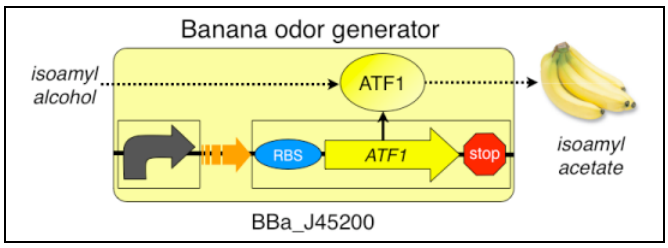
Teacher's Guide
SYNTHETIC BIOLOGY AND THE HIGH SCHOOL CURRICULUM: LAB 1
http://openwetware.org/wiki/SynBio_and_the_HS_Curriculum_Teacher's_Resource_Room:_Lab_1



LAB 1: Eau that smell
Comparing 2 competing designs to optimize system performance

Needed Materials

Teacher Provides

- Innoculating loop or sterile toothpicks and bunsen burner
- Large and small glass tubes for growing cells
- Cuvettes to measure absorbances if spectrophotometer is not fitted for glass tubes
- Pipetmen and tips (P1000, P200, P20)
- 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
- Fume hood for measuring isopentyl alcohol

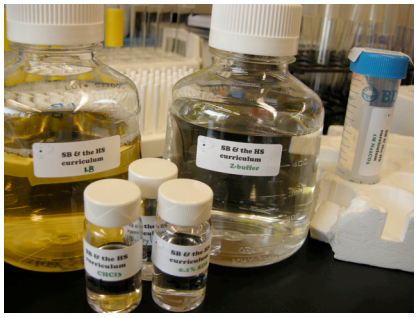
Kit Provides

- 4 strains

Strain #	Plasmid	plasmid description	Cells	cells description
1-1 (NB376)	BBa_ J45250	sigma 54 directing transcription of ATF1, AmpR	BBa_J45999 (NB370)	indole- chassis, CamR
1-2 (NB377)	BBa_ J45990	sigma 54 plus tetR-4 part inverter directing transcription of ATF1, AmpR	BBa_J45999 (NB370)	indole- chassis, CamR
1-3 (NB378)	BBa_ J45200	sigma 70 directing transcription of ATF1, AmpR	BBa_J45999 (NB370)	indole- chassis, CamR
1-4 (NB379)	pUC18	no promoter, no ATF1 gene, AmpR	BBa_J45999 (NB370)	indole- chassis, CamR

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.

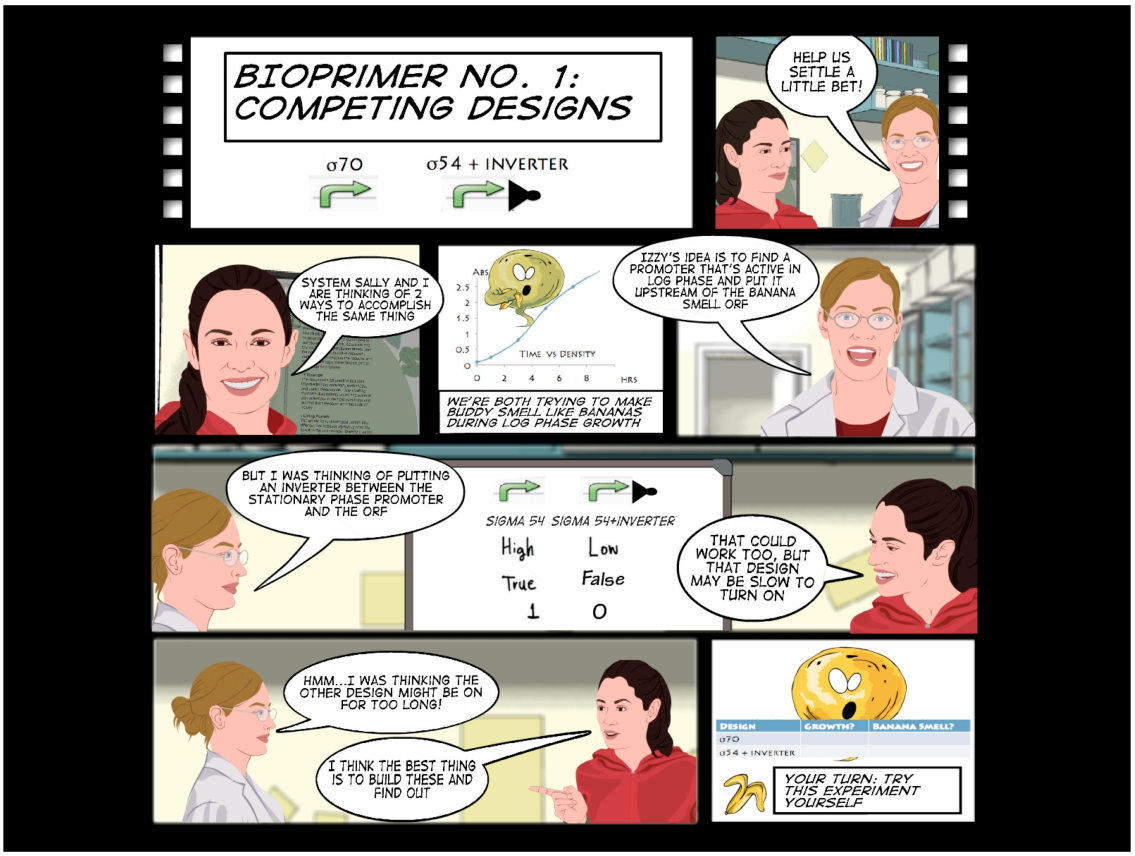
4° (fridge)
- 2 ml Amp (100 mg/ml in H2O, filter sterilized)
- 4 LB + Amp plates

Chemical Hood
- 1 ml isopentyl alcohol

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 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 isopentyl alcohol (day 3) that should be done in the fume hood by the teacher.

Day 1

Streak out stabs onto LB+amp plates. Incubate 37° overnight. If your class will test the whole set, there will be 4 strains to streak out. Strains can also be streaked out on LB+amp+cam if you'd like to verify the indole- strain background.

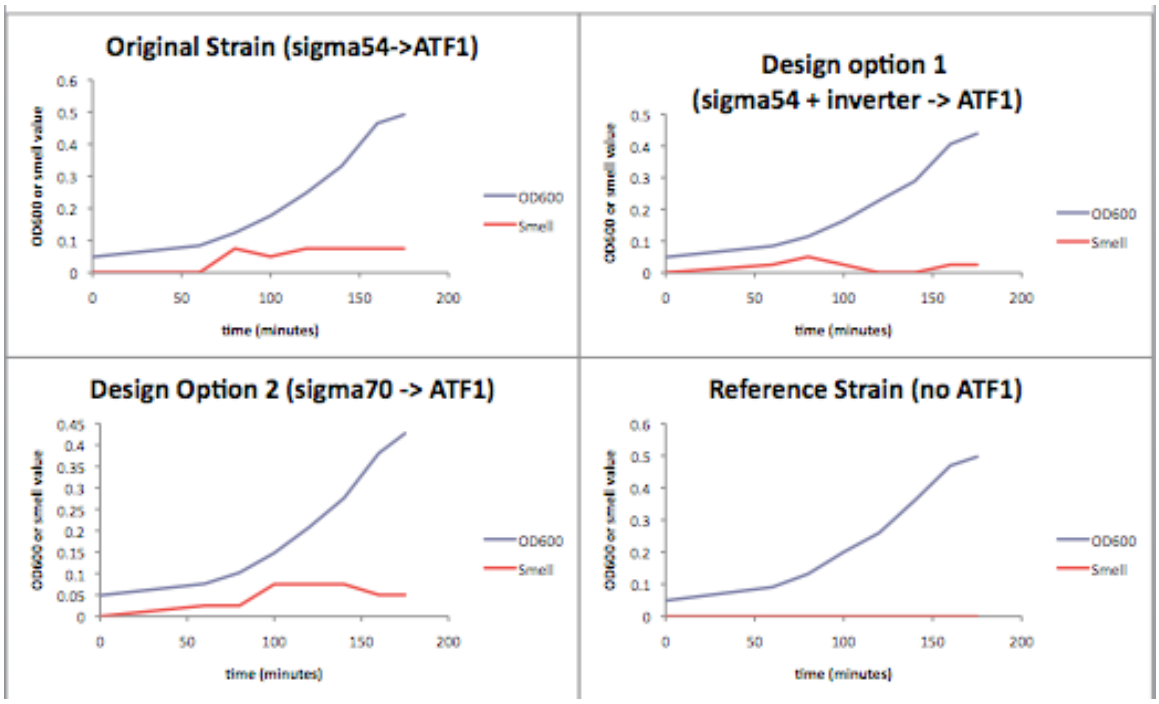
Day 2

- 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) but will take considerably longer to start growing on the day you subculture (~3 hours rather than 1).

Day 3

- Teacher should dilute strains using 100 ul into 2 or 2.5 ml of fresh media supplemented with amp (1:1000 dilution) and isoamyl alcohol (1.5 ul per 2 ml of media, added in hood). Note isoamyl alcohol is also called isopentyl alcohol—same thing.
- Measured OD600 should be about 0.05 if reading in the tubes in the spec. If you need to move aliquots of the cells to cuvettes to read the density, then use larger volumes that you'll have to determine as you pre-run the labs.
- Students can take the first reading one hour after the teacher has started the subcultures. The experiment can continue with readings/smellings every 20 minutes for at least 2 hours--ideally this can be done with back to back class groups of one hour each.

Sample Data Set



Note that the original strain is suppose to smell like bananas only during stationary phase but we have found that it actually smells throughout the measurements we've made...perhaps because stationary phase activity starts earlier than we think.

Assessment
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.
Thanks!

Variations to try
Next version of this series will have BBa_J45400, a 3-methylbutanal generator to allow the strain to convert its own leucine into the ATF1 precursor.

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