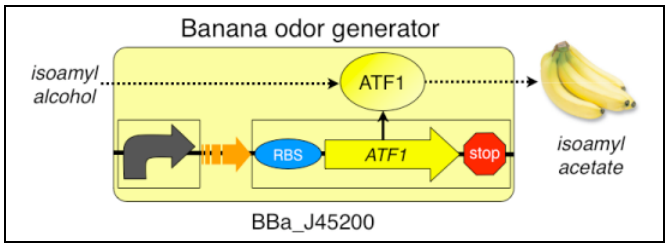
SYNTHETIC BIOLOGY AND THE HIGH SCHOOL CURRICULUM: LAB 1
http://openwetware.org/wiki/Synthetic_Biology_and_the_High_School_Curriculum:_Lab_1



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

Objectives

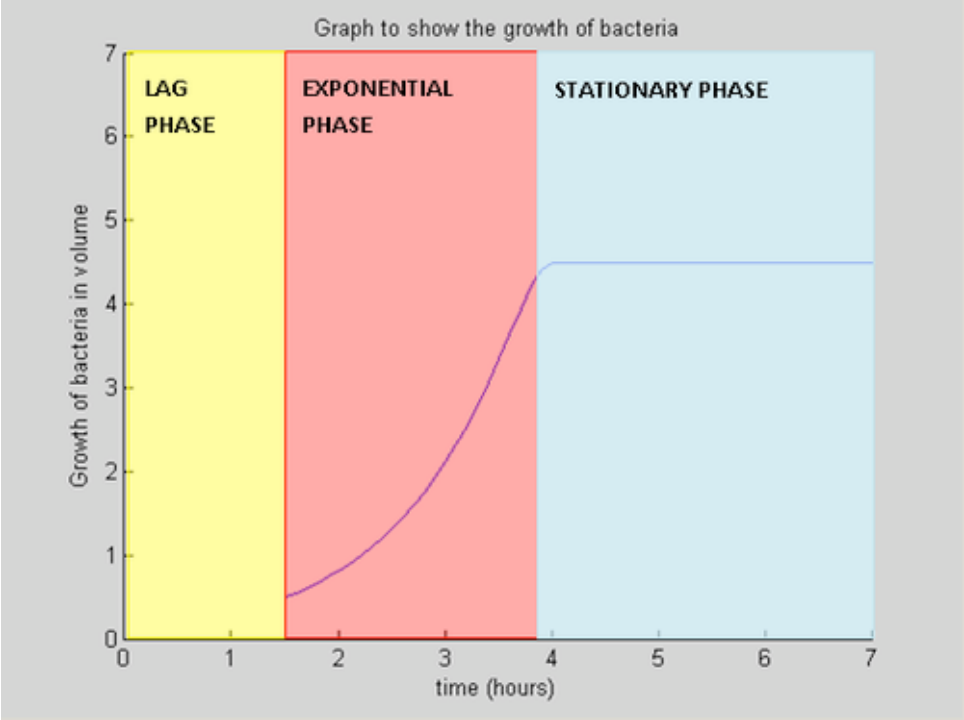
By the conclusion of this laboratory investigation, the student will be able to:

- 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.

Introduction

For the 2006 iGEM competition, MIT students designed eau d' e coli, E. coli that smell like bananas when their population is in the stationary phase. They did this by inserting 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.

It has been suggested that a device that generates the banana smell during the bacteria's log (or exponential) phase of population growth will be helpful. There are two ways to accomplish this. Both methods will continue to use the banana smell device but alter the function of the promoter. One method involves coupling the banana smell device to a new part, a log phase promoter. The other method involves using the same promoter but adding an inverter. Synthetic biologists have constructed these devices for us and transformed bacteria with them.



We have been sent four different E. coli colonies. Each contains a different device:

Sample 1. The original Eau d' Coli device

Sample 2. The banana smell generator coupled to the log phase promoter

Sample 3. The original Eau d' Coli device but with an inverter added between the promoter and the RBS.

Sample 4. A strain of E. coli that has no smell generating devices.

Our task will be to grow these bacterial populations and test for the banana smell as the population moves through the log phase and into the stationary phase. We will determine the population growth by using a Spec 20 to measure the density of the bacteria in liquid culture. As the population increases we can assess the increasing banana smell.

Procedure

Part 1: Culturing Bacteria

We will be receiving our bacteria with the plasmid already inserted. This culture will come in the form of a "stab" or "slant", a test tube with a small amount of bacteria on a slanted media. To continue the experiment we will have to further culture the bacteria.

Day 1:

1. Using a sterile toothpick or inoculating loop, gather a small amount of bacteria from the stab and transfer it to a petri dish containing Luria Broth (LB) agar plus ampicillin medium.
2. Repeat with the remaining stab samples, streaking out each onto a different petri dish.
3. Place these cultures in a 37°C incubator overnight.

This video illustrates the technique used for this transfer:
http://www.youtube.com/watch?v=QydH5ZoD_Aw

Day 2:

1. Using a sterile inoculating loop, transfer a bacterial colony from one of the petri dishes to a large sterile culture tube containing 5 ml of Luria Broth and 5 µl of ampicillin.
2. Repeat for each strain you will inoculate.
3. Place the culture tubes in the roller wheel in the incubator at 37°C overnight. Be sure to balance the tubes across from each other to minimize stress on the roller wheel.

This video illustrates the idea of this technique, though you'll be transferring cells from the petri dish to the Luria Broth:
http://www.youtube.com/watch?v=0odxJy0nR9s&NR=1

Part 2: Measuring bacterial population growth

1. Prepare a large sterile culture tube with
    - 10 mL Luria broth
    - 10 µL Ampicillin
    - 7.5 µL isopentyl alcohol
2. Pipet this solution up and down two times to mix it.
3. Move 2 mL of this mixture into a small sterile culture tube. Set this aside, it will be the blank for the spectrophotometer.
4. Move 2 ml of the broth solution to another small sterile tube and add 100 µL of bacteria from one of the overnight cultures. This will be one of your the sample tubes.
5. Repeat the mixing of 100 µL of bacteria with 2 ml of broth for each of the overnight cultures. If you are testing all 4 samples you should now have 5 small test tubes (4 with bacterial dilutions and one blank).
6. Prepare the spec 20 by setting it to OD600.
7. Note the time and take an "initial" density reading for the bacterial samples.

8. Place the tubes with bacteria into the roller wheel in the incubator. Make sure they are balanced across from one another.
9. After 60 minutes, remove the culture tubes.
10. Read the blank and adjust the % Absorbance to zero.
11. Read the sample tubes and record the % Absorbance.
12. Sniff the culture tube for any evidence of a banana smell and record your data.
13. Return the bacterial cultures to the roller wheel.
14. At 20 minute intervals repeat steps 10-13.
15. Between time points, you can calculate the bacterial population: 1OD600 unit = 1 x 10^9 bacteria.

Data Table

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

SAMPLE_____

Time	OD600	cells/ml*	Banana smell (+/-)
Initial			
60 minutes			
80 minutes			
100 minutes			
120 minutes			
140 minutes			
160 minutes			
180 minutes			

- 1 OD600 unit ~ 1 x 10^9 bacterial cells/ml

Use
"-" for no banana smell
"-/+" for maybe detected
"+/-" for detectable
"+" for strongly detected

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

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? Why are we using optical density to measure the population?
- Presume that a reader of your lab report has not read the assignment.
- Explain each phase of the bacterial population growth curve.

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

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.

Discussion:
- Describe the results: Were we able to measure the population growth? Were we able to smell bananas?
- 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 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 genetic system, what might you construct? What would you need to do?

Citations and references:
- Be sure these are of good quality.
- Embed citations.
- Follow proper reference format.