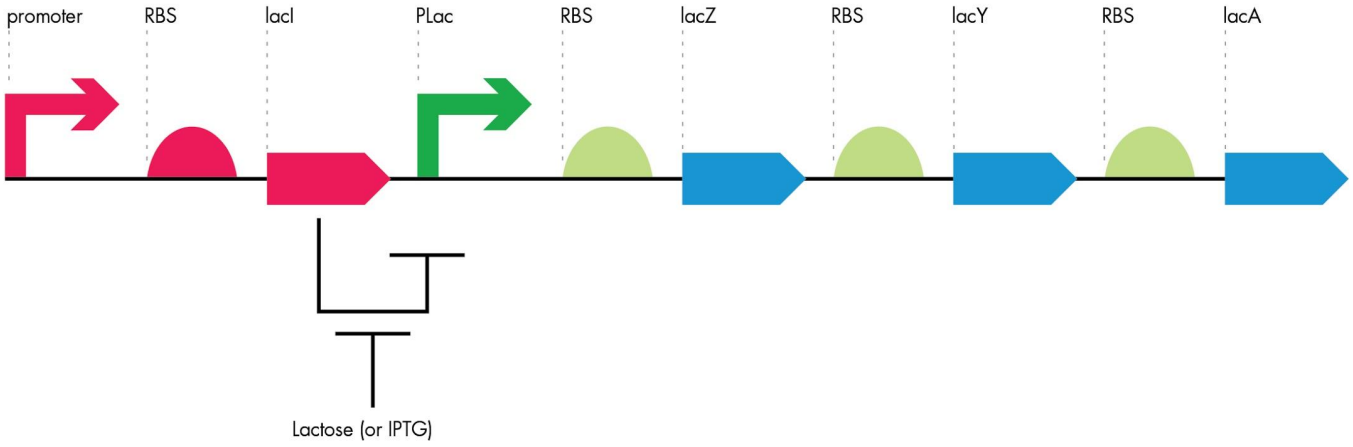
In the 1960s, Dr. Francois Jacob and Dr. Jacques Monod identified foundational principles of gene regulation through their studies of lactose transport and metabolism in bacteria. The genes for lactose metabolism are clustered in the lac operon. The lac operon consists of a single promoter (pLac, green arrow) controlling three downstream RBS–ORF pairs (green semicircles and blue arrows, respectively). A single mRNA is transcribed from the lac operon's promoter, giving rise to the multiple protein products needed for lactose metabolism and transport. Translation of each product can occur from the single mRNA thanks to the RBSs that are associated with each ORF.



Introduction to Gene Regulation (con't)

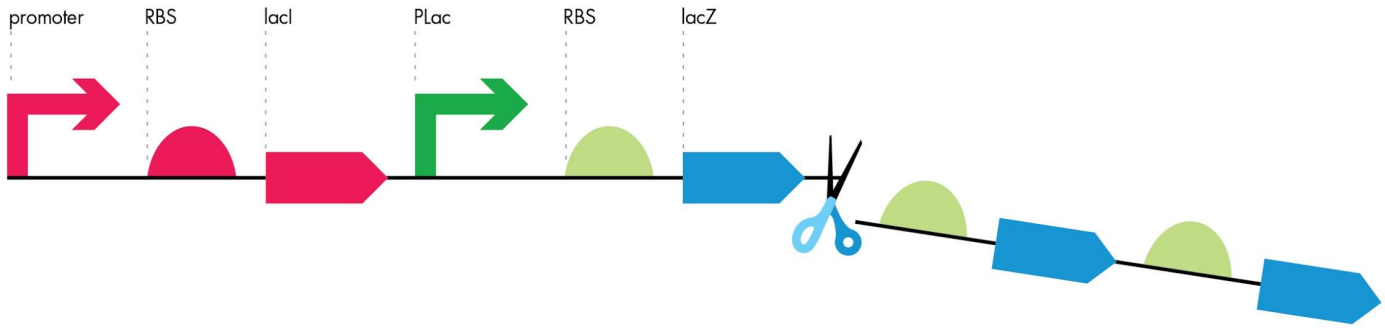
Bacteria conserve energy by transcribing the lac operon genes only when glucose is absent. When glucose is present, a transcriptional repressor protein encoded by the LacI gene turns off transcription of the lac operon. When lactose is present and glucose is absent, the lac repressor protein switches behaviors and allows for transcription of the operon so lactose can be transported into the cell and metabolized.

In this lab, the cells will be grown in the presence of IPTG, which will artificially remove the lac repressor protein from the Plac promoter. In this way we can be sure that measurements reflect the fully de-repressed promoter.



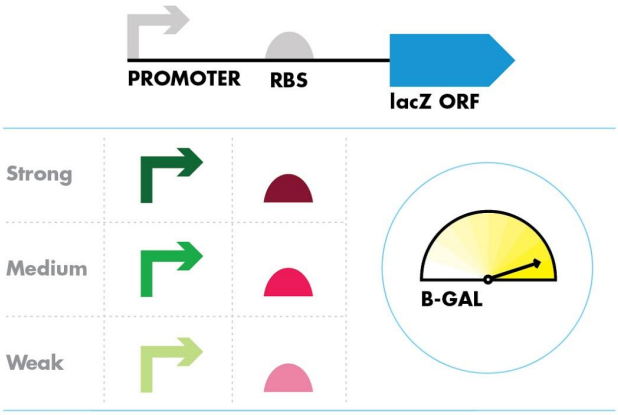
The key protein for lactose metabolism is an enzyme called β-galactosidase, often abbreviated β-gal, and it is encoded by the ORF called lacZ. The β-gal enzyme cleaves lactose into glucose and galactose, which can be used by the cell to power its other functions. Researchers found that β-gal reacts with a variety of molecules similar to lactose, including synthetic analogs such as ONPG, which you will use in the iTune Device lab.

In this lab, you will react the cells with ONPG, which will turn the solution yellow according to how much β-gal the cells are making. More yellow → more β-gal!



While the other ORFs in the lac operon, lacY and lacA, are important for lactose transport and metabolism in wild-type bacteria, they are dispensable for BioBuilder's iTune Device experiment. Your measurements of lacZ expression in this experiment will be dependent only on the Plac promoter and ribosome binding site.

BIOBUILDER'S iTUNE DEVICE ACTIVITY



BioBuilder's *iTune Device* activity emphasizes the "test" phase of the design-build-test cycle. Standard biological parts were combined to build several variants of an enzyme-generating genetic circuit. The circuits have small differences in their DNA sequences, which are expected to change the amount of enzyme the cells produce.

What predictions can be made about the output for the genetic circuits?

It seems reasonable that a "strong" promoter would give rise to more enzyme activity than a "weak" promoter. But can we predict just how much more? Examining the DNA sequences of the parts gives some clues to the activity of the parts because the most commonly found sequence for each part is called the "consensus sequence." Changes from the consensus will affect the efficiency of transcription for the promoter parts and of translation for the RBS parts -- though it's hard to know by how much.

Even more difficult to anticipate is how modular and how insulated the parts are from one another. Perhaps a medium promoter and a strong RBS will give greater activity than a strong promoter and a medium RBS. Luckily, the output of each genetic circuit can be measured with an enzyme assay, allowing predicted and actual behaviors to be compared quantitatively.

MEASURING ENZYME ACTIVITY

The assay for β-gal activity is carried out on cells that have been grown in LB with Ampicillin and IPTG. The Ampicillin selects for the plasmids carrying the genetic circuits. The IPTG insures that the lac repressor protein is not binding to the promoter region of the circuits.

Once the cells are grown, their density is measured using a spectrophotometer set to 600 nm. The spectrophotometer measures the amount of light scattered by the cells. More cells lead to more scattering and a higher absorbance. If no spectrophotometer is available, then it is possible to use MacFarland standards, made with barium chloride and sulfuric acid.

The cells are then lysed with detergent to release the β-gal enzyme from the cell , reacted with ONPG for a measured amount of time, and then the reactions are stopped by changing the pH of the solutions which inactivates the enzyme. The amount of yellow color in the reactions is measured with the spectrophotometer set to 420 nm. If no spectrophotometer is available, then it is possible to use yellow paint samples, as described on the BioBuilder website.

Reference measurements

To compare data collected by different laboratory groups, you will use a "reference" promoter:RBS:lacZ sequence. This reference is known to generate some intermediate amount of enzyme, so you can use it to calibrate all the other measurements you make. The reaction run without any cells can serve as one negative control as well as the blank for the spectrophotometer.

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.

B-galactosidase (β-gal) is an enzyme that normally cleaves the disaccharide, lactose, into the monosaccharides, glucose and galactose. For the enzymatic measurements we make in this lab, we use ONPG rather than lactose. Why?
ONPG is structurally similar to lactose but when it is cleaved by β-gal one of its products is o-nitrophenol, which is yellow-colored. This way we can measure the amount of enzyme by measuring how much yellow color is formed in a given amount of time. When β-gal cleaves lactose, both products are colorless so we can't know if the enzyme has worked.

In this lab, we are varying the amount of enzyme that a cell can produce by varying the strength of two gene expression elements. Name them, the proteins they bind and processes they control.

Part	Binds	Process controlled
Promoter	RNA Polymerase	transcription
RBS (ribosome binding site)	Ribosome	translation

Why can't we just assume that the strongest promoter and the strongest ribosome binding site, when combined, will generate the most β-galactosidase?
Biologically speaking: the cell's resources might get used up making so much enzyme, resulting in equal or even less enzyme than in a case with a combination of weaker parts. Speaking as an engineer: the ability to predict the functional properties of assembled parts is a goal but not a reality yet since we don't always know everything about a cell that can influence the behavior of devices.

To a synthetic biologist, what is a part?
A part is a DNA-encoded human defined genetic function, for example a promoter is a part since it is a sequence of DNA that binds RNA polymerase to initiate transcription.

The reactions you will perform must be started at precisely timed intervals. Why do you not start all the reactions at the same time?
It's not possible to start them all at exactly the same time, and we want each reaction to proceed for exactly the same amount of time.

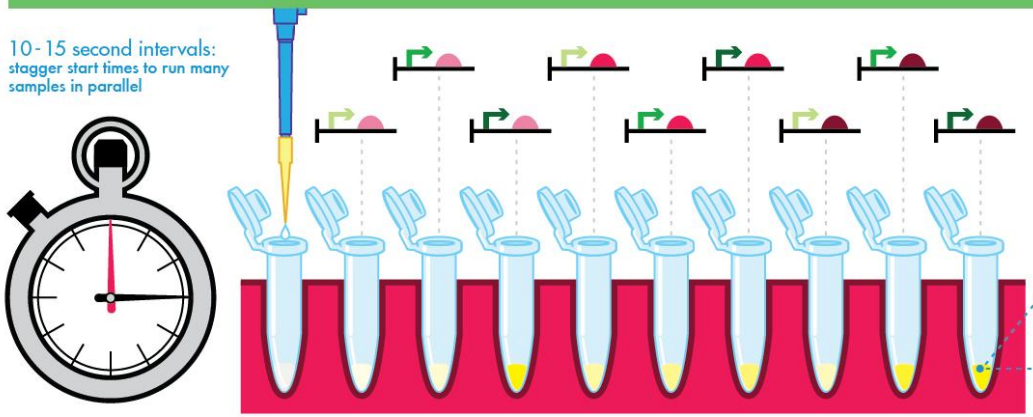
The reactions include a step when you add detergent. Why is it added?
The detergent lyses the cells to release the β-gal enzyme into solution where it can be measured.

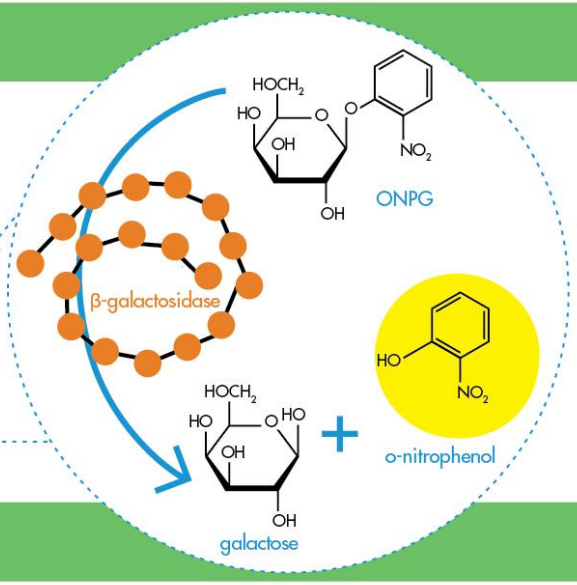
The reactions are stopped by the addition of sodium bicarbonate. Why does this work?
The pH of the solution is changed so the enzyme is no longer functional.

iTUNE DEVICES

MAKING THE INVISIBLE VISIBLE

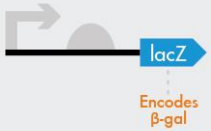
10-15 second intervals:
stagger start times to run many samples in parallel

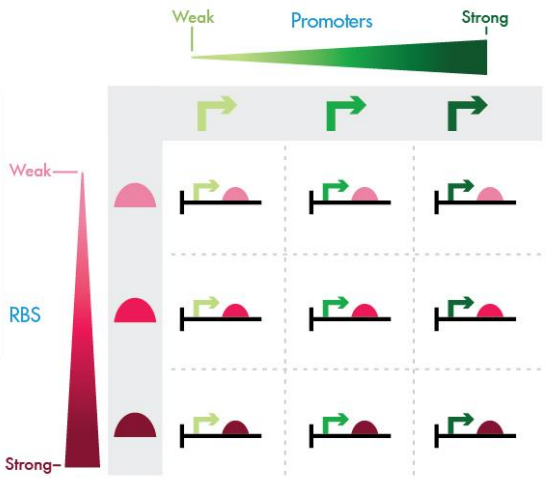


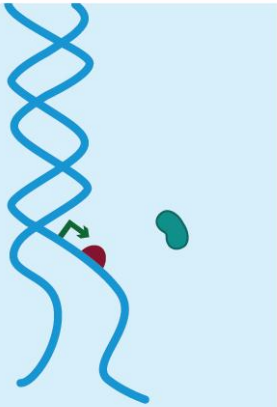


UNDER THE HOOD

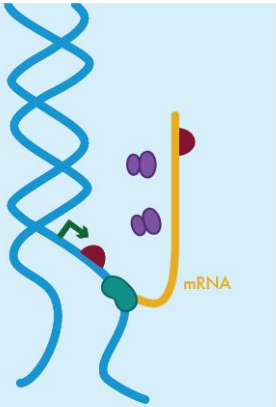
Mix and match Parts
(RBS & Promoters)
to make the iTunes library:



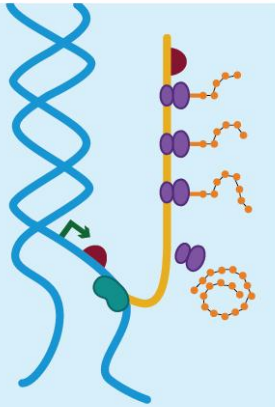




RNA polymerase binds to promoter and makes mRNA



Ribosomes bind to the RBS and make the β-gal enzyme



The more β-gal enzyme is made, the more enzymatic activity results

BioBuilder
Educational Foundation

CHECKLIST FOR KIT CONTENTS

- ☐ Bacterial strains (10 stabs)
- ☐ Luria Broth (3 bottles, 200 ml/bottle)
- ☐ 10 mg/ml Ampicillin (1 vial, 4 ml/vial)
- ☐ IPTG (1 vial, 24 mg/vial)
- ☐ ONPG (1 vial, 40 mg/vial)
- ☐ Sodium bicarbonate (3 bottles, 75 ml/bottle)
- ☐ Sodium carbonate (3 bottles, 75 ml/bottle)
- ☐ SDS (1 vial, 10ml
- ☐ 15 ml conical tubes (2 packs, 25 tubes/pack)
- ☐ Cuvettes (2 packs, 100 cuvettes/pack)
- ☐ Sterile inoculating loops (1 pack, 30/pack)
- ☐ 13x100 mm glass tubes (250)
- ☐ Test tube racks (6)
- ☐ 1.5 ml microfuge tubes (3 packs, 30/pack)

Unpacking your kit

- Store the Luria Broth, bicarbonate, carbonate and SDS on the shelf until the day of the experiment
- Store the Ampicillin, IPTG and ONPG in the fridge (4°C)
- The bacterial stabs can be kept at room temperature or in fridge

Up to one month in advance of lab

Dissolve the IPTG: add 1000 ul of clean or (ideally) sterile water to the vial of IPTG and vortex to dissolve. Store the dissolved IPTG in the fridge until you are ready to set up the overnight cultures. **Dissolve the ONPG:** mix the 40 mg of ONPG in 40 ml of water and vortex to dissolve. Store frozen for several months or in the fridge up to one month.

Up to one week in advance of lab

Prepare growth media by mixing 3 ml of LB with 30 ul of ampicillin and 30 ul of IPTG in 15 ml conical tubes. Prepare 10 tubes total, one for each strain to be grown. Using a sterile inoculating loop to transfer some of each bacterial strain from the stabs to the media. Grow on a roller wheel at 37°C overnight. If there is no roller wheel or incubator available, you can increase the volume for each starter culture to 10 ml LB+amp+IPTG, and you can grow the samples in small Erlenmeyer flasks with a stir bar at room temperature. You should grow them this way for at least 24 hours to reach saturation.

Alternative materials

You can dissolve 1 g Arm & Hammer baking soda in 50 ml water to make the 2 % sodium bicarbonate.
You can dissolve 5.3 gram soda ash in 50 ml water to make the 1M carbonate solution.
You can use 1 "squirt" clear liquid dish soap in 50 ml water as an alternative to the SDS

Each student bench will need: bacterial cultures, 1.5 ml SDS, 1.5 ml ONPG, 35 ml bicarbonate, 15 ml sodium carbonate

The lab will need

- Vortex
- Spectrophotometer (optional)
- Biohazardous waste disposal
- 37° incubator, though strains can be grown on stir plates at room temperature for one day longer
- Timers
- Micropipets and tips
- Sharpies
- Latex gloves
- Roller wheel (optional)

QUICK GUIDE: ITUNE DEVICES

IN ADVANCE

Grow liquid overnight cultures of the 10 strains to be tested** Don't forget to add ampicillin and IPTG to growth media!

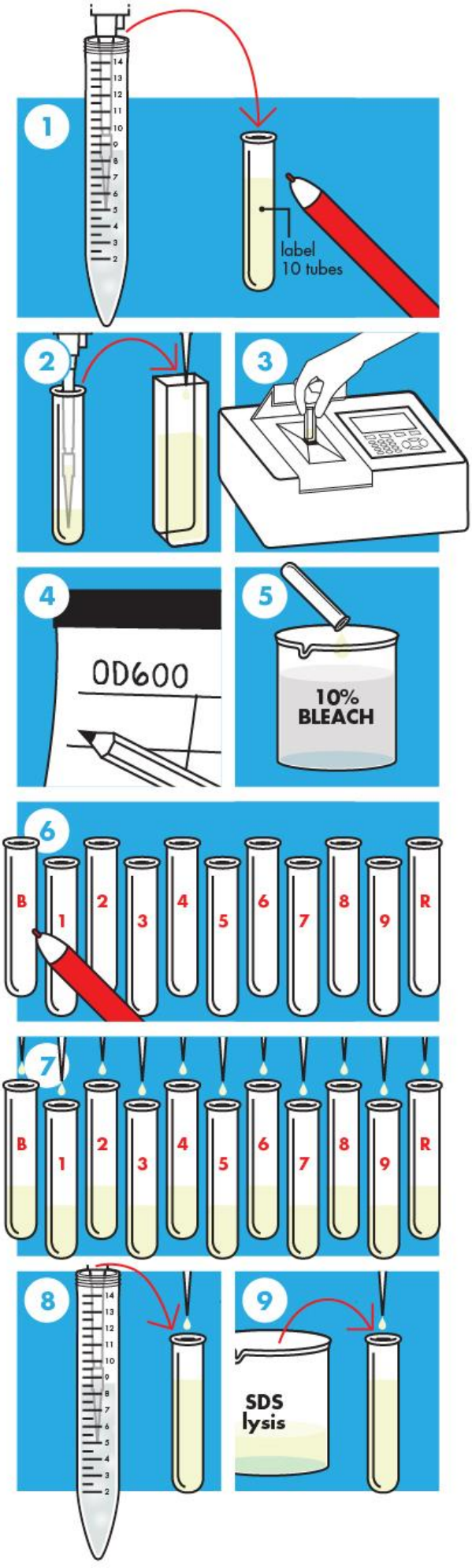
DAY OF LAB

Cell Density Measurements:

1. Label 10 glass tubes "**1**" through "**9**" and "**R**" for reference. Make a 1:10 dilution of each overnight culture by mixing 300 µl of cells with 2.7 mL of bicarbonate buffer.
2. Transfer each sample to a cuvette, filling the cuvettes about three-quarters full.
3. Read and record the absorbance value for each sample. Start by zero-ing the spectrophotometer set at **600** nm using bicarbonate buffer or water.
4. Record the value x 10 in your data table under the **OD600** heading.
5. Discard all dilutions in 10% bleach.

Enzyme Reactions:

6. Label 11 glass tubes "**1**" through "**9**" as well as "**B**" for blank and "**R**" for the reference.
7. Add 1.0 mL bicarbonate buffer to each tube.
8. Transfer 100 µl of cells from the UNDILUTED overnight cultures to the appropriate tube. Transfer 100 µl of bicarbonate to the blank.
9. Add 100 µl of SDS lysis solution to each tube, including the blank.



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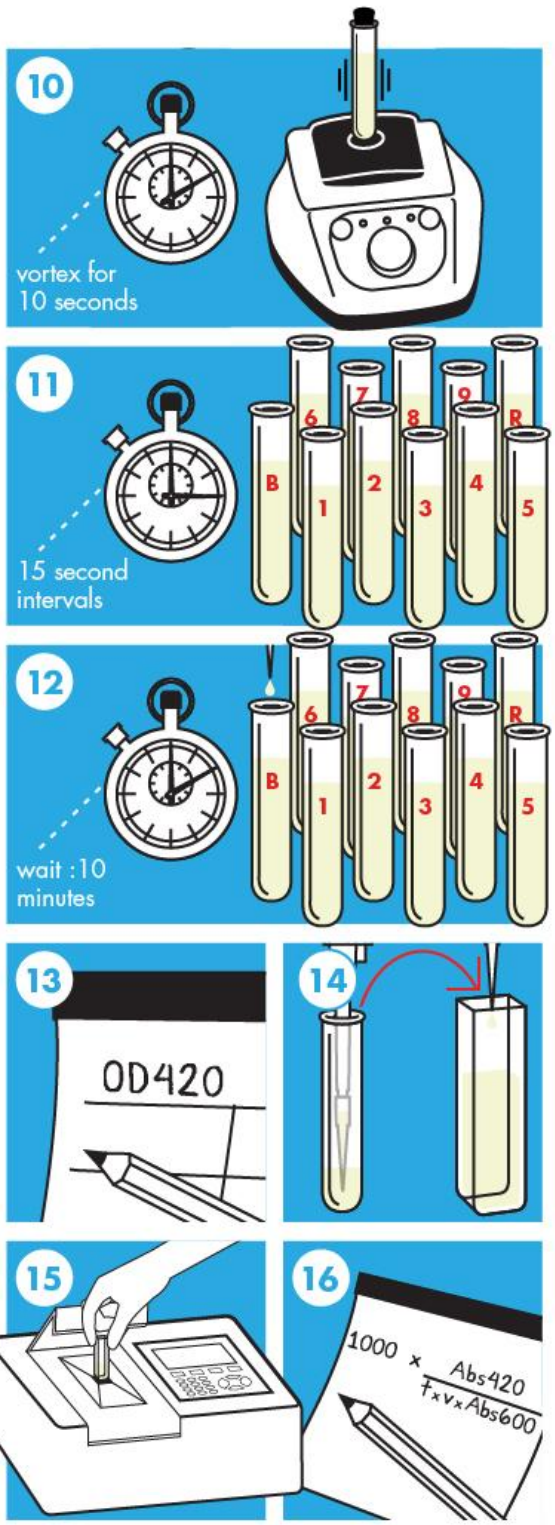

10. Vortex each tube, including the blank, for EXACTLY 10 seconds.

11. Start the timer when you add 100 µl of ONPG solution to the first tube. Then at 15 second intervals, add 100 µl of ONPG solution to each tube, including the blank.

12. When 10 minutes have elapsed from the time you started the reaction in the first tube, you should stop that reaction by adding 1 mL of the soda ash solution. Add the quench solution at 15 second intervals to all the tubes in the order that you started the reactions, including the blank.

13. Reactions can be refrigerated for later analysis or absorbance data can be collected immediately.

14. Transfer each sample to a cuvette, filling the cuvettes about three-quarters full. (OPTIONAL: Spinning down samples in a centrifuge to pellet cell debris will increase the reproducibility of your absorbance measurements.)

15. Read and record the absorbance value for each sample. Start by zero-ing the spectrophotometer set at **420** nm using the blank reaction.

16. Calculate Miller units for all of your sample data using the following formula:

$$\text{β-gal production in Miller units} = 1000 \times \frac{\text{Abs 420}}{t \times v \times \text{Abs 600}}$$

t is the time in minutes
v is the volume in mLs

** VIDEO OF PROCEDURE AVAILABLE ONLINE

UNDERSTANDING THE RESULTS

Students will notice some general trends in the data, e.g. the strength of the RBS is difficult to assess in the context of the weak promoter since all combinations give units that are all very low. They will also notice some unanticipated things. For example, the combination of a medium strength promoter and a strong RBS often give higher units than the strong/strong combination.

The reference sample is a good place to start the class discussion of the results because it often varies from group to group due to small differences in experimental technique. If the reference is orders of magnitude different, it is reasonable to recheck the calculations.

Teaching Tips

- The volume of cells you'll need to grow will depend on how you are setting up your student's work. If each student or student team is to test every strain, then 2.5 ml of each culture for each team will be more than enough.
- In many cases, teachers only use a subset of the samples, e.g. 2-R plus 2-3, 2-6, 2-9, to measure the impact of changing one part (the RBS) in the context of the same strong promoter.
- If you would like students/student teams to share the cultures that are grown when they perform "Part 2: Beta-galactosidase assay," then insure that there is at least 1 ml of bacteria for every assay to be performed.
- If the reactions are not noticeably yellow after the 10 minute reaction time, the students can allow the reactions to proceed for longer (even hours!). The reaction time is taken into account in the units calculation.
- If the reactions are turbid, 1.3 ml of the reactions can be moved to 1.5 ml microfuge tubes and centrifuged for one minute and then 1 ml can be transferred to cuvettes.
- The reaction mixtures are stable once the reactions have been stopped. They can be stored overnight in the fridge and read the next day. Just allow the tubes to warm to room temperature so condensation isn't collecting on the tubes when they are being read in the spectrophotometer.
- Students often have questions about the calculations of units. The 1:10 dilution of the cells must be taken into account for the OD600 value. The volume of cells should be 0.1ml, not the total volume of the reactions. If the protocol is followed as written then the denominator of the equation multiplies the 10 minute reaction time and the 0.1ml volume of cells, resolving to 1 and leaving the calculation simply a ratio of OD420 to OD600. Students sometimes forget to multiply this ratio by 1000, resulting in units that are orders of magnitude different from everyone elses.
- If a spectrophotometer is not available, then MacFarland turbidity standards can prepared using $BaCl_2$ and H_2SO_4 as described in Chapter 6 of the BioBuilder book. These standards can be prepared well in advance of lab and in any volume. To use these standards, aliquot them to a glass tube with a cap and visually compare then to the turbidity of the bacterial samples, also in glass tubes. It can be helpful to compare how well the samples obscure a black line on a card behind them.
- Some spectrophotometers (Spec-20s for example) allow the reactions to be read directly in the glass reaction tubes.
- Clean up: containers can be provided at each workstation for biological waste such as pipet tips and tubes. Follow hazardous waste procedures recommended by your school or municipality. Generally it is safe to soak the biohazardous materials in 10% bleach for 20 minutes and then dispose as regular trash or liquid waste.
- You can find a lab report assignment and grading rubric at the biobuilder.org website.

POST-LAB QUESTIONS

What is expected for the enzymatic reactions in each of the following cases:

a. We forgot to add detergent

We would measure little or no β-gal activity since the cells would not be lysed.

b. We added twice as many cells to one of the tubes but didn't include that change in the calculation of units

The activity measured in that tube would be twice its actual value.

c. We added twice as much ONPG to one of the tubes

There would be no difference in the measurement since ONPG is in excess already.

d. We measured these genetic devices in a strain with a wild type lac operon in the genome

All would show some level of activity from the operon that is expressing β-gal.

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 accuracy of reaction timing, different range of yellow color measured making spectrophotometer differently sensitive, cell debris in tubes confusing measurement, or condensation on outside of tubes or cuvettes, etc.

If the reference strain behaved identically for each group, would it be sensible to compare the experimental strains that each group tested? What about if the reference strain didn't behave identically?

In both cases the reference strain can be used to enable comparisons between groups. When the reference does not match, a correction should be made between the experimental samples.

Why might a collection of well-characterized genetic parts that behave in a predictable way be a useful resource for synthetic biologists?

One goal of synthetic biology is to reliably assemble genetic programs from scratch. Just as a mechanical engineer would like a rich collection of standard and well-characterized materials to build with, so would a synthetic biologist like a collection of standard biological parts to assemble into novel living systems.

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

Answers will vary

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

TEACHER MANUAL

BioBuilder

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