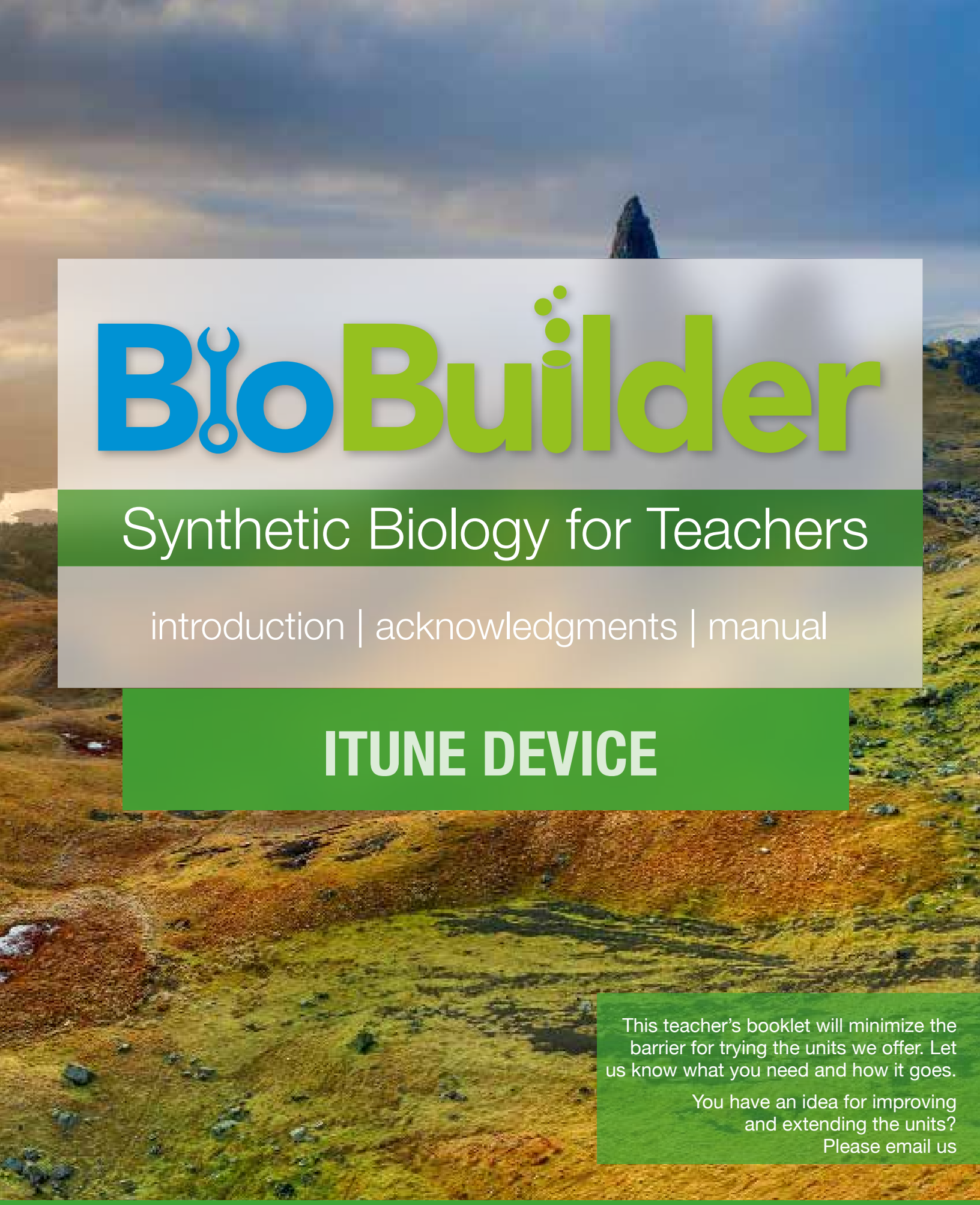


BioBuilder

Synthetic Biology for Teachers

introduction | acknowledgments | manual

ITUNE DEVICE

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

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.

The 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 statistical analysis of data.

The iTune device

Predict β-galactosidase activity

context | check lists | lab protocol

This lab focuses on predictable design. Students will examine the role of parts, such as promoters and ribosome binding sites, in predicting the output of a genetic device.

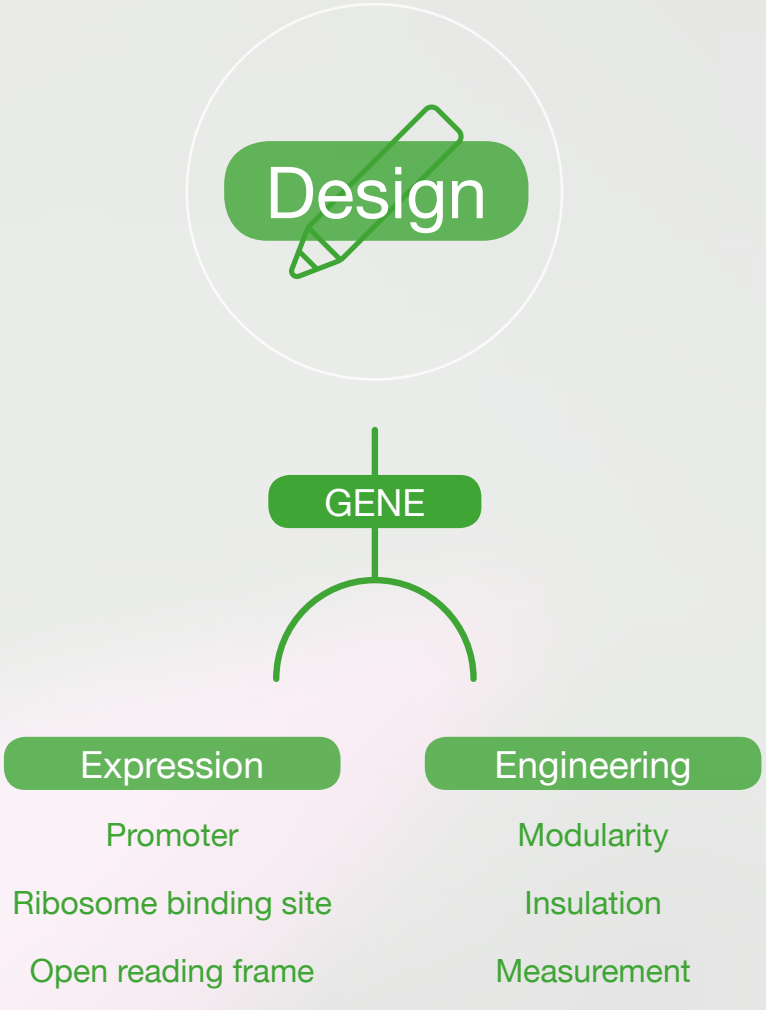
The students measure β-galactosidase enzymatic activity as the device's output, thereby looking through the lens of molecular genetics to predict and then evaluate a device's behavior.

In this manual

The iTune device lab emphasizes the “measure” phase of the engineering design-build-test cycle.

This lab focuses on the proteins and DNA sequences needed to express a gene such as promoters, RNA polymerase, Ribosome Binding Site (RBS), Open Reading Frames (ORF) and consensus sequence. The iTune Device lab also serves as an introduction to basic enzymology. The engineering concepts of modularity, insulation, and measurement will be explored by analyzing nine gene regulatory designs.

Each design has a unique combination of promoter and RBS controlling the expression of an enzyme, beta-galactosidase (β-gal). Spectrophotometric analysis and enzyme kinetic assays are the main biotechnology skills emphasized in this lab.

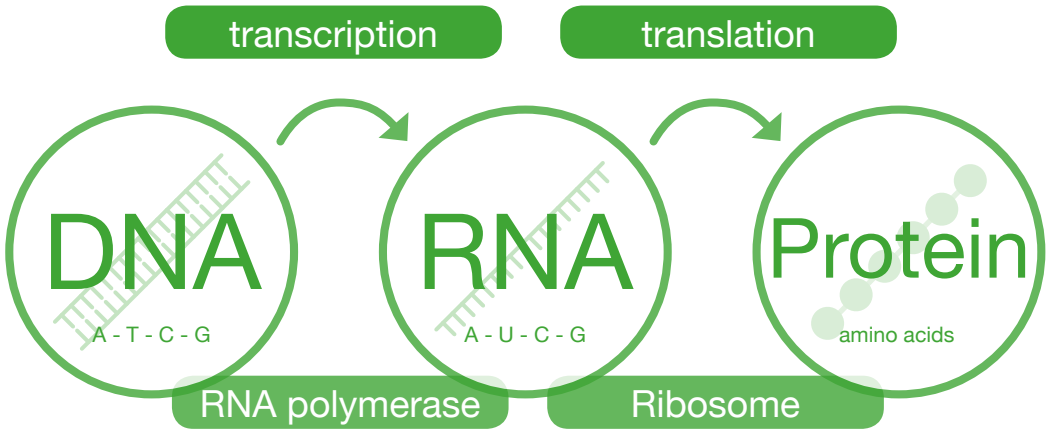


Gene expression

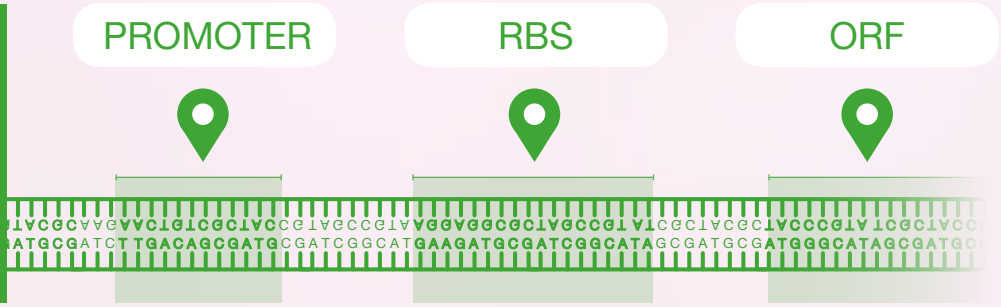
This lab explores some gene regulation designs, and so this manual presents a short recap of how gene expression works at the DNA level.

The central dogma of gene expression, the mantra “DNA makes RNA makes protein” is shorthand for the tenet that proteins are assembled by translation of RNA sequences, and RNA sequences are transcribed from DNA sequences.

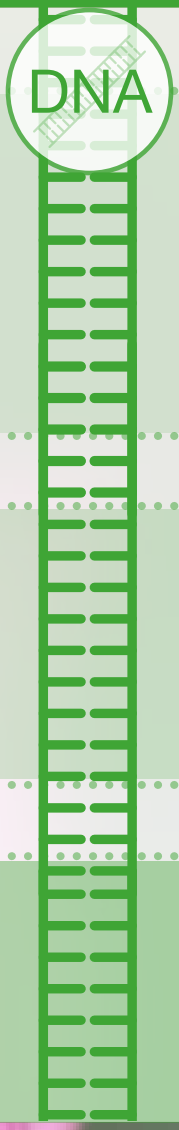
Central Dogma



Genetic parts



DNA parts in details



Promoter

A promoter is a DNA sequence where the RNA polymerase can bind and initiate the formation of RNA chains based on the DNA sequence that follows

Ribosome binding site - RBS

A ribosome binding site is a DNA sequence that is recognized by the ribosome to begin protein synthesis from an RNA template

Open Reading Frame - ORF

An Open Reading Frame is a DNA sequence that can be transcribed into an mRNA that can be decoded into amino acids that then fold into a protein

About the consensus sequence

For both the promoter and the RBS, the DNA sequence varies around a most common used sequence through out the entire genome of the organism.

This is called a “consensus sequence”. Varying from it will regulate the efficiency of transcription for the promoter sequence and of translation for the RBS.

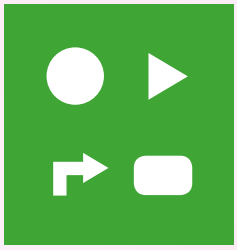
Engineering

Established engineering disciplines rely on modular components that can be functionally assembled in a variety of ways, making it easy to customize combinations according to an individual's needs. The pieces not only need to be physically connected but, when connected, they also must behave according to specification. To put it simply, the parts must function as expected when they are assembled.

Modularity, insulation, and measurement of parts are crucial components toward enabling such functional assembly.

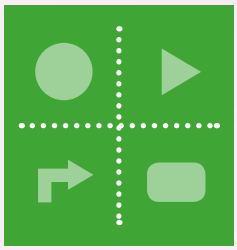
Modularity

Modularity refers to the idea that engineers can design and generate systems by combining functional units, or "modules." Now, in an era of genetic engineering and interchangeable genetic parts, many genetic sequences encoding specific functions are known and can be precisely manipulated.



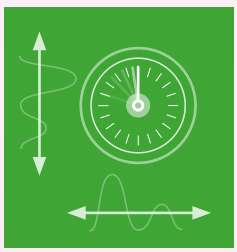
Insulation

As modular materials are mixed and matched, new complications arise, including the increased chance that the modules can interact with one another in undesirable ways. One tool to minimize unanticipated interactions between modules is to insulate the behavior of the parts.



Measurement

By measuring the actual performance of synthetic systems, composed of well-characterized parts, and comparing the measurements to what was predicted, biobuilders can assess their designs and move closer to correctly anticipating the success or failure of future designs.



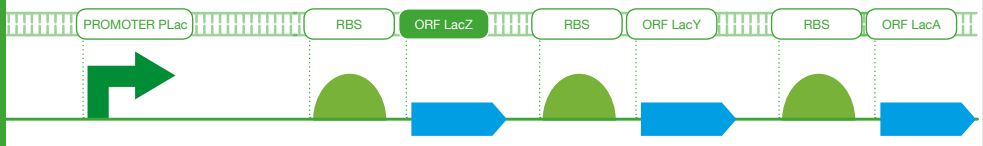
Lactose metabolism - the *lac* operon

In the 1960s, Dr. Francois Jacob and Dr. Jacques Monod identified foundational principles of gene expression through their studies of lactose transport and metabolism in bacteria. The genes for lactose metabolism are clustered in the *lac* operon, but the bacteria conserve energy by turning on these genes only when glucose is **not present**.

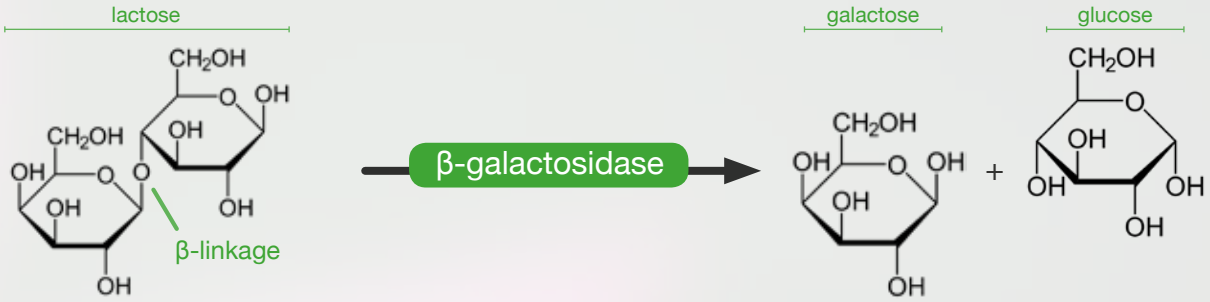
When glucose **is present**, regulatory factors **turn off transcription** of the downstream ORFs. If lactose is present and glucose is absent, those same transcription regulatory factors switch their behaviors, and transcription of the operon leads to transport and metabolism of lactose.

The key protein for lactose metabolism is an enzyme called β-galactosidase, often abbreviated β-gal, and it is encoded on the DNA by the Open Reading Frame (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.

Symbolic representation of 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). The operon produces the enzymes necessary to metabolize sugars only when glucose is not present.



Researchers have also 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.

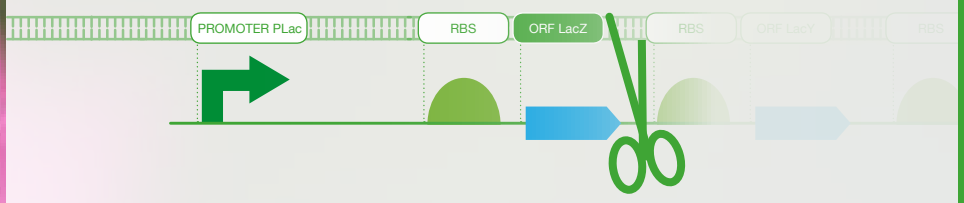
Visit Biobuilder.org for an introductory powerpoint for this lab and more:
http://biobuilder.org/teacher/itune-device-teachers/

EXPERIENCE OVERVIEW

Design choices

In contrast with the naturally occuring lac operon, the genetic architectures of the iTune Device gene circuits are simpler structures, with one promoter and one RBS controlling one ORF.

To alleviate any impact of positive and negative regulatory factors on the iTune Device measurements, the cells are grown in rich media and without the cell's positive regulatory protein, and in the presence of IPTG, a molecule that inhibits the negative regulatory protein. We can customize the behavior of the iTune circuits because of the modular behaviors of each DNA part.



The iTune Device's parts have discrete functions and performance characteristics, which synthetic biologists use to their advantage for biodesign.

Experimental question

Which combination of promoter + RBS results in the greatest production of β-gal?

HOW?

Students measure the activity of the LacZ gene product, β-gal, to assess the performance of different promoter and RBS combinations.

iTune Device
CONTEXT - EXPERIENCE OVERVIEW

1 - Methods

In BioBuilder's iTunes lab activity, β-gal activity is measured because it is a good reflection of the performance of each circuit. β-gal activity is measured using a substrate called ONPG, a colorless compound that is chemically similar to lactose.

β-gal cleaves ONPG just as it would normally cleave lactose. The products of the reaction with ONPG are a yellow compound, o-nitrophenol, and a colorless product, galactose.

2 - Positive & negative controls

Positive control
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.

Negative control
For this lab, we have not included a specific strain to use as a negative control. The reaction run without any cells can serve as one negative control as well as the blank for the spectrophotometer.

3 - Results

When ONPG is cleaved by β-gal, it produces a yellow compound. The intensity of yellow color can be used to calculate the amount of β-gal expressed by the different circuits.

This lab requires 10 bacterial strains

Ten bacterial strains in BioBuilder's iTune Device activity must be grown in liquid media consisting of growth media, LB, and ampicillin to select for the plasmid carrying the promoter:RBS:LacZ construct, and IPTG to relieve inhibition of the LacZ gene.

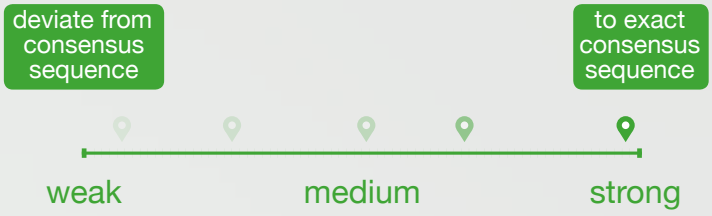
Students will begin the experiment by measuring the cell density of each overnight liquid culture, either using:

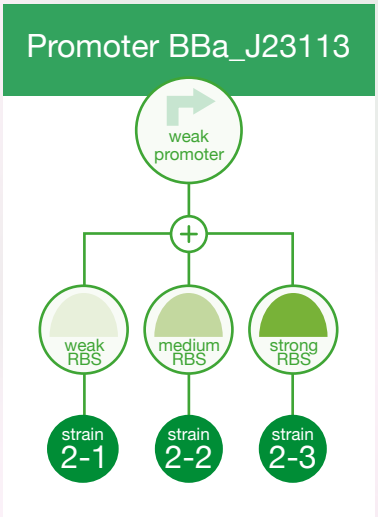
- a spectrophotometer to measure the OD600 or
- the MacFarland turbidity scale.

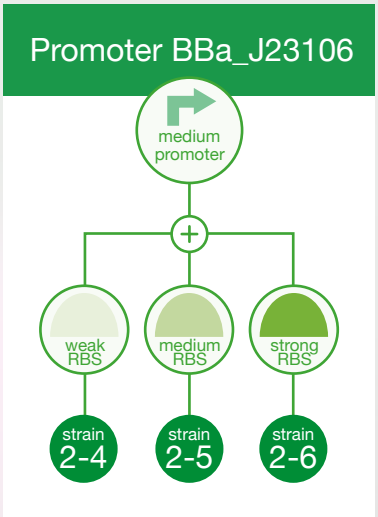
After reacting the cells with ONPG, students will measure the yellow absorbance of each solution to calculate the β-gal activity.

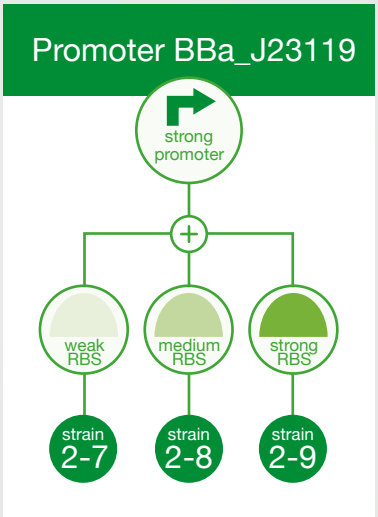
Strain #	Promoter - Registry #	RBS - Registry #	Relative strenght Promoter/RBS
2-R	BBa_J23115	BBa_B0035	Reference/reference
2-1	BBa_J23113	BBa_B0031	Weak/weak
2-2	BBa_J23113	BBa_B0032	Weak/medium
2-3	BBa_J23113	BBa_B0034	Weak/strong
2-4	BBa_J23106	BBa_B0031	Medium/weak
2-5	BBa_J23106	BBa_B0032	Medium/medium
2-6	BBa_J23106	BBa_B0034	Medium/strong
2-7	BBa_J23119	BBa_B0031	Strong/weak
2-8	BBa_J23119	BBa_B0032	Strong/medium
2-9	BBa_J23119	BBa_B0034	Strong/strong

All strains are constructed in the "TOP10" E. coli strain of genotype: F– mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara leu) 7697 galU galK rpsL (StrR) endA1 nupG









SUGGESTED WORKFLOW

On the day of the lab, students will lyse the cells to release the β-gal enzyme and then carry out a timed enzymatic reaction.

The reaction mixtures are stable once the reactions have been stopped. The reactions can be stored overnight 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.

You may want students to rehearse this procedure prior to the actual experiment.

The enzyme activity associated with each genetic construct can be calculated any time after the data is collected.

Visit BioBuilder.org for an introductory powerpoint for this lab and more:
http://biobuilder.org/teacher/itune-device-teachers/

Advanced preparation		1 day or more in advance Grow the 10 strains to be tested
Before	Introduce synthetic biology	Prepare materials, review lab protocol
	Pre-lab Q&A **Checklists & Lab preparations**	
Day 1	Day of the lab	Students perform measurements for β-gal activity
	Lab protocol - Part 1 **Lab protocol - Part 2**	
Day 2	Data Analysis	Students complete measurements, calculations and data analysis
	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. β-galactosidase 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.

3. 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
Ribosome Binding Site (RBS)	Ribosome	translation

4. 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 cells 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.

5. 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.

6. 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 allat exactly the same time, and we want each reaction to proceed for exactly the same amount of time.

7. 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.

8. 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.

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

iTune Device
DAY 1 - Kit checklist

WHAT I HAVE

Checklist of the kit content

For a class of 24 students - 6 groups of 4

Item	Amount	✓
Stabs of 10 bacterial strains	1	
Luria Broth	3 x 200 ml	
Ampicillin, 10 mg/ml	4 ml	
IPTG	1 x 24 mg	
ONPG	1 x 40 mg	
Sodium bicarbonate solution	75 ml	
Sodium carbonate solution	75 ml	
SDS	10 ml	
50 ml screw capped tube (sterile)	1 x 25 tubes	
15 ml screw capped tube (sterile)	3 x 25 tubes	
Sterile innoculating loops	1 x 30/package	
Glass tubes, 13x100mm	250	
Test tube rack	6	
1.5 ml microcentrifuge tubes	3 x 30	
Cuvettes, 100/package	2 packages	
Teacher + Student Instructions	downloadable	

Will arrive in the "perishable" supplement that can be ordered up to a month before running the lab.

Something missing?
Contact us: info@biobuilder.org

WHAT I NEED

A few things to prepare before the lab

For a class of 24 students - 6 groups of 4

The bacterial strains you will be receiving for this experiment already carry plasmid DNA encoding genetic circuits to be tested. The plasmids also confer resistance to the antibiotic, ampicillin. The bacteria will arrive as a “stab” or “slant,” which is a test tube with a small amount of bacteria on a slanted media.

The students or the teacher can prepare these starter cultures up to one week prior to the experiment itself and then store to cultures on the bench or in the fridge. Each group of students can prepare their own starter cultures or larger volumes can be prepared and shared between groups.

2 days in advance

PREPARE MATERIALS

! max 1 month in advance

2 days in advance

SOLUTIONS - BUFFERS

! max 7 days in advance

1 day in advance

LIQUID CULTURES

! max 7 days in advance

iTune Device
BEFORE - Pre-lab procedures

2 days in advance

PREPARE MATERIALS

1 Store the ampicillin

You should recieve the Ampicillin as a 10 mg/ml stock that can be stored in the fridge up to one month. This stock will be diluted 1:100 to a final concentration of 100 ug/ml in the LB + ampicillin + IPTG that you prepare.

2 Prepare the IPTG

You should add 1000 ul of clean or (ideally) sterile water to the vial of IPTG and vortex to dissolve the powder. This will make a 0.1M stock of IPTG that can be stored in the fridge up to one month. This stock will be diluted to 1:100 in the LB + ampicillin + IPTG that you prepare.

! max 1 month in advance

2 days in advance

SOLUTIONS - BUFFERS

Ortho-nitrophenyl-β-galactoside, ONPG (0.01 % solution)
Dissolve 40 mg ONPG in 40 ml water to make a 0.01 % solution. Store frozen for up to several months or in the fridge for a week.

Bicarbonate buffer (2 % solution)
As an alternative to the sodium bicarbonate, you could dissolve 1 g Arm & Hammer baking soda in 50 ml water to make 2 % bicarbonate solution.

Carbonate buffer (1M solution)
As an alternative to the sodium carbonate you recieve, you could dissolve 5.3 gram soda ash in 50 ml water to make a 1M carbonate solution.

Lysis buffer (1 % SDS solution)
As an alternative to the SDS, use 1 “squirt” clear liquid dish soap in 50 ml water.

1 day in advance

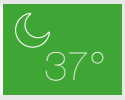
LIQUID CULTURES

1. Using a sterile inoculating loop or toothpick or pipet tip, transfer a bacterial colony from one of the petri dishes to a large sterile culture tube containing 3 ml of LB, 30 µl of ampicillin, and 30 µL IPTG.

   This volume is more than enough for each strain that students must use.

2. Repeat for each strain you will inoculate.

3. Place the culture tubes in the roller wheel in the incubator at 37°C overnight. *Balance the tubes across to minimize stress on the roller wheel.*



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.

max 7 days in advance

Experimental 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.

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.

Watch a video for how to prepare the overnight liquid cultures (though this video skips the IPTG addition): https://goo.gl/7JFGk5

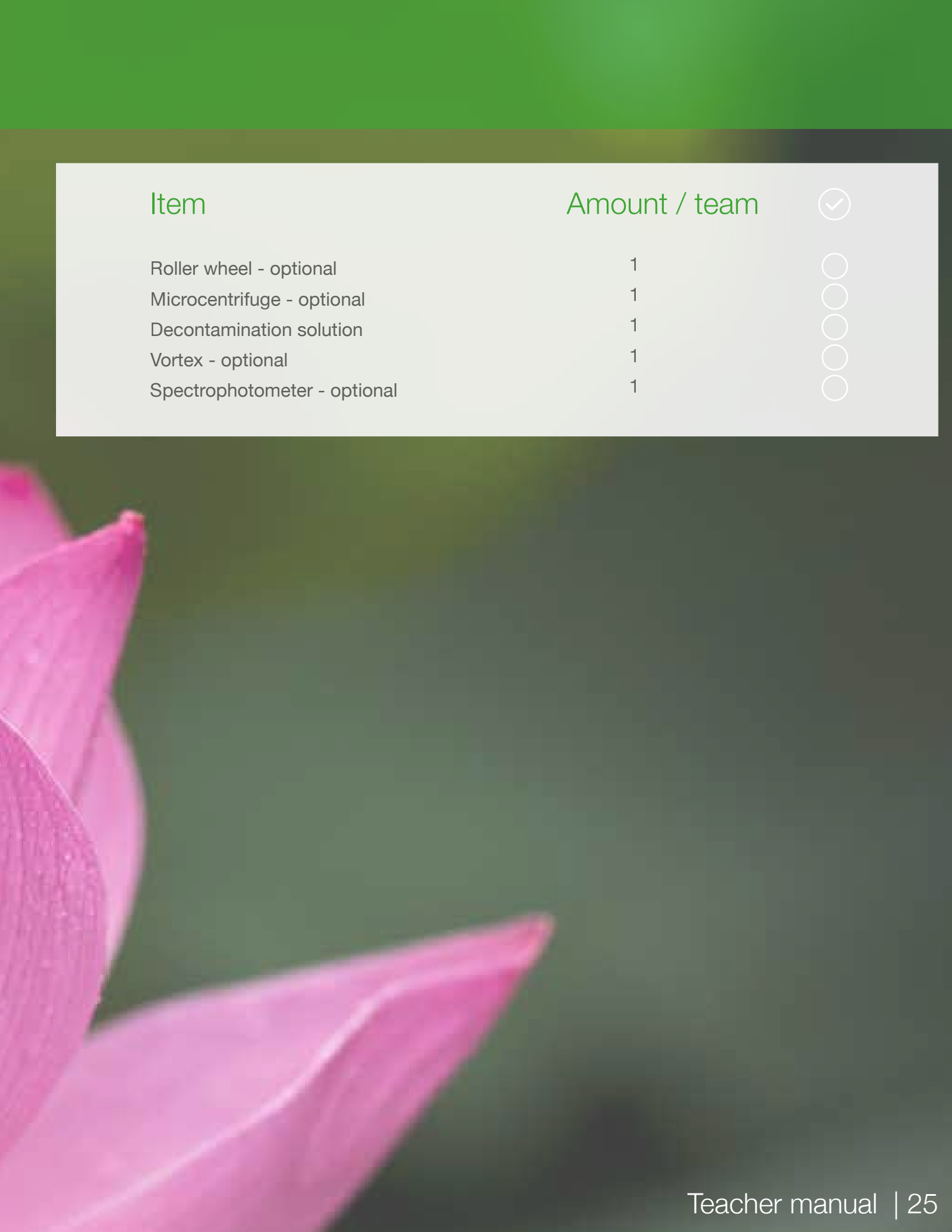
Workstation checklist

What each student team will need

The experiment has been optimized with the equipment listed but can be modified to do without some accessories.

Item	Amount / team	✓
Test tubes with bacterial cultures	10	○
SDS	1.5 ml	○
ONPG solution	1.5 ml	○
Bicarbonate buffer	50 ml	○
Sodium carbonate buffer	15 ml	○
Micropipets and tips to measure 100 µl	1	○
Micropipets and tips to measure 1 ml	1	○
Glass tubes for reactions	22	○
Test tube rack	2	○
Microcentrifuge tubes - if spinning samples	12	○
Waste receptacles/decontamination solution	1	○
Container for contaminated waste	1	○
Timer	1	○
Sharpie	1	○

Item	Amount / team	✓
Roller wheel - optional	1	○
Microcentrifuge - optional	1	○
Decontamination solution	1	○
Vortex - optional	1	○
Spectrophotometer - optional	1	○

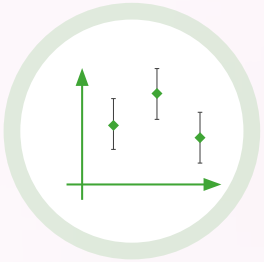


DAY 1
LAB PROTOCOL

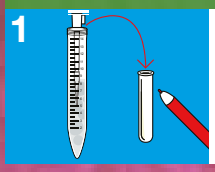
These are the same instructions as included on the student manual but with special notes added for you.

Experimental Tips

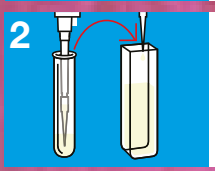
Your class can try to perform replicate assays of each sample so each strain gets measured two or three times. Then pool your class data to gain some confidence in the values you measure. A data table is included to help you organize your assay, but you can make one of your own if you prefer.



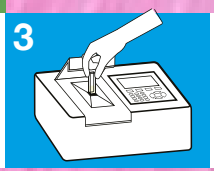
PART ONE Cell density measurements

1 

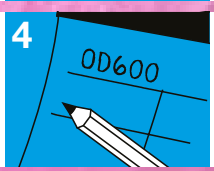
Label 10 glass tubes "**1**" through "**9**" and "**R**" for reference.
Make a 1:10 dilution of each overnight culture by mixing 300 µl of cells with 2.7 mL of bicarbonate buffer.

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.

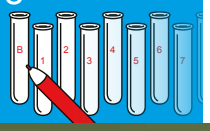
5 Discard all biological materials after decontaminating with 10% bleach.

Pipet tips, eppendorf tubes, spreaders, innoculating loops, and plates

PART TWO Enzyme reactions measurements

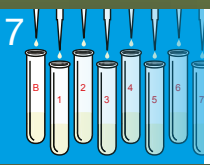
With this assay you will determine the amount of β-galactosidase activity associated with each sample of cells.

6



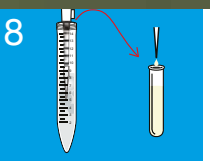
Label 11 glass tubes
"**1**" through "**9**"
"**B**" for blank
"**R**" for the reference.

7



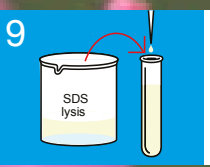
Add 1.0 mL bicarbonate buffer to each tube.

8



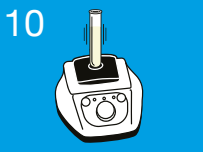
Transfer 100 µl of cells from the UNDILUTED overnight cultures to the appropriate tube.
Transfer 100 µl of bicarbonate to the blank.

9



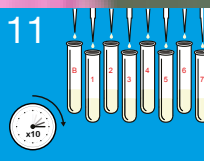
Add 100 µl of SDS lysis solution to each tube, including the blank.

10



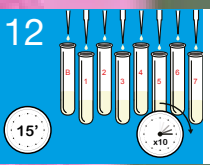
Vortex the tubes for 10 seconds each.
You should time this step precisely since you want the replicates to be treated as identically as possible.

11



Start the reactions by adding 100 µl of ONPG to each tube at 15 second intervals, including your blank.

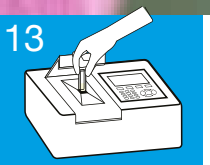
12



After 10 minutes, stop the reactions by adding 1 ml of stop solution to each tube at 15 second intervals. Ten minutes is sufficient to provide reliable results with the spectrophotometer, best between 0.1 and 1.0.
Usually this color is approximately the same as that of a yellow tip for your pipetman.
Don't be surprised when the stop solution makes the reactions look more yellow.

Reactions are now stable and can be set aside. If stored in the fridge, let them warm up before reading them to reduce the condensation that collects on the outside of the tubes.

13



Read the absorbance of each sample tube at 420nm (OD 420). These values reflect the amount of yellow color in each tube. If you do not have a spectrophotometer and are comparing the color to paint chips instead, follow the instructions in the next section.
If a microfuge is available, transfer some of the reaction mixture to a microfuge tube, spin the eppendorf tube for one minute, and transfer that cleared solution to a cuvette to read the OD 420. If you must use the larger glass tubes allow time for the debris to settle.

14

Discard all biological materials after decontaminating with 10% bleach.

Pipet tips, eppendorf tubes, spreaders, innoculating loops, and plates

Download the Quick Guide

iTune Device
Alternative protocol - No spectrophotometer

If no spectrophotometer is available

Prepare turbidity standards, where 1 OD 600 ~ 1 x 109 cells/ml

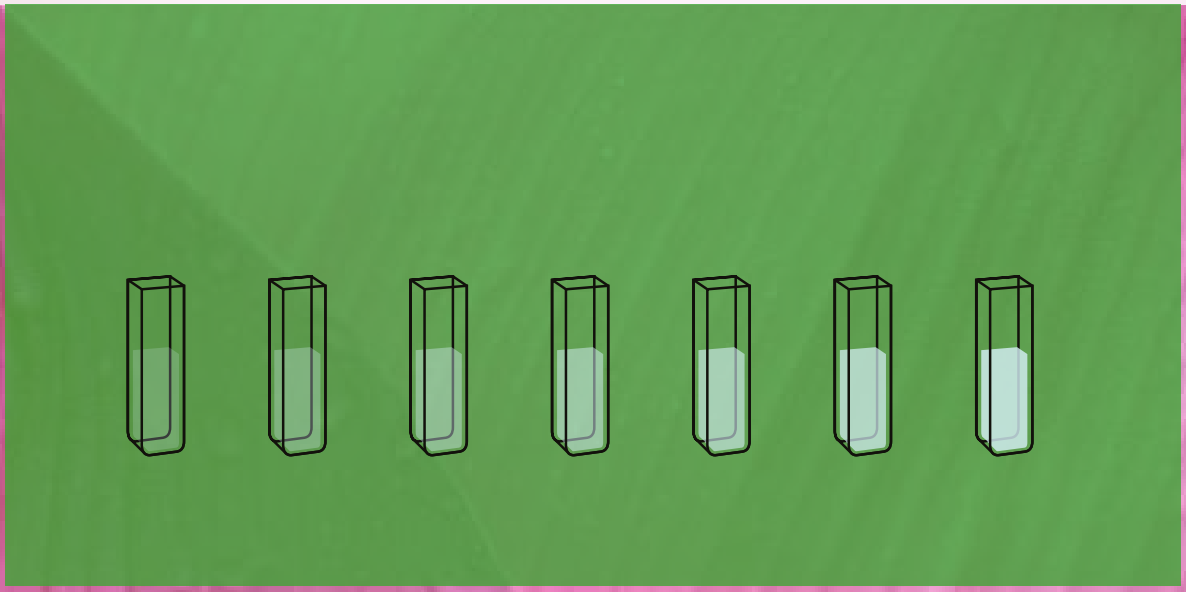
These standards can be prepared well in advance of lab and are useful if you are running the protocols without access to a spectrophotometer.

The standards can be made in any volume but then should be suspended and aliquoted to a small glass tube with a cap.

The size of the tubes and the volume of the standards you put in them doesn't matter.

Turbidity scale	OD 600	1% $BaCl_2$ in 1% H_2SO_4
0	0	0.0/10
1	0.1	0.05/9.95
2	0.2	0.1/9.9
3	0.4	0.2/9.8
4	0.5	0.3/9.7
5	0.65	0.4/9.6
6	0.85	0.5/9.5
7	1.0	0.6/9.4

[1% $BaCl_2$ can be prepared from 0.2M $BaCl_2$ (5%)
1% H_2SO_4 is approximately 0.1M H_2SO_4]

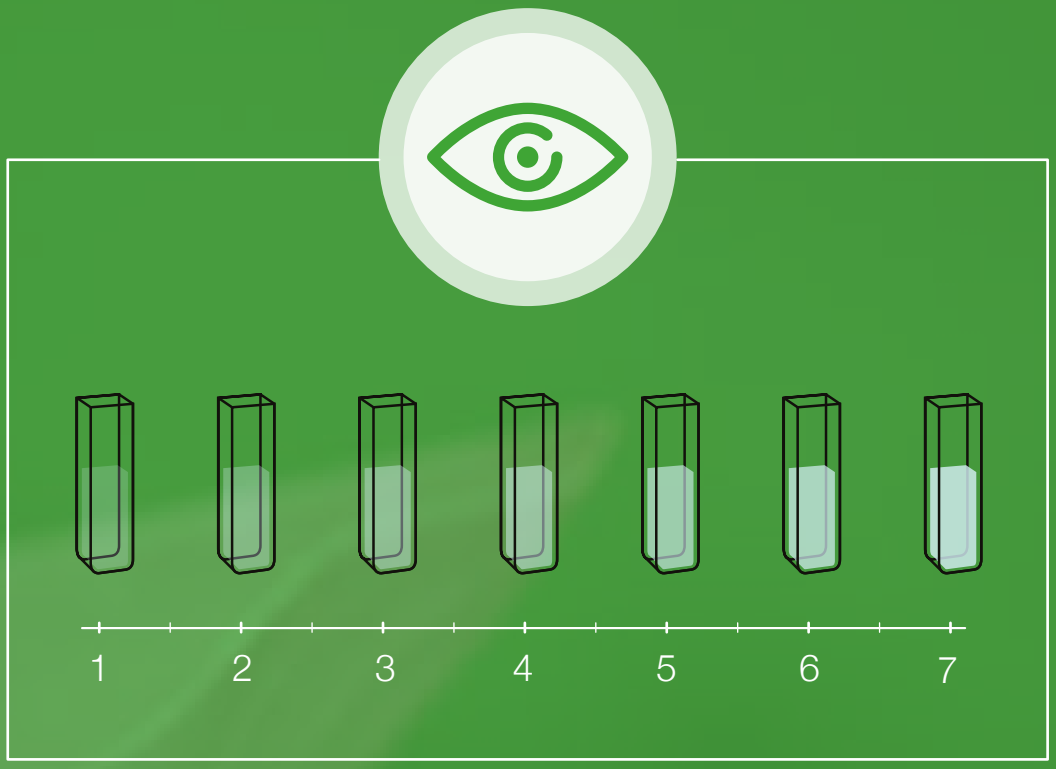


About the turbidity standards

Students will compare the turbidity of these standards to the turbidity of their bacterial samples by transferring some of the bacteria to the same sized glass tubes and identifying which turbidity standard comparably obscures dark markings placed behind the tubes with the standards.

> Wikipedia
>
> The McFarland Turbidity Scale are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. An example of such testing is antibiotic susceptibility testing by measurement of minimum inhibitory concentration which is routinely used in medical microbiology and research.
>
> https://en.wikipedia.org/wiki/McFarland_standards

It is likely that each stationary phase samples will have a turbidity greater than McFarland standard 7. The students can use 7 as the value in that case. You may advise the students that they can use half values (e.g. 1.5). This will also lead to interesting questions about the nature of data.



DAY 2
DATA COLLECTION

On the second day the students will collect the data in order to understand how each tested strain has behaved according to the strength of its promoter and RBS. Students will report the cell growth (OD600), the yellow absorbance (OD420), and the calculated β-gal activity.

Expected results

Using the protocol presented here, we generally find that the activity increases with increasing promoter strength and, to a lesser extent, with increasing RBS strength.

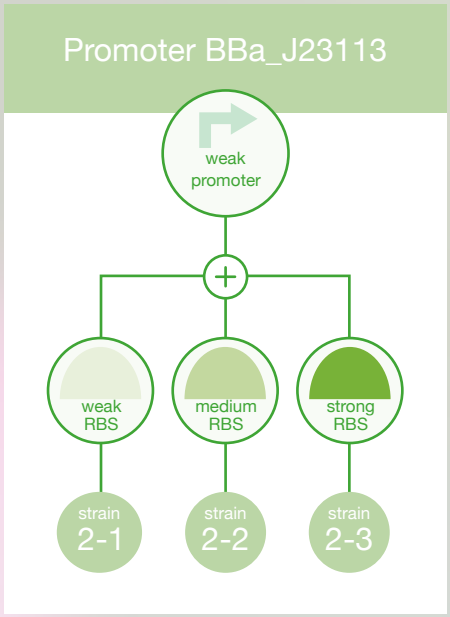
In our hands, the three strains with the weak promoter all provide small amounts of β-galactosidase activity, and we see the activity increase slightly as the RBS strength increases.

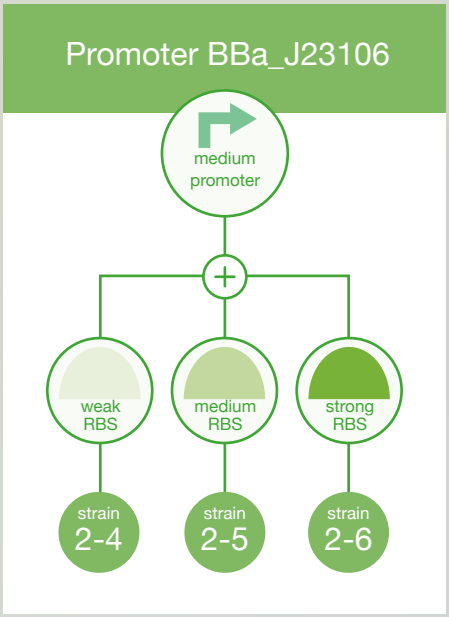
The medium promoter strength gives higher activity than the weak promoter collection and the activity increases with increasing RBS strength—very comforting data.

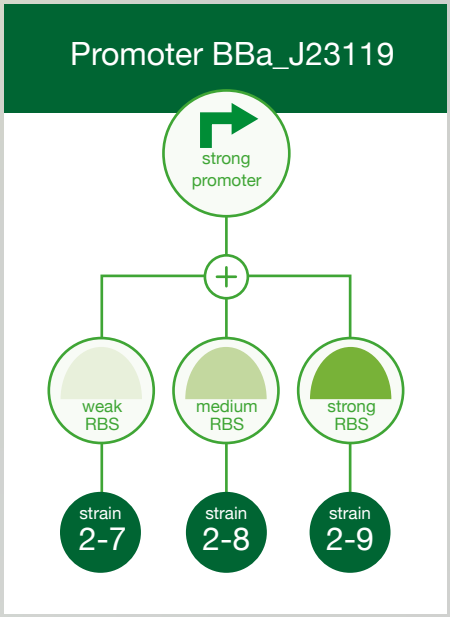
The strong promoter collection does not differ considerably with RBS strength and is not always very different from the medium promoter/strong RBS combination.

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

THE STRAINS

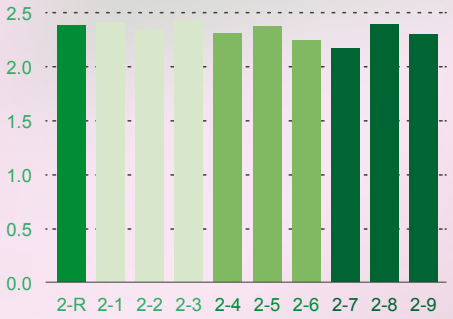




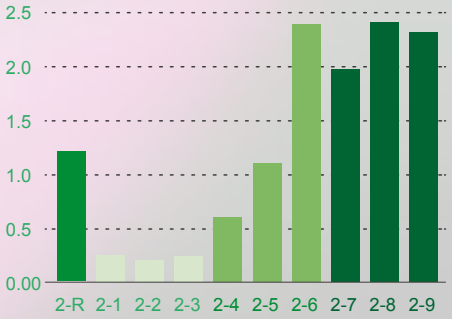


DATA SAMPLE

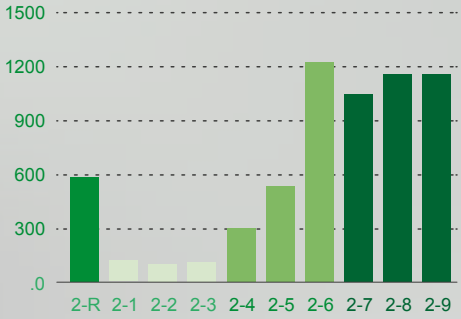
Strain	OD600	OD420	Time	Volume	β-gal units
2-R	2.370	0.961	7.000	0.100	579
2-1	2.400	0.195	7.000	0.100	116
2-2	2.330	0.156	7.000	0.100	96
2-3	2.420	0.186	7.000	0.100	110
2-4	2.300	0.477	7.000	0.100	296
2-5	2.360	0.878	7.000	0.100	531
2-6	2.230	1.902	7.000	0.100	1218
2-7	2.160	1.573	7.000	0.100	1041
2-8	2.380	1.920	7.000	0.100	1152
2-9	2.290	1.846	7.000	0.100	1152



OD600



OD420



β-gal units

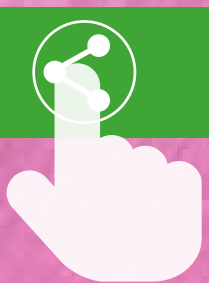
UNDERSTANDING THE DATA

1. Parts are defined as a DNA-encoded human defined functions and depending on how much basic biology you've covered, you might ask the students to define other "parts" and then look for them in the Registry of Standard Biological Parts, though it can be difficult to find things there sometimes. In this lab, the promoter, the RBS and the ORF are all "parts" that have been assembled into a "device" = higher order function, namely a gene expression unit, that's built from basic parts.

2. A major goal of synthetic biology is to assemble basic parts into a device that behaves as predicted. This is not reality yet—as can be seen from the results of this experiment. The strength of the RBS makes little difference when the promoter is strong, and the overall strength of the "strong promoter" collection is not terribly different from the "medium promoter/strong RBS" combination.

3. Sometimes the activity at very high expression levels goes down due to stress on the cell as it dedicates a lot of resources to making a lot of a particular product. Cells under this kind of stress are also likely to slow down their growth and mutate the gene that causes their slow growth.

4. Students may want to know why we "know" that the strong promoter or RBS is strong vs. medium vs weak. One explanation to offer is based on bioinformatics and how closely the sequences in question resemble the "consensus" sequences, that is the sequences that bind the RNA polymerase or ribosome binding site with greatest affinity.



5. Because this lab is based on measurement of β-galactosidase activity, it provides a nice opportunity to review the important discoveries about gene regulation that Jacob and Monod are known for, as well as a chance to review the usefulness of genetically engineered reporter constructs like these. The use of restriction enzymes to assemble the reporters could be examined if appropriate.

6. The reactions are started at known time intervals and stopped at intervals so that the time allowed for each enzyme reaction can be precisely timed. The importance of time in the activity measurement should be clear when the students perform the calculations.

7.The reference strain is included in this experiment so that the students can compare their data between groups. It provides a calibration point for analysis should groups find the values measured for their experimental strains are wildly different. It also lends itself to some nice statistical analysis if enough groups have measured the same strain.

NOTES

POST-LAB Q&A

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

1. What would be true about the enzymatic reactions if:

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.

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

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.

3. 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.

4. 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.

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

Answers will vary.

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

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