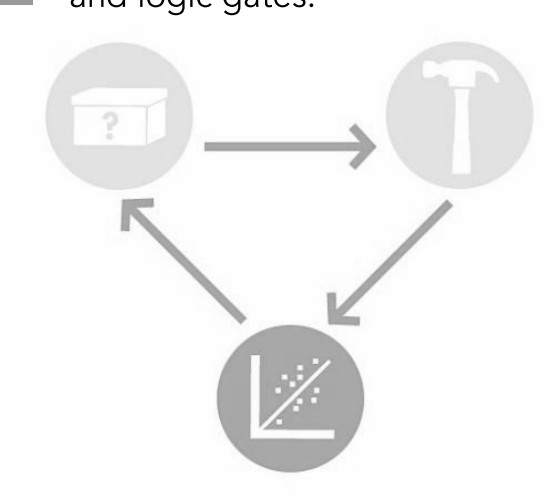
STUDENT MANUAL

BioBuilder

Synthetic Biology for Students

Eau That Smell

Normally stinky smelling bacteria are engineered to smell sweet. Lab activities include measurements of microbial growth, analysis of genes on a molecular level, and exploration of synthetic biology concepts related to system design and logic gates.



BioBuilder

Eau That Smell

Table of Contents

ABOUT EAU THAT SMELL

The engineer's process of "design-build-test" is similar to the scientist's method of "hypothesize, test, analyze." Both of these approaches are often presented as linear endeavors that start with "design" for engineers and "hypothesis" for scientists, but in reality they are iterative efforts that can begin at any point in the cycle.

The Eau That Smell activity emphasizes the "test" phase of the engineering design-build-test cycle, but also shines a spotlight on the design and re-design of the system being tested. For this experiment, two different synthetic living systems have been designed and built by other engineers. Both of the designs change the smell of normally stinky bacteria, and both designs look like they could work. In comparing the performance of the two designs, there are opportunities to explore just how synthetic biologists make design choices as well as dive into some important scientific ideas about gene regulation and cell growth.

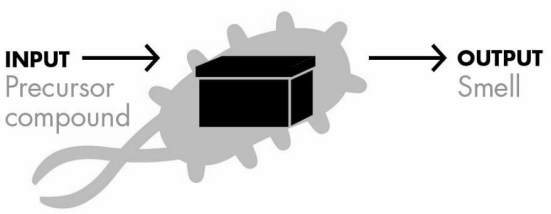
BioBuilder's Eau That Smell activity was inspired by an International Genetically Engineered Machines (iGEM) project from 2006 in which a team of undergraduates from MIT designed a new strain of bacteria they called "Eau d'coli." The MIT team built a strain of *E. coli* that gave off sweet smells, like wintergreen or ripe bananas, depending on the growth phase of the cells.

The 2006 iGEM team from MIT and their project,"Eau d'coli"

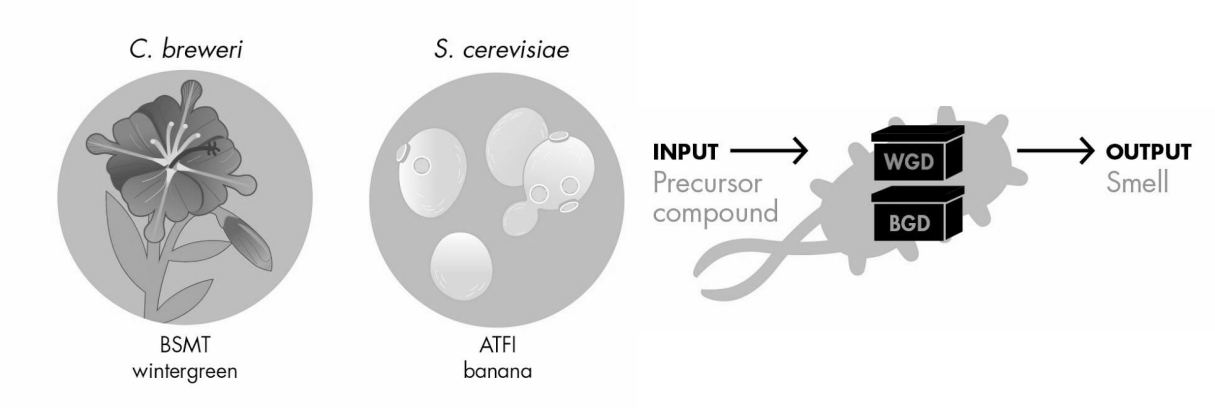
ENGINEERING Eau d'coli

The 2006 MIT iGEM team began their Eau d'coli project with a simple observation: *E. coli* smell really bad. They decided to introduce a new, more pleasant scent into bacteria so the engineered cells could smell nice instead of stinky.

Having identified the challenge they wanted to address, they could then specify a system-level design. They decided that the bacteria could be engineered to convert a precursor compound in the media into a nice smelling output.



To realize their system design, the team looked for some genetic devices that could turn a non-smelling precursor compound into a pleasant scent. They found several by searching the scientific literature, and then they tried some preliminary experiments to narrow down their ideas. They finally settled on two genetic devices: one normally found in flowers and the other from yeast cells. The genetic devices they selected could convert precursor compounds into molecules that smelled like wintergreen or bananas.



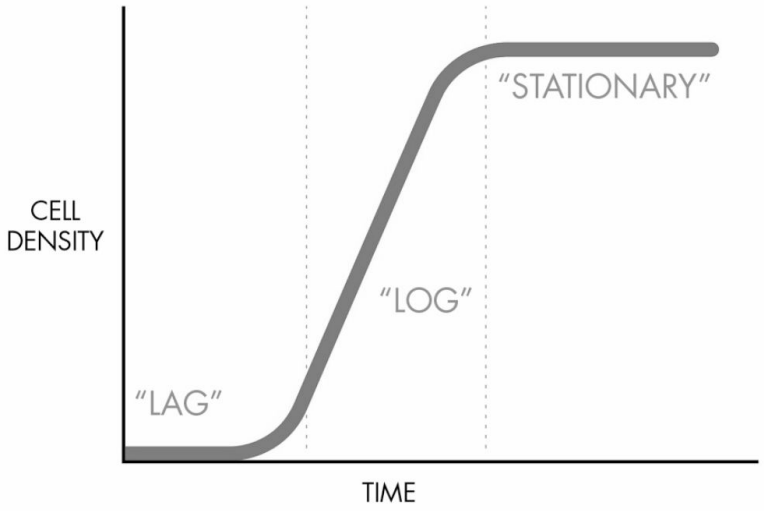
At this point, the team could have started to work on their parts-level design and then built and tested their system.

But instead, they decided to revise their system-level design so the cell could switch between the two smell outputs. The team went back to the drawing board and modified their original design goal of making a better smelling bacteria. They redesigned their system so cells would have one kind of smell when the cells were actively dividing and another smell when the cells reached their maximal density. With this modification to the system's design, the team went on to build the first synthetic living system that used scent as a reporter for cell growth.

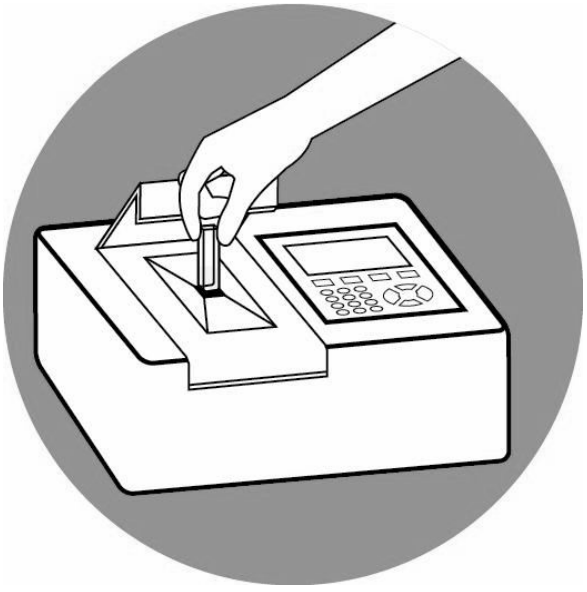
INTRO TO BACTERIAL GROWTH CURVES

Cell populations follow a predictable growth pattern. When cells are introduced into fresh media, they spend time in what's called "lag phase." During this time, the cells are getting acclimated to the new media, but they are not dividing. Consequently, the # of cells/unit of volume, aka the cell density, does not increase and the growth curve is flat.

During the lag phase, cells are being primed for growth, and when they are ready to divide, they enter "log phase" (also called "exponential phase"). During this phase of growth, the number of cells increases quickly. At 37°C, bacterial cells can double every 30 minutes or so. The log phase of the growth curve shows an increase in the density of cells over time.



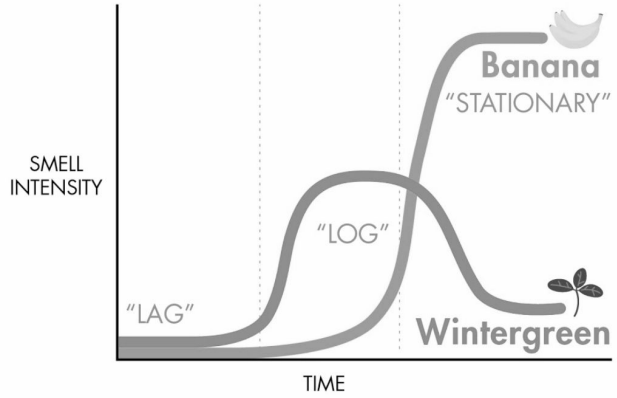
As the cells get crowded and the media gets used up, the rate of cell division slows down and then stops. During this "stationary phase" of growth, the density of the culture does not increase. The cells are alive, however, and can enter lag phase again if they are diluted into fresh media.



FYI: Researchers often measure cell density using a spectrophotometer. This instrument detects the amount of light that can pass through a sample. The difference between the amount of light that enters a sample and the amount that exits is called the optical density. The optical density increases as the density of cells increases.

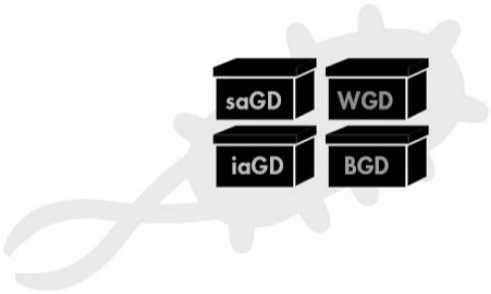
Your experiment will focus on growth control of the banana scent. In particular, you will ask how to best express the banana scent during the log phase of growth.

SYSTEM AND DEVICE-LEVEL REDESIGN



The MIT iGEM team wanted to change their system so the smell-generating devices could respond to cell growth phase. In particular they wanted the wintergreen scent to be produced in log phase and the banana scent to be produced in stationary phase.

To accomplish this new system specification, the team needed to re-engineer the cells in three ways.



First, the cells were re-engineered to include additional devices that made the smell precursor molecules. Precursors did not have to be added to the growth media since the cells could make them from natural starting materials already inside the cells.

Second, the cells were re-engineered so the scent production was controlled at the level of transcription. In particular, the banana-scent was transcribed from a promoter called P_{osmY}, which is most active during the stationary phase of growth. Similarly, the wintergreen-scent was controlled at the transcriptional level. The MIT iGEM team chose to re-use the P_{osmY} promoter to express the wintergreen scent, but they added a genetic inverter device (also called a NOT logic gate, described more on the next page). This design was intended to restrict the wintergreen scent to growth phases that are NOT stationary phase.

CHASSIS ENGINEERING

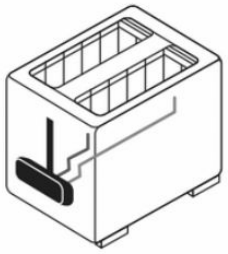
The third aspect of the system that the MIT iGEM team re-designed was the bacterial chassis itself. This redesign came about because in early tests of their system, they noticed that the natural stinkiness of the strain was overwhelming the banana and wintergreen smells. They researched the genetic origins of the strain's terrible smell and found that bacteria produce a smelly compound called indole. Deletion of the tryptophanase gene led to an indole-deficient chassis that had a much less pungent stink. This chassis was used for all later experiments.

INTRODUCTION TO LOGIC GATES

When the MIT iGEM wanted to control the wintergreen scent, they deployed a concept that is more commonly found in electrical engineering. The team used a digital inverter device, aka a NOT gate, to construct a genetic digital circuit. The operation of logic devices, also sometimes called Boolean logic gates, can be described using truth tables. These tables represent all possible combinations of inputs and specify a single output for each combination.

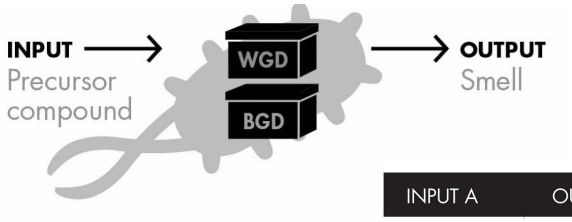
In electrical engineering, the inputs might be electrical signals from wires and the output might be a third electrical signal that does some function, such as turning on your toaster. In synthetic biology, logic gates are built out of DNA using control elements and products from natural genetic circuits. Inputs might be environmental stimuli, such as sugar, pH, heat or cell density. The output might be a fluorescent reporter such as GFP, or a molecule of interest such as a medicine, biofuel or scent.

Electrical Engineering



INPUT A	INPUT B	OUTPUT
0	0	0
0	1	0
1	0	0
1	1	1

Synthetic Biology

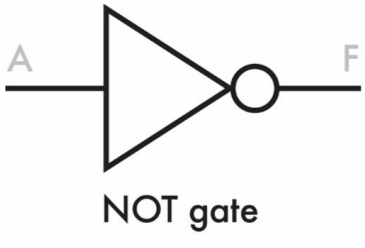


INPUT A	OUTPUT F
0	1
1	0

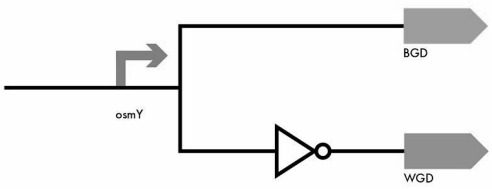
Logic gates in the context of Eau d'coli

The 2006 MIT iGEM team wanted to express the wintergreen scent in the log phase of growth. To accomplish this design, they paired the P_{osmY} stationary phase promoter with a genetic NOT gate that could invert the natural pattern of gene expression from P_{osmY}. The truth table (below) shows the on/off states for the outputs with and without the inverting NOT gate.

By combining the stationary-phase promoter and a genetic NOT gate, the wintergreen scent was expressed whenever the banana scent wasn't.



	STATIONARY	LOG
osmY	1	0
osmY + inverter	0	1

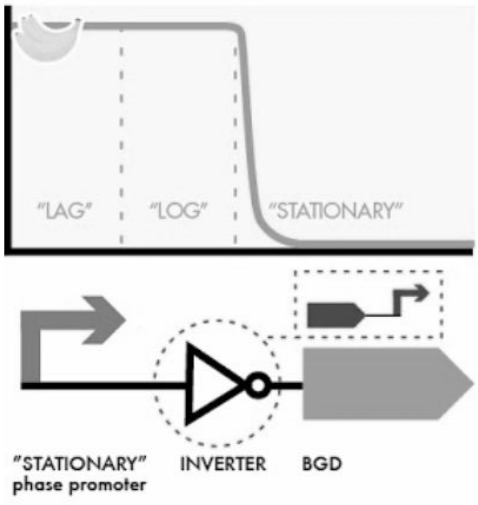


In this lab, you will compare two banana-scent generating devices that are both expected to produce scent in the log phase of growth.

BIOBUILDER'S EAU THAT SMELL ACTIVITY

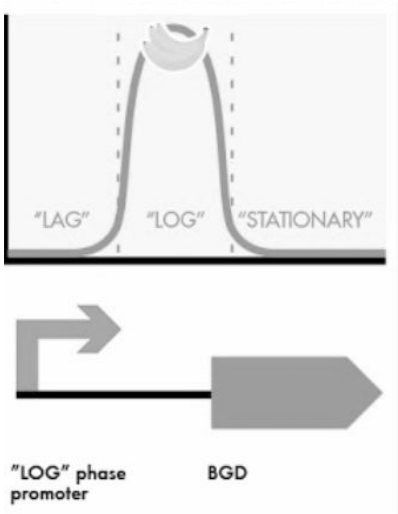
BioBuilder's Eau That Smell activity extends the work of the 2006 MIT iGEM team, looking at different ways to control the relationship between cell growth and scent generation. In this activity, the system has been re-engineered once again. The strain has no wintergreen scent production and the banana scent is re-designed to express during the log phase of growth, rather than the stationary phase. The goal with this experiment is to compare two designs for making banana scent during log phase. On paper, the two design options appear nearly equivalent, but real-world behavior often deviates from expectations, and this laboratory activity allows for direct testing of the differently designed strains.

Design 1



Expresses the banana scent using a stationary phase promoter and an inverting NOT gate

Design 2



Expresses the banana scent using a log phase promoter and no logic gate

MEASURING SCENT PRODUCTION

To simplify the genetics of the strains, the precursor molecule for the banana scent will be added to the media rather than be produced by the cell itself. This change may impact the smell of the media, but it will do so with all the strains tested.

All strains are indole-deficient to alleviate the strain's natural stink.

To measure the amount of banana scent, smell tests will be carried out at each stage of growth. The original Eau d'coli strain will provide a POSITIVE CONTROL FOR SCENT since this strain is known to produce banana scent during the stationary phase of growth. The indole-deficient strain with no scent generating devices will serve as a NEGATIVE CONTROL FOR SCENT.

Banana oil will be diluted to make banana scent standards. Because everyone will use these standards, the amount of smell can be described using a shared measurement scale.

Two protocols

The basic version of this protocol involves pre-growing the strains to lag, log and stationary phase. The activity emphasizes data analysis over data collection. A longer version of the protocol follows the strains through the three stages of growth, enabling growth curve-data collection as well as scent measurements.

PRE-LAB QUESTIONS

Briefly explain the goal of synthetic biology.

What is going on during the lag phase of growth? How can you explain the fact that there is no change in the number of cells over time? How is this different from what is going on during the log phase of growth?

The 2006 MIT iGEM team designed a cell to produce both the wintergreen scent and the banana scent. The wintergreen scent was controlled with a stationary phase promoter and an inverter device, leading to production of the wintergreen scent during lag and stationary phase. Complete the truth table to show when the wintergreen smell output is produced in term of growth phase input.

Input (Stationary Phase of Growth))	Output (Wintergreen Smell).

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

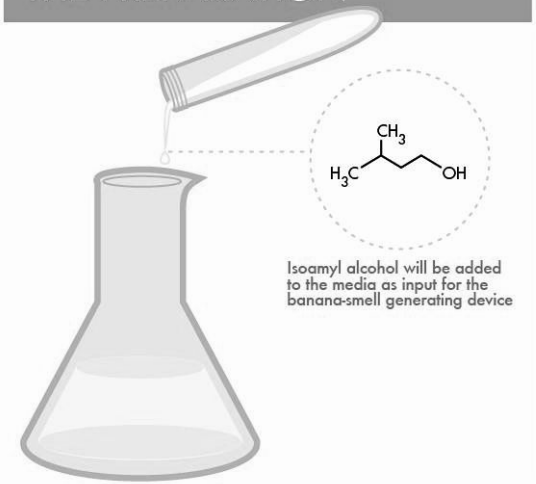
How was the chassis engineered to improve the performance of the strains in this system? What was the modification expected to do?

Isoamyl alcohol is added to the media used in this experiment. Why?

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

EAU THAT SMELL

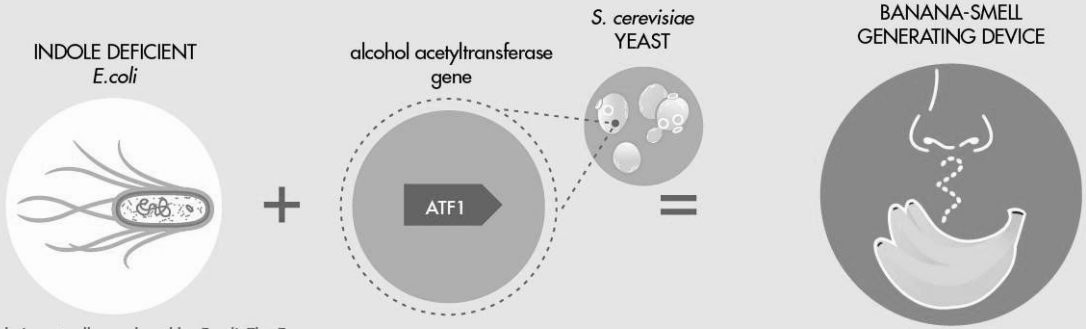
THE PREPARATION



Isoamyl alcohol will be added to the media as input for the banana-smell generating device

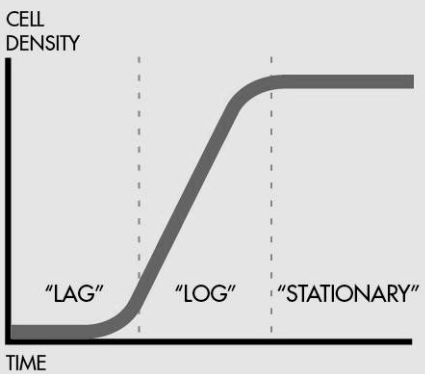
THE SYSTEM

A gene from *S. cerevisiae* converts isoamyl alcohol into isoamyl acetate, which smells like bananas.

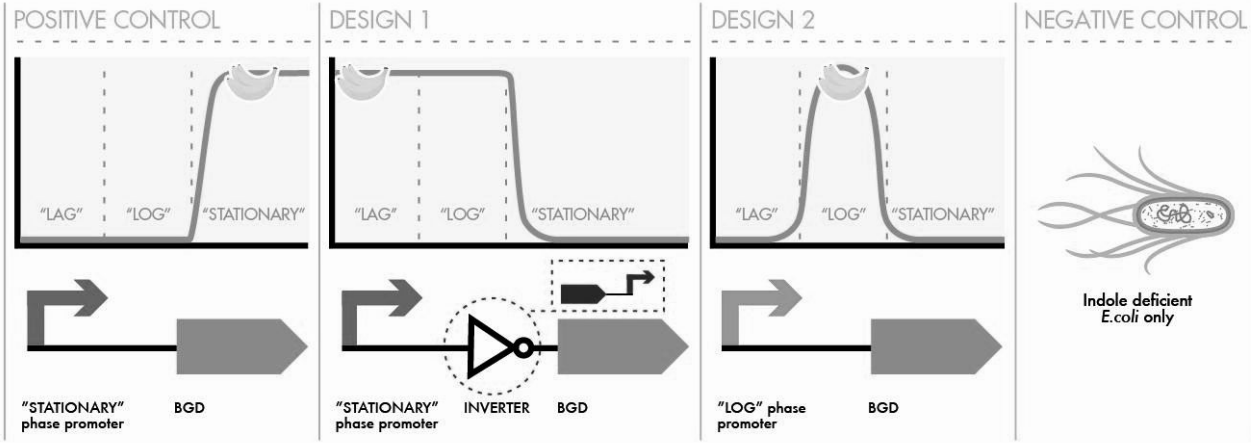


Indole is naturally produced by *E.coli*. The Eau d'coli strain was modified to eliminate indole production and its stinky smell.

GROWTH PHASES



UNDER THE HOOD



BioBuilder Educational Foundation

QUICK GUIDE: EAU THAT SMELL

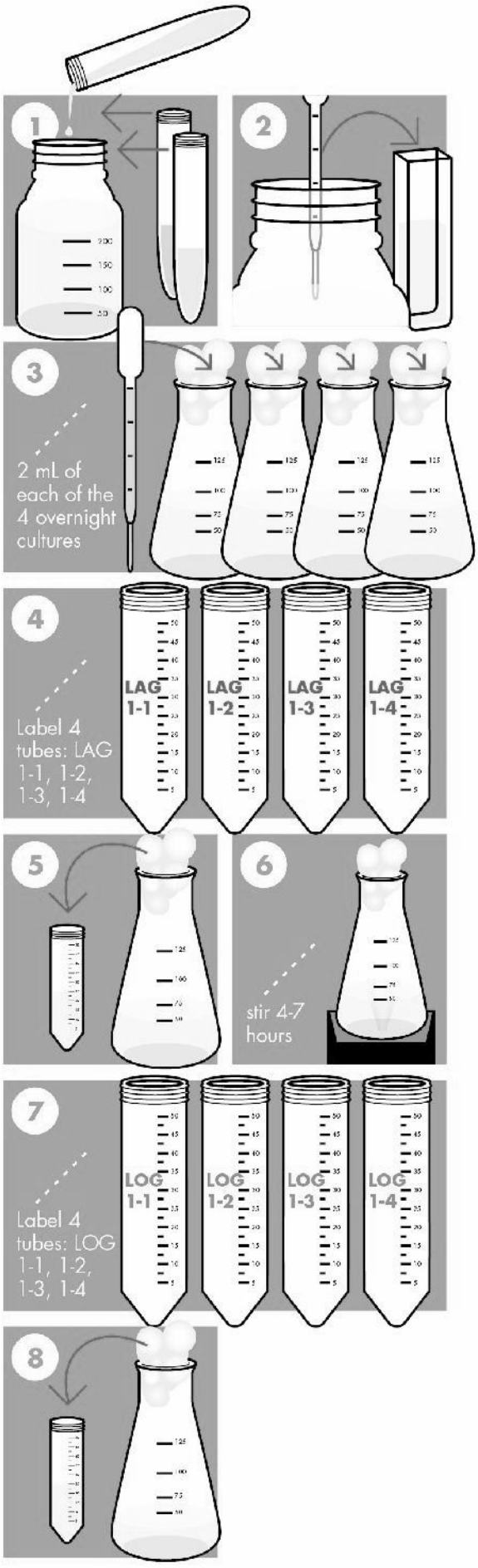
IN ADVANCE

Grow liquid overnight cultures of the 4 strains to be tested**
Mix banana smell standards

DAYS OF LAB

Day 1:

1. In a sterile bottle or flask, prepare growth media (LB + ampicillin + isoamyl alcohol).
2. Remove 1 mL of media and store in the refrigerator. This will be used to blank the spectrophotometer.
3. Transfer 75 mL of growth media to 125 mL sterile Erlenmeyer flask, and add 2 mL of one overnight culture**, e.g. strain 1-1. Repeat with remaining 3 strains.
4. Label 4 x 50 mL conical tubes with the word "LAG" and the strain name, 1-1 or 1-2 or 1-3 or 1-4.
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. Label 4 x 50 mL conical tubes with the word "LOG" and the strain name, 1-1 or 1-2 or 1-3 or 1-4.
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.



BioBuilder
Educational Foundation

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. Label 4 x 50 mL conical tubes with the word "STATIONARY" and the strain name, 1-1 or 1-2 or 1-3 or 1-4. Transfer the grown cultures to these tubes. Store the tubes in the refrigerator until you are ready to make the Day 2 measurements.

Day 2:

11. Invert the "LAG" phase conical tubes several times to completely mix the cells with the media.

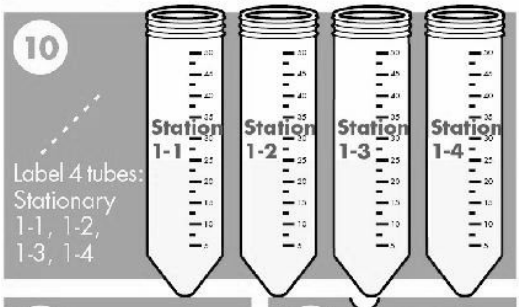
12. Transfer 1 mL from each "LAG" sample to cuvettes.

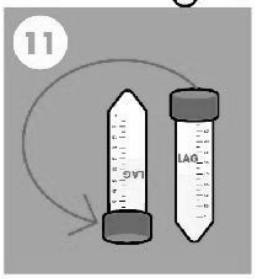
13. Read and record the O600 of each sample. Start by zero-ing the spectrophotometer set at 600 nm using the uninoculated media you saved on Day 1.

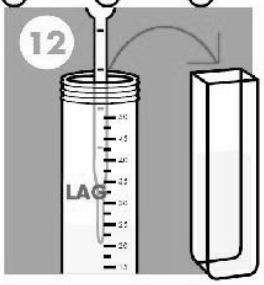
14. Waft the air above the conical tubes towards your nose to test for any evidence of banana smell. Compare the intensity of the banana smell to the banana smell standards.

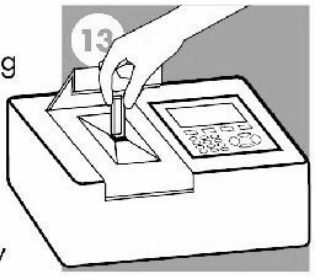
15. Repeat steps **11-14** with the "LOG" and the "STATIONARY" phase cultures.

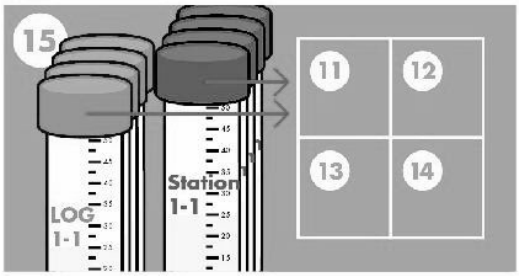
16. Discard all biological materials after decontaminating with 10% bleach











** VIDEO OF PROCEDURE AVAILABLE ONLINE

POST-LAB QUESTIONS

What is expected in each of the following cases:

a. The banana scent generator is controlled by a stationary phase promoter but the cells have been diluted into fresh media?

b. The banana scent generator is expressed in a strain that also makes indole?

c. The banana scent generator is controlled 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. The banana scent generator is controlled by a stationary phase promoter that is followed by TWO inverter devices?

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

What are some applications you can imagine with this synthetic system that can make a chemical or an enzyme when it reaches a particular growth stage?

What is synthetic biology and what are some examples of what you can do with this field?

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

Ideally, the interpretation of these results should encourage more experimentation, provide ideas for improved designs, and build excitement to explore and do more.

STUDENT MANUAL

BioBuilder

Do you have an idea for improving and extending the units? Please email us: **info@biobuilder.org**