BioBuilder

Synthetic Biology for Students

introduction | acknowledgments | manual

EAU THAT SMELL

WELCOME

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

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

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

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

- You, for example -

What is synthetic biology?

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

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

A little bit more about

BioBuilder.org

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

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

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

Share your science
on
- www.biobuilder.org -

Acknowledgments
This lab was developed with materials and guidance from the MIT 2006 iGEM team, as well as technical insights and help from Ginkgo Bioworks.

EAU THAT SMELL

A test of banana scent generators

context | check lists | lab protocol

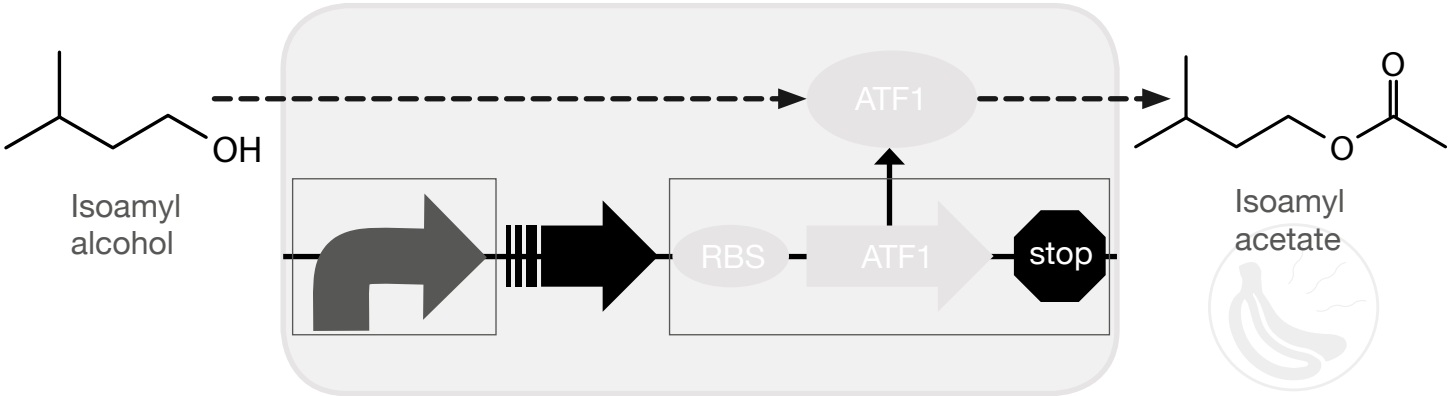
This lab provides an effective introduction to microbial growth and population dynamics as well as to the proteins and DNA sequences needed to express a gene. The engineering concept of abstraction is also emphasized through the analysis of two competing designs. Finally, biotechnology skills such as sterile technique, standard curves, and spectrophotometric analysis are embedded in the work.

CONTEXT OF THE LAB

The Eau That Smell lab emphasizes the "test" phase of the engineering design-build-test cycle.

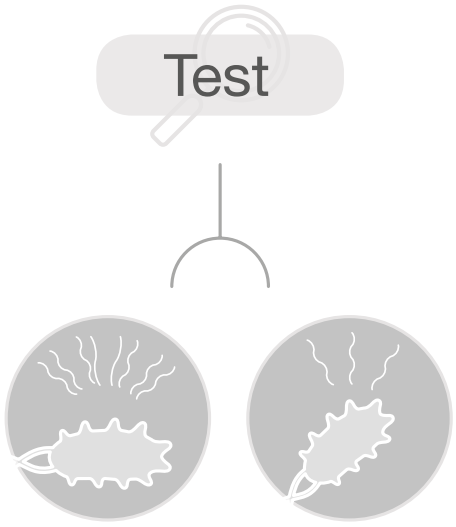
You will test two different synthetic living systems, already designed and built by other engineers. There are two design options for you to compare. Both of these designs change the smell of normally stinky bacteria, and both look like they could be "right." In comparing the designs, there will be opportunities to explore how synthetic biologists make design choices as well as to learn and teach some important scientific ideas about gene regulation and cell growth.

Banana odor generator



BBa_J45200

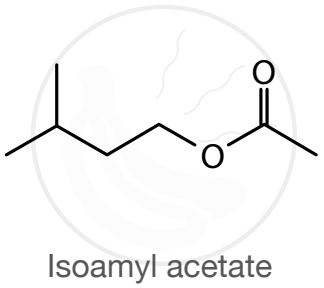
There are two ways to perform this lab. No matter which protocol you try, it will provide a valuable opportunity to learn microbiology techniques, population growth dynamics, molecular genetics and basic synthetic biology concepts in a meaningful, real world way. The analysis of the lab will provide you a chance to do meaningful error analysis and examine the difference between quantitative results and qualitative results.



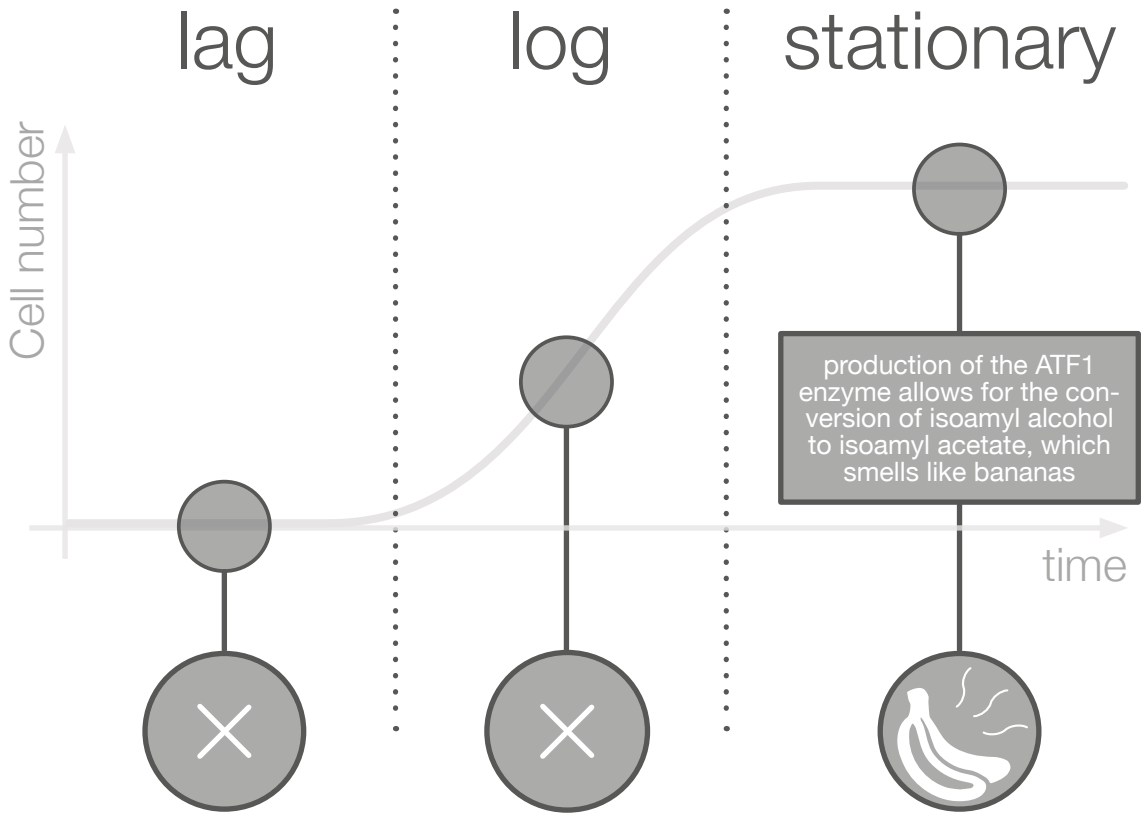
The iGEM project

For the 2006 iGEM competition, MIT students designed Eau d'coli, *E. coli* that smell like bananas when their population is in the stationary phase.

They did this by inserting a device that contains a stationary phase sensitive promoter coupled to a banana smell device, a device that contains a ribosome binding site (RBS), an open reading frame (ORF) that codes for the ATF1 enzyme, and terminator sequences. The ATF1 enzyme converts isoamyl alcohol to isoamyl acetate, the molecule that gives bananas their characteristic smell.



Isoamyl acetate



This activity

In this investigation, you'll try to generate the banana smell during the bacteria's log phase of population growth. There are two ways (at least!) you could accomplish this. Both approaches use the original banana smell generator device (an RBS, the ATF1 gene and a transcriptional terminator).

One approach uses the stationary phase promoter that was used in the original MIT project but adds a genetic inverter between the stationary phase promoter and the banana smell generator device. The other approach uses the banana smell generator in combination with a new part, a log phase promoter.

Approach 1

banana smell generator

stationary phase promoter + inverter

Approach 2

banana smell generator

log phase promoter

EXPECTED RESULTS IN BOTH APPROACHES

lag phase	log phase	stationary phase

Your task

Grow liquid cultures of these bacteria and measure the intensity of the banana smell as the population moves from lag phase through log phase and into stationary phase.

You will use 4 strains

STRAIN 1-1

The original Eau d'coli device.

Approach 1

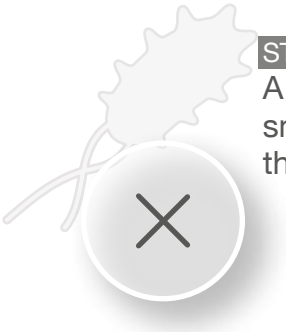
STRAIN 1-2

The original Eau d'coli device but with an inverter added between the promoter and the RBS.

Approach 2

STRAIN 1-3

The banana smell generator coupled to the log phase promoter.



STRAIN 1-4

A strain of *E. coli* that has no smell generating devices. It is the negative control.

The intensity of the banana smell will be compared to dilutions of banana extract.

The population growth can be measured at each of the phases using a spectrophotometer or the McFarland Turbidity Standards.

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

Explain how synthetic biology as an engineering discipline differs from genetic engineering.

Explain the population growth curve of bacteria.

Culture bacteria using proper microbiology methods.

Measure the growth of a bacterial population.

Define and properly use synthetic biology terms: Part, Device, Inverter.

Define and properly use molecular genetics terms: Promoter, Ribosome Binding Site (RBS), Open Reading Frame (ORF), Terminator, Plasmid.

And that's awesome!

EXPERIENCE OVERVIEW

Design choices

With this experiment, you will investigate the growth and smell patterns produced when the banana smell–generating device is controlled by a log-phase promoter versus a stationary-phase promoter and an inverter.

Both of these designs are expected to produce the banana smell during log phase, but one design might have advantages over the other. For example, the log-phase promoter might remain slightly active even when the cells begin to enter stationary phase, whereas the inverter-based design might be active in the lag phase of the growth curve, as well. Which will give the most intense banana smell in log phase is anyone's guess, and until the computer-aided design tools for synthetic biology mature, the best way to evaluate these system designs is to do an experiment.

Experimental question

Does a *log-phase promoter* or a *stationary-phase promoter plus an inverter* provide better log-phase specific banana smell?

HOW?

This experiment uses smell and turbidity measurements to compare four different strains of *E. coli* that are already designed with the genetic circuits we want to compare.

PRE-LAB Q&A

1. Briefly explain the field of synthetic biology.

2. What explains the slow rate of growth when bacteria are in lag phase? How are these factors different from what's going on when a bacteria is in log phase?

3. The 2006 MIT iGEM team put both a wintergreen smell and banana smell in *E. coli* bacteria as a way to reveal the state of growth of the cell. The wintergreen smell was made with a stationary phase promoter and an inverter device, leading to that smell being generated during lag and log phase.
Write a truth table to show when the wintergreen smell (output) is produced, in terms of the growth phase (input).

Input	Output

4. Why do we compare the intensity of the banana smell during growth to a set of dilute banana oil standards?

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

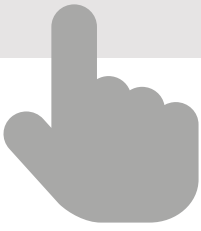
6. Why was the indole-producing gene deleted from the genome of the cells?

7. Isoamyl alcohol is added to the media we use in this experiment. Why is it there?

8. The negative control we will use is _____, which has _____.

9. Do you have any way to know in advance how the strains 1-2 and 1-3 will compare for banana-smell being generated during log phase? In other words, can you predict which will produce a stronger smell or which will produce a log-phase-specific smell?

Online you can find a rubric and score sheet for a lab report assignment: http://biobuilder.org/eau-that-smell/



DAY 1
LAB PROTOCOL

Here is the protocol you will have to follow to start the lag phase culture of the 4 strains that have been provided to you in order to perform the first measurements of banana smell and turbidity.

This part might have already been performed by your teacher.

Watch this procedure https://goo.gl/bIYNyE

Prepare a stock growth solution with

- 600 ml Luria broth
- 3 ml Ampicillin (final concentration 50 mg/liter)
- 500 µl isoamyl alcohol

While isoamyl alcohol is safe enough for lab use, it is best if this is added by the teacher.

1

Prepare the growth media by mixing 600 **ml** LB, 3 **ml** ampicillin solution and 500 **µl** isoamyl alcohol.

Most likely by adding 1 ml amp and 170 µl isoamyl alcohol to each of three bottles of 200 ml LB

2

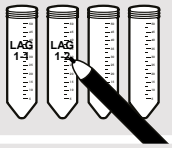
Set aside 1 ml of this mixture for each student group into a small sterile culture tube.
This aliquot will serve as the blank for the spectrophotometer.

Teachers If you are using a small plastic cuvette, a 1 ml sample will be sufficient while 2 ml will be needed for a small test tube that fits into most Spec 20 spectrophotometers

3

In duplicate, aliquot 75 ml of the broth solution to 125 ml sterile Erlenmeyer flask and add 2 ml of bacteria from one of the overnight cultures, e.g. strain 1-1.
Repeat the addition of 2ml of bacteria to 75 ml of broth in the Erlenmeyer flasks for each of the overnight cultures. Cover the flasks with foil and start them gently stirring on the stir plates.

4



For each set, label 4 x 50 mL conical tubes with the word “LAG” and the strain name, 1-1, 1-2, etc.

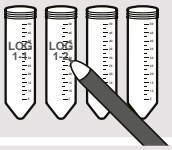
5

Transfer 25 mL of inoculated growth media from each flask into the appropriate conical tube.
Store these tubes in the refrigerator until you are ready to make measurements.

6

Grow remaining volumes of each culture in Erlenmeyer flasks with stirring at room temperature or 37°C for 4-7 hours. Be sure to record how long the cells grow.

7



For each set, label 4 x 50 mL conical tubes with the word “LOG” and the strain name, 1-1, 1-2, etc.

8

Transfer 25 mL of cell culture from each flask into the appropriate conical tube.
Store these tubes in the refrigerator until you are ready to make measurements.

9

Grow remaining volumes of each culture in Erlenmeyer flasks with stirring at room temperature or 37°C overnight. Be sure to record how long the cells grow.

10

For each set, label 4 x 50 mL conical tubes with the word “STATIONARY” and the strain name, 1-1, 1-2, etc. Transfer the grown cultures to these tubes. Store the tubes in the refrigerator until you are ready to make the Day 2 measurements.

Download the Quick Guide
http://goo.gl/XjRLEZ

DAY 2
LAB PROTOCOL

The samples you set up yesterday were placed in the fridge overnight to slow their growth and then warmed up in the morning for class. These warm up hours allow the population of cells to reach log phase and should be taken into account in the "time" column of your data table, whereas the time in the fridge overnight does not get counted. The cultures will be allowed to grow at room temperature overnight to reach stationary phase for Day 3.

One measure per sample will provide sufficient data points for construction of the population growth curve. Just make sure that you are tracking the time accurately from the initial reading.

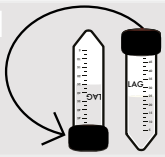
> If you are dividing the growth curve into several short lab periods, be sure to store the cells in the fridge (~4°C) until the next session.

The tips of BioBuilder

We have found that smelling small cups of coffee grounds between measurements can be used to reset everyone's sense of smell between samples.

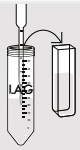
If you notice that the banana smell dissipates a bit while the tube is open then close the tube for a minute and then re-shake to bring back the smell.

1



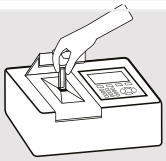
Invert the “LAG” phase conical tubes several times to completely mix the cells with the media.

2



Transfer 1 mL from each “LAG” sample to cuvettes.

3

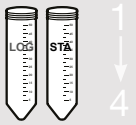


Read and record the O600 of each sample. Start by zero-ing the spectrophotometer set at 600 nm using the uninoculated media you saved on day 1.

4

Waft the air above the conical tubes towards your nose to test for any evidence of banana smell. Compare the intensity of the banana smell to the banana smell standards.

5



1
4

Repeat steps 1-4 with the “LOG” and the “STATIONARY” phase cultures.

6

Discard all biological materials after decontaminating with 10% bleach.

Clean-up instructions

Provide containers at each workstation for biological waste such as pipet tips and tubes. Be sure to follow hazardous waste procedures as set forth by your school or municipality. Generally, it is safe to soak the material in each container with a 10% bleach solution for 20 minutes. Materials can then be discarded into the regular trash. You can find more information about microbiology lab safety at the BioBuilder website.

If no spectrophotometer is available

Prepare turbidity standards, where 1 OD 600 ~ 1 x 10^9 cells/ml

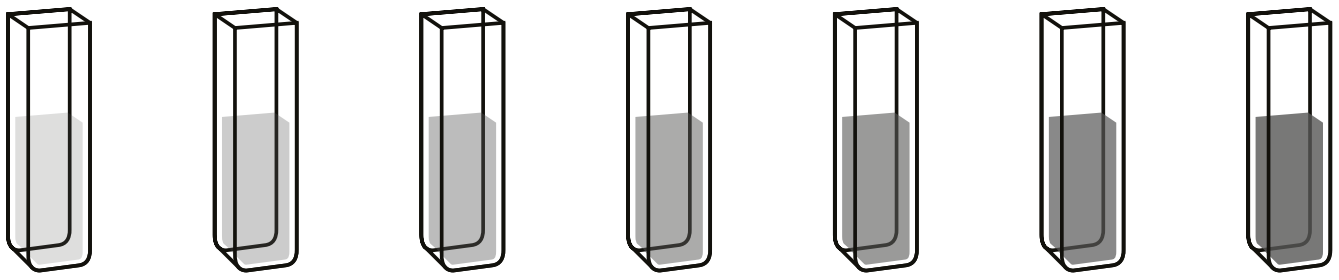
These standards can be prepared well in advance of lab and are useful if you are running the protocols without access to a spectrophotometer.

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

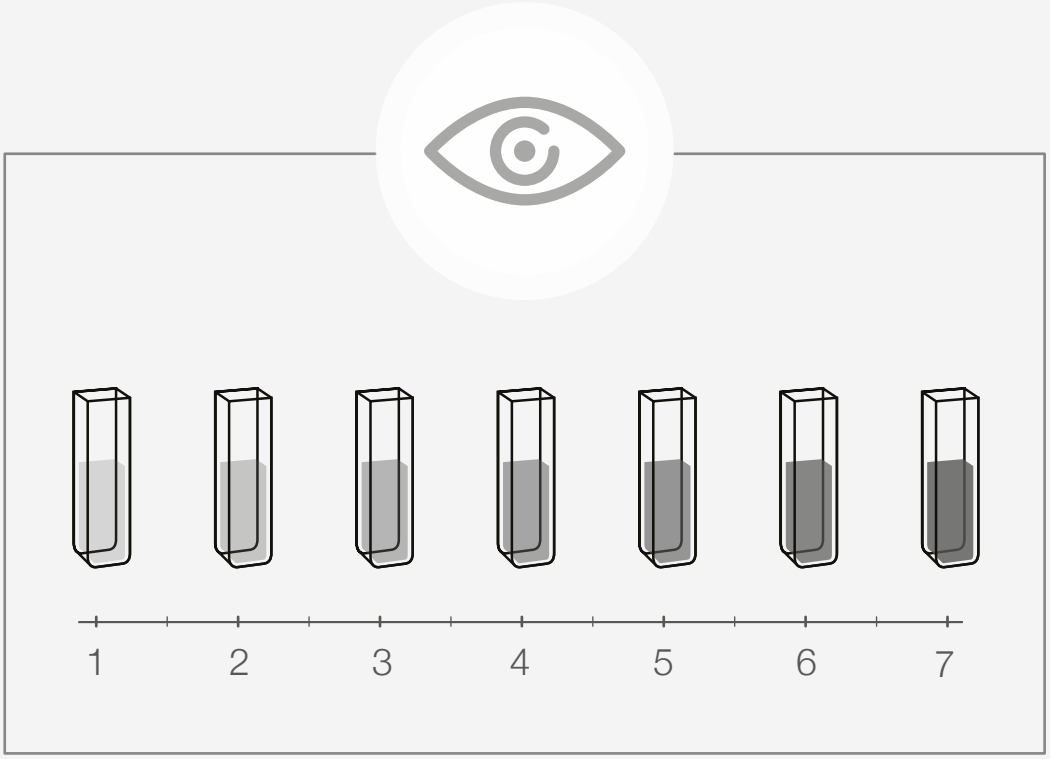


About the turbidity standards

You 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. You can use 7 as the value in that case. You may use half values (e.g. 1.5). This also leads to interesting questions about the nature of data.



DAY 3
DATA COLLECTION

The experimental strains represent the two designs we want to compare:
- stationary phase promoter + inverter + banana-smell generating device
- log phase promoter + banana-smell generating device

By comparing the smell and growth of these two seemingly equivalent designs, we can ask the experimental question: does a log-phase promoter or a stationary-phase promoter plus an inverter design provide better log-phase specific banana smell?

In your lab notebook, you will need to construct a data table as shown below for each of the samples.

Time	OD600	Cells/ml	Banana Smell
T_0			
T_0+____			
T_0+ ___			
T_0+ ____			
T_0+ ____			
T_0+ ____			
T_0+ ____			

LAB REPORT

As you write, be sure to define and properly use all highlighted terms throughout the introduction and other parts of the lab.

I. Introduction

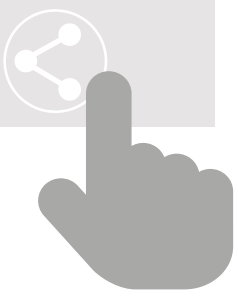
- Provide a brief introduction describing the field of synthetic biology.
- Briefly describe the purpose of the lab. What are we trying to do here?
- Explain how the banana smell generator functions.
- Why are we using optical density to measure the population?
- Explain each phase of the bacterial population growth curve.
- Presume that a reader of your lab report has not read the assignment.

II. Methods

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

III. Results

- Present the data tables in clear format.
- Draw population growth curves of the class mean data for each sample.
- Indicate on each curve when you could smell bananas.



IV. Discussion (possible questions to address are here, you are not required to address them all)

• Describe the results: Were we able to measure the population growth? Were we able to smell bananas? Did each device produce the same results? Did the genetic systems affect the growth curve of the bacteria? Explain your answers.
• Analyze the data: Be sure to discuss how each part of the experiment adds to your conclusion.
• Discuss errors and other reasons for data variability.
• How confident are you in the results? Are you equally confident in both the growth data and the smell data? Explain.
• Is using your sense of smell a valid way to measure the intensity of the banana scent? why or why not?
• What methods did you use to increase your confidence in the results?
• How might we try to change this system so that we can quantify the banana smell? Would we be better off using a different kind of signal? If so, what would you suggest?
• If you could construct a different genetic system, what might you construct? What would you need to do?

V. Citations and references

• Be sure these are of good quality.
• Embed citations.
• Follow proper reference format.

Q&A

1. What is expected if the banana-smell generator is:
   a. controlled by a stationary phase promoter but the cells have been diluted into fresh media?

   b. expressed in a strain which still also makes indole?

   c. expressed by a log phase promoter that is stronger than the one we used, i.e. more RNA polymerase activity is associated with that stronger promoter?

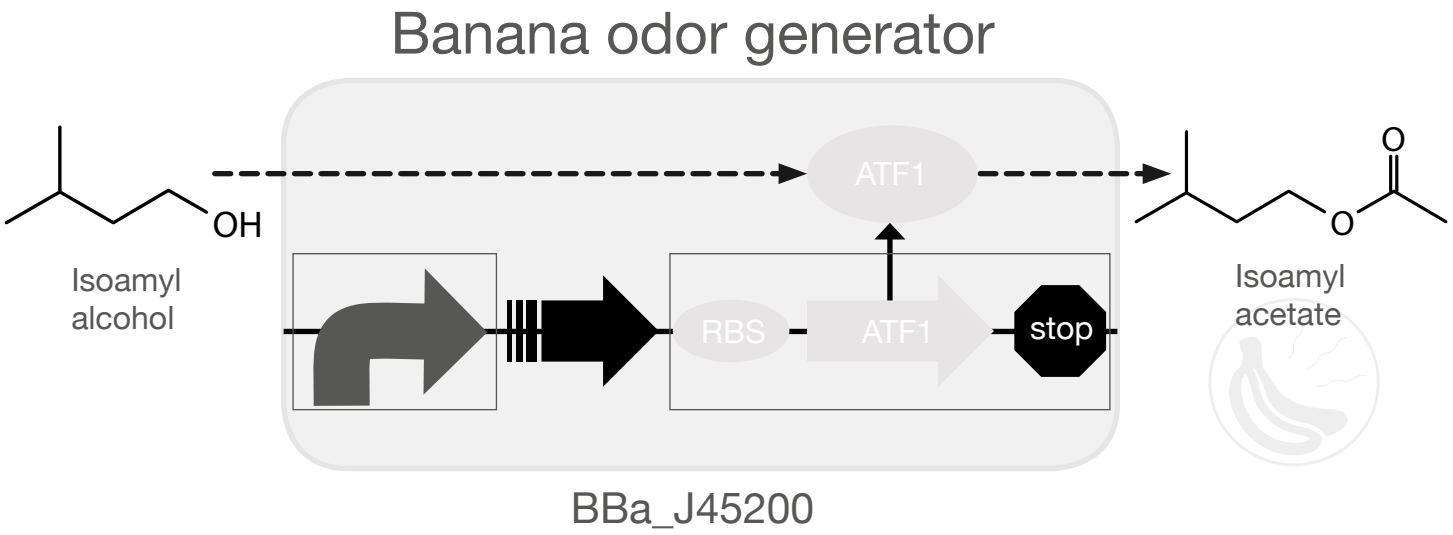
   d. expressed from a stationary phase promoter that is followed by TWO inverter devices?

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

3. If the banana-smell gene was expressed from an inverter device that was controlled by a log phase promoter, what growth stage(s) would you expect to smell bananas?

4. What are some applications you can imagine with this synthetic system, namely a cell that makes a chemical or an enzyme when it reaches a particular growth stage?

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?



Online you can find a rubric and score sheet for a lab report assignment: http://biobuilder.org/eau-that-smell/

MORE SYNTHETHIC BIOLOGY ON

BioBuilder.org

What a Colorful World	**The iTune Device**	**Picture This**
Eau that Smell	**Golden Bread**	

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

BioBuilder
all rights reserved