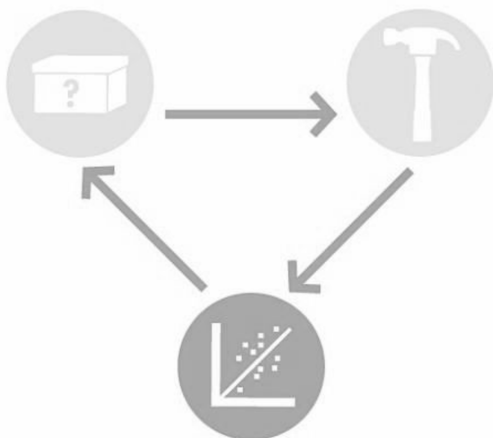
TEACHER MANUAL

BioBuilder

Synthetic Biology for Teachers

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.



This teacher's booklet is meant to help support you and your students with the BioBuilder units. Let us know what you need and how it goes. Email us: **info@biobuilder.org**

BioBuilder

Eau That Smell

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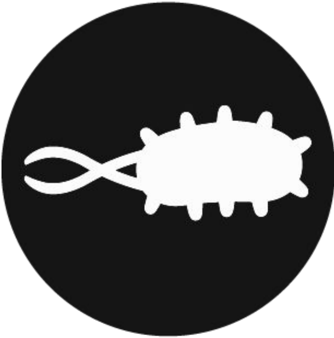
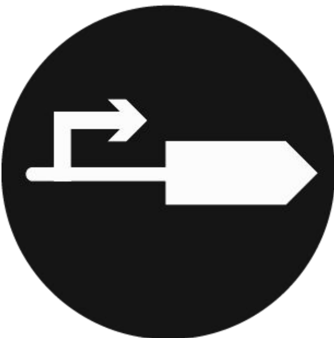


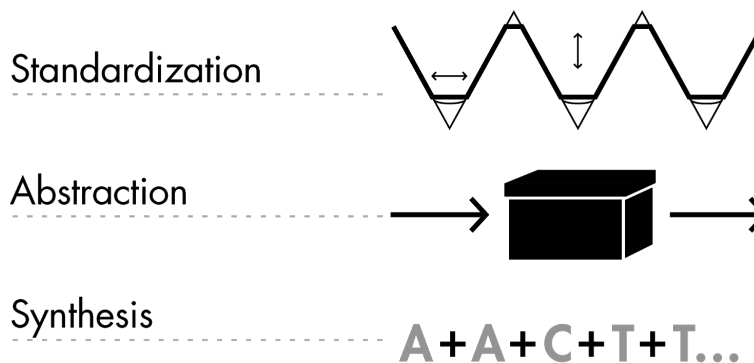


About Synthetic Biology

For the last decade, teachers have introduced genetic engineering techniques to students. It is becoming commonplace for students in Biology and AP Biology courses to conduct a standard set of "experiments" using gel electrophoresis and bacterial transformation techniques. Students who perform these experiments learn several basic techniques, but that is where the laboratory experience ends. There is little room for student inquiry or creativity. The students are more technicians than scientists.

A solution to this limitation comes not from biology but from a relatively new field, Synthetic Biology. Synthetic biologists apply engineering principles and extend genetic engineering techniques to construct synthetic living systems. The synthetic biology approach familiarizes teachers and students with molecular biology, genetic engineering and microbiology methods in an engineering setting. The students learn designing, building or testing designs of engineered biological systems. In addition, this approach provides science teachers with a means of fulfilling state and national teaching standards that are hard to address in most biology classes.

Using synthetic biology to teach engineering



BioBuilder's engineering approach focuses on two important principles: abstraction and standardization, and relies on enabling technologies such as DNA synthesis. These principles and technologies extend the teaching of molecular techniques into real world, authentic applications. In the way that physics teachers can have students create functioning circuits and computer teachers can have students create 3-D animations, biology teachers can have students safely design, construct and analyze engineered biological systems.

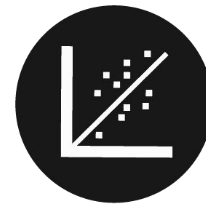
The BioBuilder Curriculum

BioBuilder provides educational materials for students and teachers to explore the underpinnings of synthetic biology. All the material is modular and can be taught completely, in any order, or piecemeal, as individual exercises to supplement an existing program. BioBuilder's curriculum includes both classroom lessons and laboratory activities. Biodesign and Bioethics lessons can be carried out in any sized classroom and with many age groups. The laboratory investigations provide standard protocols as well as modifications to meet local situations and needs.

Biology teachers can use our materials to lead engineering challenges with students. Students gain first-hand experience with the engineering paradigm:

DESIGN

BUILD



TEST

Students are motivated to understand the underlying science within an authentic context of engineering challenges. BioBuilder students become more than technicians; they become engineers.

What A Colorful World

Examines the role of the cellular chassis in system performance. Students transform different strains of *E. coli* with DNA that turns the cells several bright colors. Students then observe how different the color intensity can be from strain to strain, despite being encoded by the same DNA sequence.

iTUNE Device

Examines the role of parts, such as promoters and ribosome binding sites, in predicting the output of a genetic device. The students measure β-galactosidase enzymatic activity as the device's output, thereby looking through the lens of molecular genetics to predict and then evaluate a device's behavior.

Picture This

Three activities to explore the role of modeling in circuit design. These activities include a downloadable program to computationally vary the parameters of a genetic circuit, an exercise to mimic a genetic circuit with electronic parts, and an opportunity to send a stencil that will be turned into a bacterial photograph.

Eau That Smell

Compares two alternative genetic designs. Both programs should make the cells smell like ripe bananas as the cells grow.

Golden Bread

Explores the science, engineering and bioethics of a yeast that's genetically modified to make a vitamin-enriched food. Lab activities include PCR, yeast transformation, codon shuffling and quantitative analysis of data.

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.

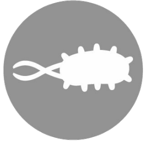
The Eau That Smell activity emphasizes the "test" phase of the engineering design-build-test cycle, but also shines a spotlight on the design and re-design of the system being tested. For this experiment, two different synthetic living systems have been designed and built by other engineers. Both of 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.

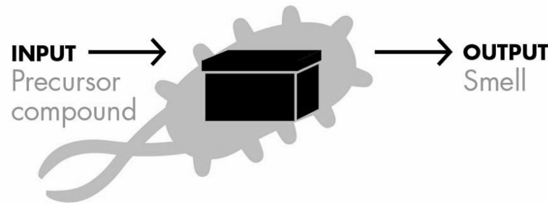
The 2006 iGEM team from MIT and their project,"Eau d'coli"

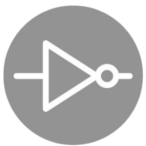
ENGINEERING Eau d'coli

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.

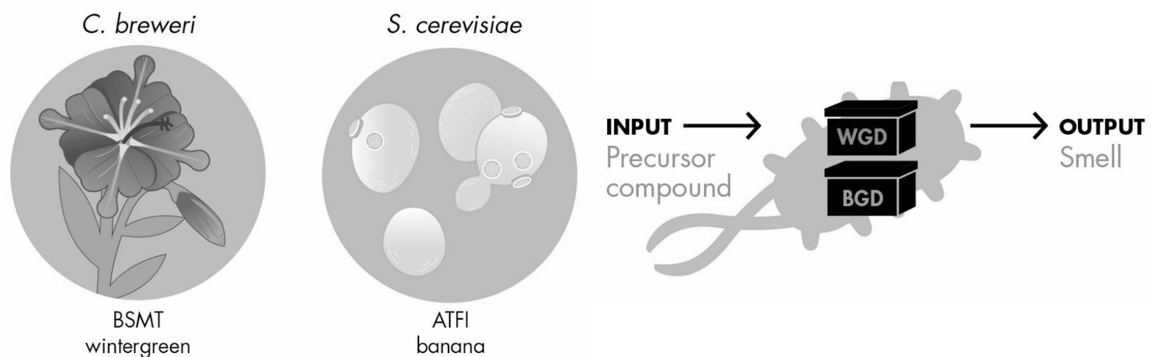


Having identified the challenge they wanted to address, they could then specify a system-level design. They decided that the bacteria could be engineered to convert a precursor compound in the media into a nice smelling output.





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.

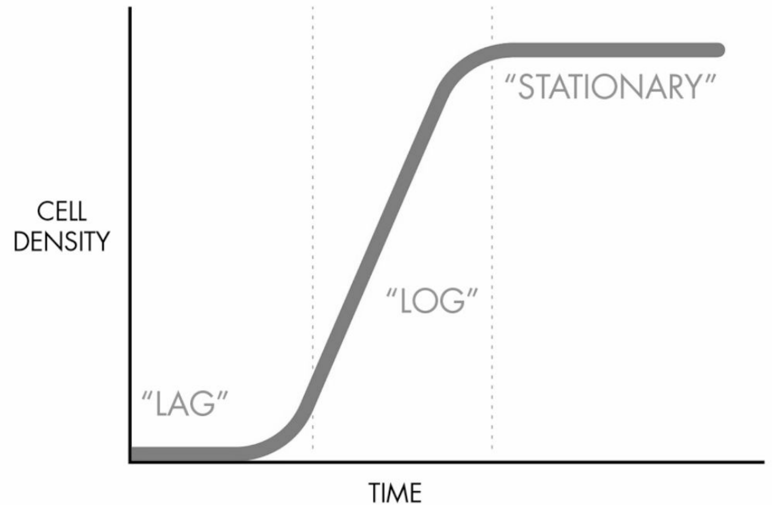


At this point, the team could have started to work on their parts-level design and then built and tested their system.

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.

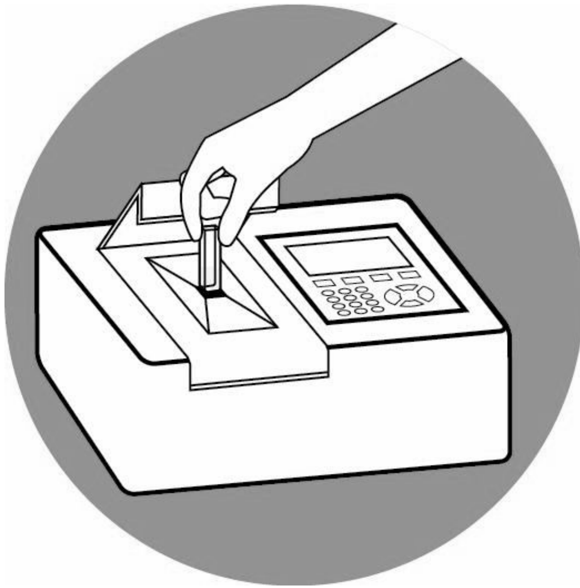
INTRO TO BACTERIAL GROWTH CURVES

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.

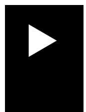


During the lag phase, cells are being primed for growth, and when they are ready to divide, they enter "log phase" (also called "exponential phase"). During this phase of growth, the number of cells increases quickly. At 37°C, bacterial cells can double every 30 minutes or so. The log phase of the growth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

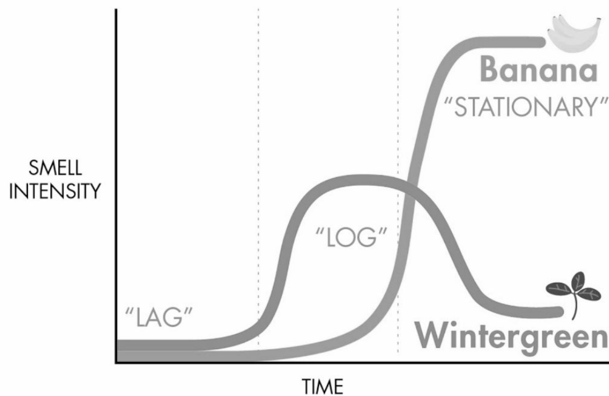


FYI: Researchers often measure cell density using a spectrophotometer. This instrument detects the amount of light that can pass through a sample. The difference between the amount of light that enters a sample and the amount that exits is called the optical density. The optical density increases as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



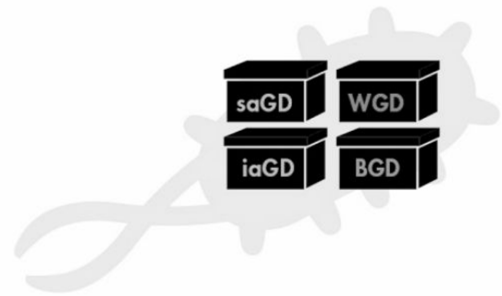
Your experiment will focus on growth control of the banana scent. In particular, you will ask how to best express the banana scent during the log phase of growth.

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

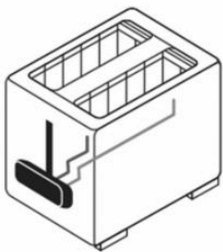
The third aspect of the system that the MIT iGEM team re-designed was the bacterial chassis itself. This redesign came about because in early tests of their system, they noticed that the natural stinkiness of the strain was overwhelming the banana and wintergreen smells. They researched the genetic origins 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.

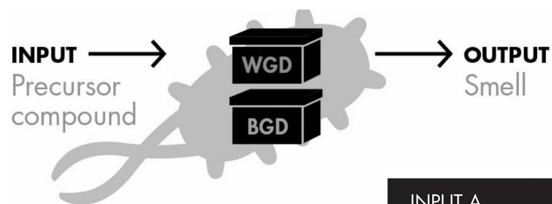
In electrical engineering, the inputs might be electrical signals from wires and the output might be a third electrical signal that does some function, such as turning on your toaster. In synthetic biology, logic gates are built out of DNA using control elements and products from natural genetic circuits. 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

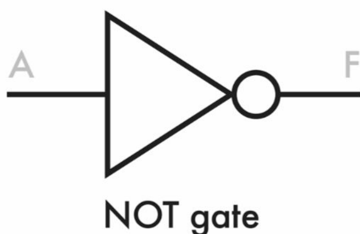


INPUT A	OUTPUT F
0	1
1	0

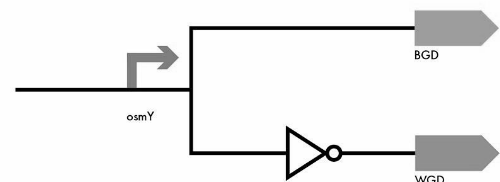
Logic gates in the context of Eau d'coli

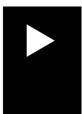
The 2006 MIT iGEM team wanted to express the wintergreen scent in the log phase of growth. To accomplish this design, they paired the P_{osmY} stationary phase promoter with a genetic NOT gate that could invert the natural pattern of gene expression from P_{osmY}. The truth table (below) shows the on/off 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



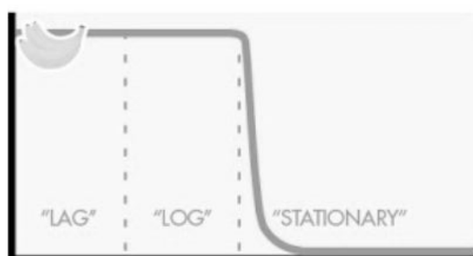


In this lab, you will compare two banana-scent generating devices that are both expected to produce scent in the log phase of growth.

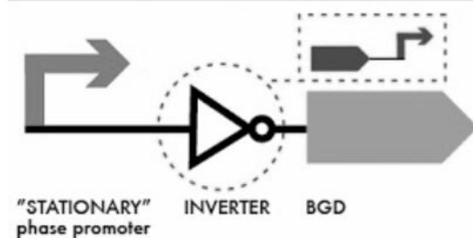
BIOBUILDER'S EAU THAT SMELL ACTIVITY

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.

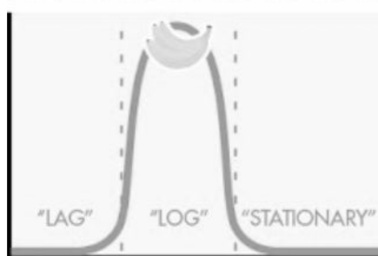
Design 1



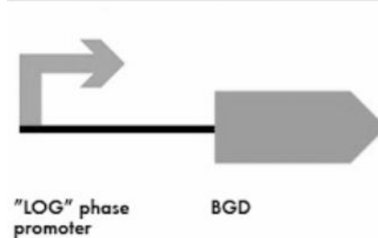
Expresses the banana scent using a stationary phase promoter and an inverting NOT gate



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.

Synthetic biology involves construction of novel living machines in order to solve problems and improve people's lives.

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?

Bacteria that have recently been moved from stationary phase to fresh media enter the lag phase of growth. It takes the bacteria some time to sense the change in nutrients that are available and to make the proteins needed for rapid division. During this time there is no change in cell number. However once the proteins and raw materials for cell division are replenished, the cells can divide quickly, giving rise to 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)
1	0
0	1

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

By using a set of "smell standards," we can compare the intensities of smells we measure to the smells that are measured by other groups. Without the standards, we'd be left to describe them as "strong" or "really strong" but not know how those descriptions compare to the measurements of others.

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

The indole-producing gene was deleted from the genome of the cells. The deletion of the gene to produce indole is expected to make the cells smell less terrible and allow the banana smell to be more easily detected.

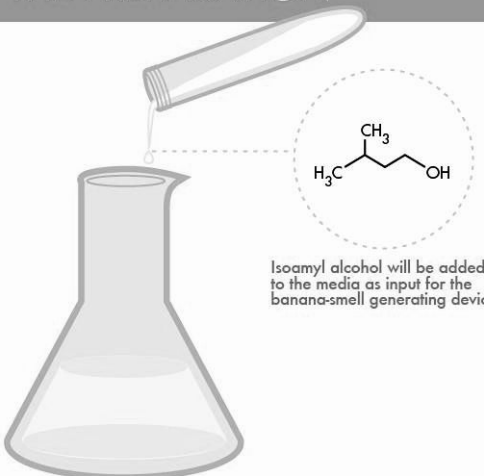
Isoamyl alcohol is added to the media used in this experiment. Why?

Isoamyl alcohol is the substrate for the banana scent generator that is produced by the bacterial cells. When the enzyme that's made by the banana scent generator reacts with isoamyl alcohol, it produces isoamyl acetate, which smells like bananas.

The negative control we will use is ____ strain 1-4 ________, which has _______no smell generating plasmid ___.

EAU THAT SMELL

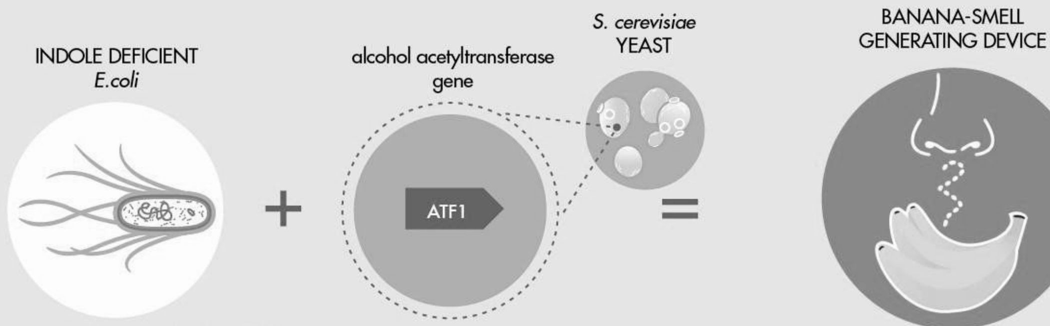
THE PREPARATION



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

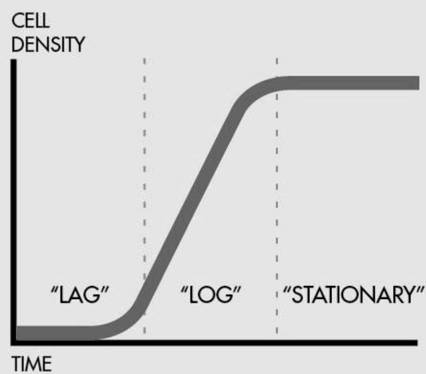
THE SYSTEM

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



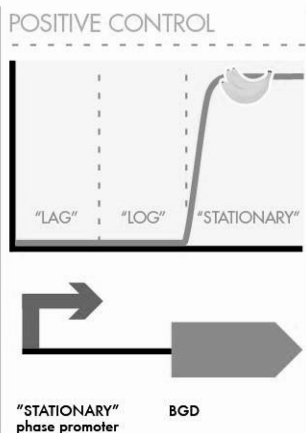
Indole is naturally produced by *E.coli*. The Eau d'coli strain was modified to eliminate indole production and its stinky smell.

GROWTH PHASES

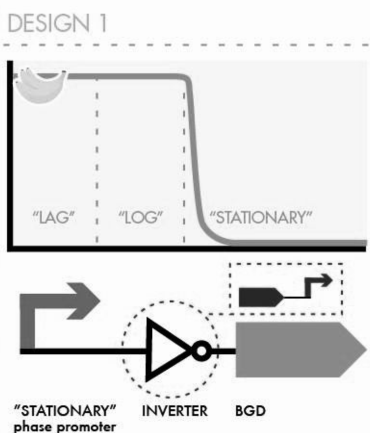


UNDER THE HOOD

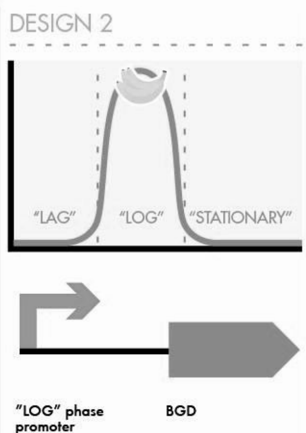
POSITIVE CONTROL



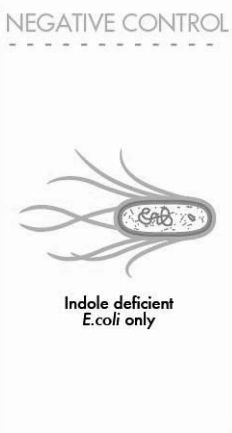
DESIGN 1



DESIGN 2



NEGATIVE CONTROL



BioBuilder Educational Foundation

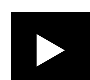
CHECKLIST FOR KIT CONTENTS

- ☐ 50 ml Conical Tubes (1 package, 50/pkg)
- ☐ 15 ml Conical Tubes (4 tubes)
- ☐ Sterile inoculating loops (1 package, 30/pkg)
- ☐ Disposable Transfer Pipets (1 package, 44/pkg)
- ☐ Disposable Cuvettes (1 tray, 100/tray)
- ☐ Luria Broth (5 vials and 3 bottles)
- ☐ 10 mg/ml Ampicillin (1 vial, 4 ml/vial)
- ☐ Isoamyl Alcohol (1 vial, 1 ml/vial)
- ☐ Banana Extract (4 vials, 2 ml/vial)
- ☐ Bacterial Strains 1-1 through 1-4

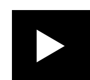
Unpacking your kit

- Store the Luria Broth, the isoamyl alcohol and the banana extract (= amyl acetate, or store bought banana extract) on the shelf until the day of the experiment
- Store the Ampicillin in the fridge (4°C)
- The bacterial stabs can be kept at room temperature or in fridge

Up to two weeks in advance of lab

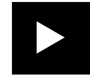


Prepare the media for growth by pouring 15 ml of LB from the vials into a 50 ml conical tube, using the markings on the side of the tube to measure the volume. Add 150 ul Ampicillin solution to the tube and invert to mix. Label four of the 15 ml conical tubes as 1-1, 1-2, 1-3 or 1-4 then aliquot ~3 ml of the LB + Ampicillin media to each tube, using the markings on the side of the tube to measure the volume.
Using a sterile loop, touch one of the four tubes of bacteria that will arrive growing in the stab or slant vial, picking up a small but noticeable amount of cells and swirling the loop into the media for the appropriately labeled tube. Incubate at 37° overnight on a roller wheel or nutator. If you do not have a roller wheel and incubator, you can increase the volumes of each culture to 10 ml and grow them in small Erlenmeyer flasks with stir bars, stirring them slowly on a stir plate.



Prepare the banana scent standards by mixing increasing amounts of banana extract with 25 ml of tap water in 50 ml conical tubes. Standard "0" should be only water. Standard "1" should have 25 ul of extract, "2" should have 50 ul, "3" should have 100 ul and so on. Double the volume of extract for each standard until the extract is used up. Students can prepare the standards as part of the lab activity. The kit contains enough material for 4 sets of standards.

Day 1 of Lab



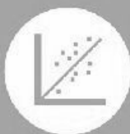
Prepare the growth media by mixing 600 ml LB, 3 ml of Ampicillin, and 500 ul of isoamyl alcohol -- most likely done by adding 1 ml Ampicillin and 170 ul isoamyl alcohol to each of the three bottles of LB. Refrigerate 1 ml of this mixture for each student group. This aliquot will serve as the blank for the spectrophotometer.
Aliquot 75 of the LB+Amp+isoamyl solution to 4 Erlenmeyer flasks labelled "1-1" "1-2" "1-3" or "1-4." Add 1.5 - 2 ml of bacteria of the appropriate bacteria to the flasks and swirl. The kit provides enough material to make two of each sample for data collection.

The lab will need

- 4-8 Erlenmeyer flasks, ideally sterile
- Spectrophotometer (optional)
- Biohazardous waste disposal
- 37° incubator, though strains can be grown on stir plates at room temperature for one day longer
- Micropipets and tips
- Sharpies
- Latex gloves
- Coffee pods, for refreshing between smell tests

ATCG...

QUICK GUIDE: EAU THAT SMELL



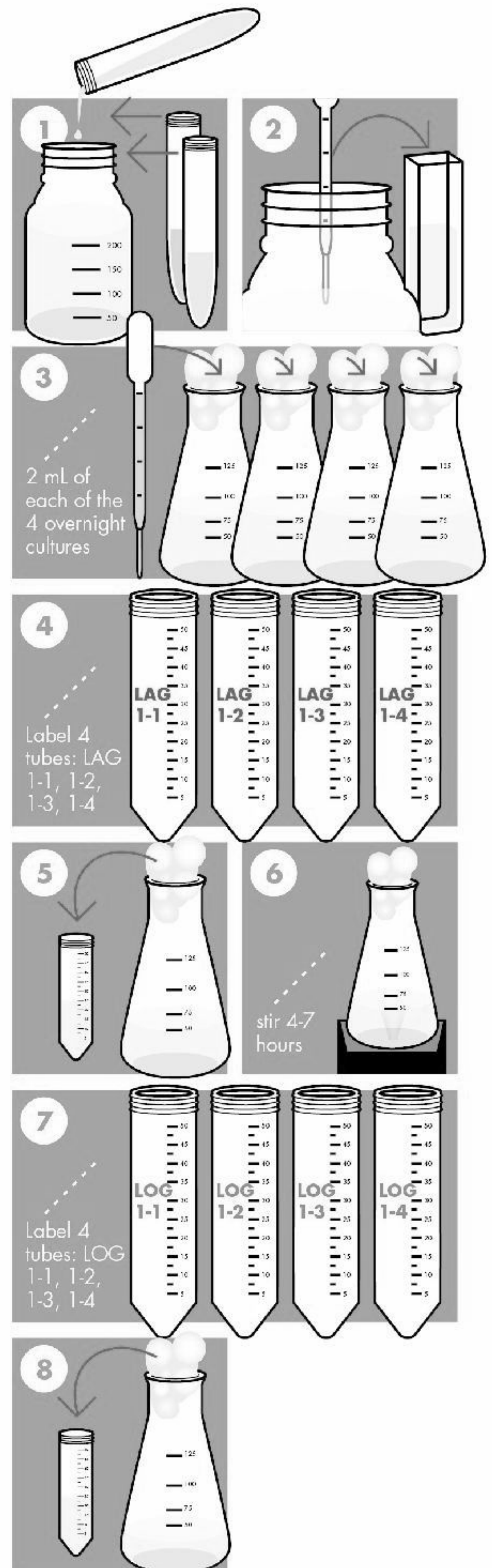
IN ADVANCE

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

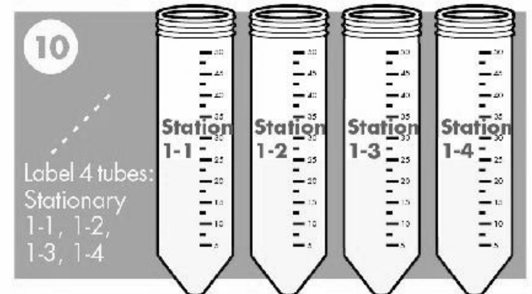
DAYS OF LAB

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.

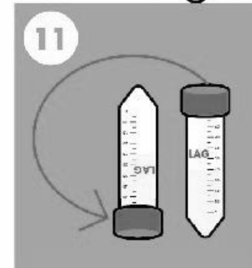


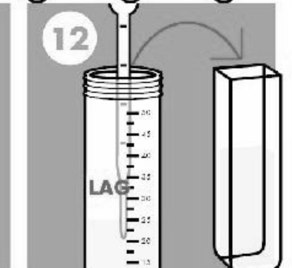
9. Grow remaining volumes of each culture in Erlenmeyer flasks with stirring at room temperature or 37°C overnight. Be sure to record how long the cells grow.



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.

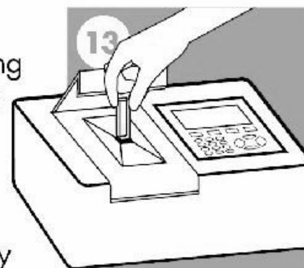
Day 2:





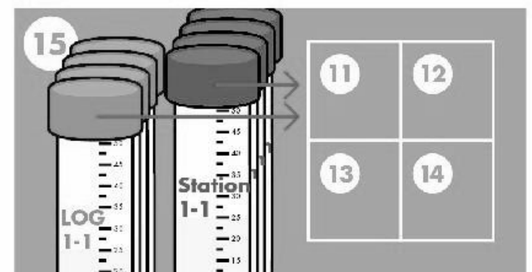
11. Invert the "LAG" phase conical tubes several times to completely mix the cells with the media.

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

UNDERSTANDING THE RESULTS

Students will notice the subjective nature of smell as they try to determine the intensity value for each sample. The qualitative nature of the banana smell assay, as opposed to the quantitative measurement of cell density, will lead to interesting discussions about confidence in data.
Using the protocol presented here, we generally find that

- Strain 1-1 expresses the banana scent most strongly during stationary phase but also during log phase.
- Strain 1-2 is active throughout the growth curve and generates a stronger banana smell than Strain 1-3.
- Strain 1-3 is most “log phase” specific.
- Strain 1-4 still has a noticeable stink, even with the indole deficiency.

The cell cultures smell like bananas because a gene has been added to the strains. The gene comes from yeast and it encodes the enzyme ATF1. When we grow the bacteria carrying the gene in media that contains the precursor molecule (isoamyl alcohol), the enzyme converts it to isoamyl acetate which smells like bananas!

The “black boxing” of functions such as the inverting NOT logic gate is a way that engineers can manage complexity of the system and form the basis of a cellular programming language. If you have explained the use of repressor proteins to control gene expression, then the black box can be opened and the details of how the inverter work might be interesting and useful.

Teaching Tips

- It will take about 4 class periods in a typical biology or biotechnology class for the students to do all the bacterial culturing and data collection. If instead the teacher prepares in advance the samples as outlined in Day 1 of the lab, then the students can conduct the smell tests and population measurements in 1 or 2 class periods.
- At room temperature, it will take around 4-7 hours for the samples to enter log phase. After ~24 hours, they will reach stationary phase.
- 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.
- Students may note that the banana smell dissipated a bit while tube is open. They can close the tube for a minute and then re-shake to bring the smell back.
- If a spectrophotometer is not available, then MacFarland turbidity standards can prepared using $BaCl_2$ and H_2SO_4 as described in Chapter 6 of the BioBuilder book. These standards can be prepared well in advance of lab and in any volume. To use these standards, aliquot them to a glass tube with a cap and visually compare then to the turbidity of the bacterial samples, also in glass tubes. It can be helpful to compare how well the samples obscure a black line on a card behind them.
- Clean up: containers can be provided at each workstation for biological waste such as pipet tips and tubes. Follow hazardous waste procedures recommended by your school or municipality. Generally it is safe to soak the biohazardous materials in 10% bleach for 20 minutes and then dispose as regular trash or liquid waste.
- You can find a lab report assignment and grading rubric at the biobuilder.org website.

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?

We expect little or no banana smell since the cells are in lag phase.

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

We expect to the banana scent to be masked by *E. coli's* natural stink

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?

We expect stronger banana smell to be detected in log phase, but that the smell will still diminish or go away during stationary phase.

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

We expect the banana smell to be produced during stationary phase.

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

Answers will vary but may include problems with accuracy of smell measurement, variation in cell growth rates between the strains, background smell of the media, or dissipation of the smell due to the number of times the flasks were opened.

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?

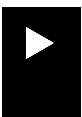
Answers will vary but might be along the lines of bacterial deodorant, or improved biomanufacturing of foods or medicines, detection devices that start to smell when enough cells have reached a stage of growth or flavored yogurts.

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

Synthetic biologists construct novel cellular machines that function to solve problems and improve lives. Examples of synthetic biology include bacteria that can sense heavy metals in the environment and improve yields in biomanufacturing of medicines and other compounds.

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?

Answers will vary



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

TEACHER MANUAL

BioBuilder

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