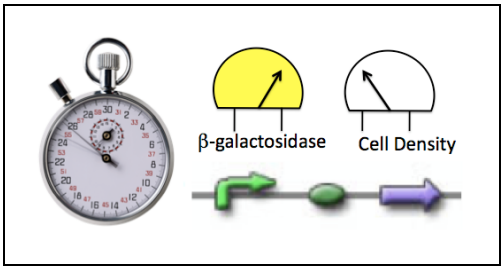
Teacher's Guide
SYNTHETIC BIOLOGY AND THE HIGH SCHOOL CURRICULUM: LAB 2
http://openwetware.org/wiki/SynBio_and_the_HS_Curriculum_Teacher's_Resource_Room:_Lab_2



Lab 2: iTUNE device
Evaluating promoter and RBS combinations to maximize beta-galactosidase output.

Needed Materials

Teacher Provides

- Innoculating loop or sterile toothpicks and bunsen burner
- Tubes to grow cells
- Eppendorf tubes or small glass tubes for running reactions
- Cuvettes to measure absorbances if spectrophotometer is not fitted for glass tubes
- Pipetmen and tips (P1000, P200)
- Pipets (10 ml and 5 ml) and bulbs
- Timers or stopwatches
- Sharpies
- Nitrile or Latex gloves
- rollerwheel at 37° for growing overnight cultures of bacteria
- vortex
- microfuge (optional)
- fume hood for measuring $CHCl_3$

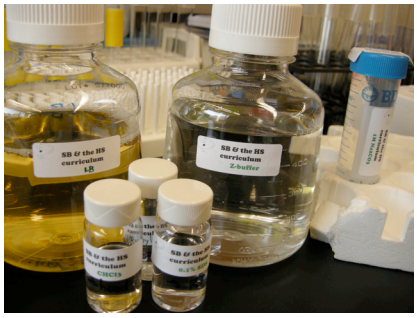
Kit Provides

- 10 strains

Promoter	Promoter #	Artificial Units	RBS	RBS #	efficiency	Strain #
Reference promoter	J23115	387	Reference RBS	B0035	?	2-R (also NB381)
weak	J23113	21	weak	B0031	0.07	2-1 (also NB391)
weak	J23113	21	medium	B0032	0.3	2-2 (also NB383)
weak	J23113	21	strong	B0034	1	2-3 (also NB384)
medium	J23106	1185	weak	B0031	0.07	2-4 (also NB385)
medium	J23106	1185	medium	B0032	0.3	2-5 (also NB386)
medium	J23106	1185	strong	B0034	1	2-6 (also NB387)
strong	I719005	uninduced T7 in BL21(DE3)	weak	B0031	0.07	2-7 (also NB395)
strong	I719005	uninduced T7 in BL21(DE3)	medium	B0032	0.3	2-8 (also NB396)
strong	I719005	uninduced T7 in BL21(DE3)	strong	B0034	1	2-9 (also NB397)

Store stabs at room temp. Store plates and liquid cultures at room temp or 4° (= fridge) for longer times.

- Chemicals



Room Temperature

- 500 ml LB (= 10 g Tryptone, 5 g Yeast Extract, 10 g NaCl per liter, plus 20g of Agar for plates). '''Keep sterile.'''
- 500 ml Z-buffer (= 8.05 g Na2HPO4*7H20, 2.75 g NaH2PO4*H2O, 0.375 g KCl, 0.123 g MgSO4*7H20 in 500 ml H20)
- 50 ml 1M Na2CO3 (= 10.6 g in 100 ml)
- 10 ml 0.1% SDS (100 ul of 10% in 10 ml H20)

4° (fridge)

- 30 ml ONPG (4 mg/ml in Z-buffer)
- 2 ml Amp (100 mg/ml in H2O, filter sterilized)

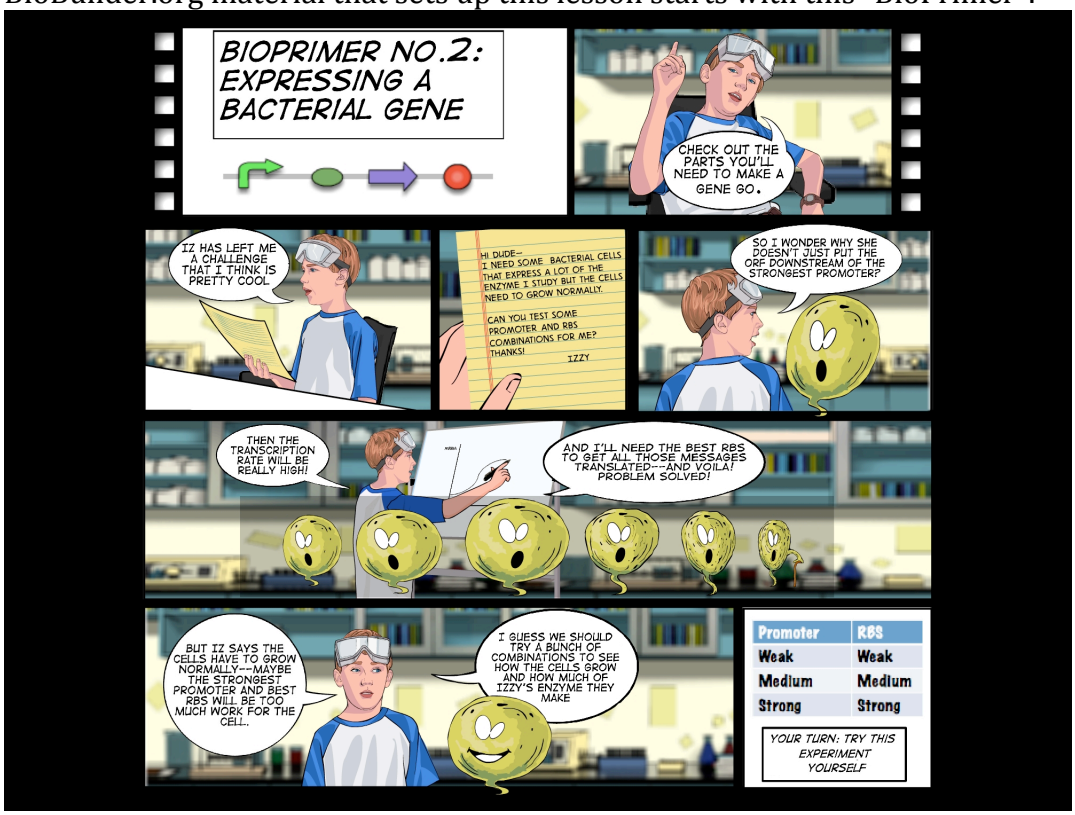
Chemical Hood

- 1 ml $CHCl_3$

Workflow

Classroom Content

BioBuilder.org material that sets up this lesson starts with this "BioPrimer":



When you are done with this lab, this link:
http://www.surveymonkey.com/s/ZP537Z3
provides a survey that you can offer the students. Thank you for helping us to improve this content.

Laboratory Content

Note that these laboratory steps can be done by the students or by you (the teacher) depending on how much time and preparation you intend to take on/delegate. The only exception is the aliquot of $CHCl_3$ (day 3) that should be done in the fume hood by the teacher.

As written here, the materials are sufficient for at least 15 groups of students.

Day 1

Streak out stabs onto LB+amp plates. Incubate 37° overnight. If your class will test the whole set, there will be 10 strains to streak out.

Day 2

- Dilute Amp 1:1000 in LB using sterile technique. You will need 2.5 ml for each strain you want to test, e.g. 25 ml if you will be testing all 10 strains.
- Colonies can be inoculated into the media with a toothpick, a loop, or a pipet tip.
- Grow 2.5 ml overnight cultures in large sterile glass tubes with loosely fitted caps on 37° roller wheel.
- Cultures are stable and active for a week at least (stored at room temp or in the fridge)

Day 3

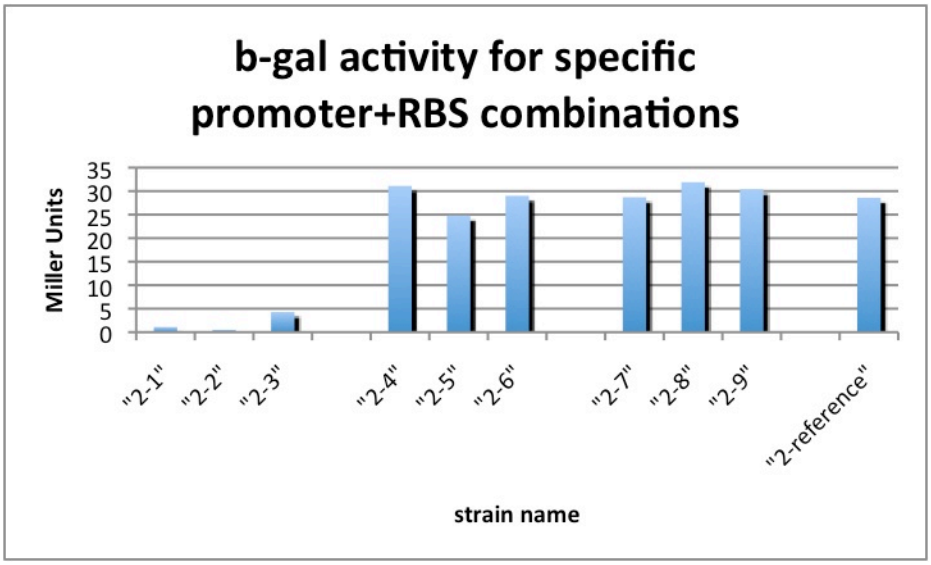
- Aliquot reagents for students as desired.
- It is recommended that all students or teams of students test the "reference strain" in addition to some number of experimental variants.
- Teacher should add 30 ul of the CHCl3 to 400 ul of Z-buffer for each b-gal assay to be performed. This cannot be done in bulk and then aliquoted since the CHCl3 will settle to the bottom of the tube immediately.
- At the start of class, students will dilute cells to measure their density (undiluted cell cultures are invariably too dense for spectrophotometers to read accurately), add 100 ul of undiluted cells to each assay tube that the teacher has prepared, add 20 ul SDS to the tubes, vortex, then start the reactions.
- If your teaching block is ~ 1 hour long, then the teacher can stop all the reactions by adding Na2CO3 to each tube (timing does not have to be precise to the second) and the tubes can stay at room temperature, '''tightly capped,''' overnight, or longer if you do not meet your students every day.

Day 4

- This day is for reading the Abs 420 and Abs 550 values and for doing the calculations. Ideally some class data should be pooled for some statistical analysis of accuracy and precision.
- These steps can be continued on Day 3 if you have more than 1 hour of time with the students.
- If you can spin the contents of the reactions in a microfuge before reading the values, then you will reduce the Absorbance at 550 nm, making the calculations more reliable. If spinning the reactions is not an option, then you may have large numbers to subtract from the Absorbance at 420. The calculation can get confusing for the weak promoters since the enzyme activity is very low--your

students may even see negative #s! If this is the case, ask them to set the 550 value to zero.

Sample Data Set



- Note that the RBS makes a difference only when the promoter is weak. This can be a starting point to talk about rate determining steps and how transcription and translation are coupled in bacterial cells.
- Note that the current set of strains (2-1 through 2-9) have "weak" and "strong" promoters behaving nearly identically. There is a new set of "very strong" promoters being built that will offer a greater range of units. This can be a way to illustrate the "design-build-test" paradigm in engineering and a way to illustrate the utility of scientific understanding to help guide the redesign cycles.

Assessments

See wiki for rubrics

Survey Monkey Link

To help us improve the labs, you can send the students to
http://www.surveymonkey.com/s/ZP537Z3
where they can offer anonymous feedback.

Variations to try

- Measure output in log phase rather than stationary. Perhaps there will be different growth rates vs production rates?
- Model RBS strength using [http://www.voigtlab.ucsf.edu/software/instructions.html RBS calculator.] Explore ways to model/measure promoter function. Consider other aspects of transcription/translation that may affect output.

Feedback

We're always looking to hear back from you if you've thought about this unit, tried it, or stumbled across it and want to know more. Please email us through BioBuilder info@biobuilder.org