CHAPTER TWELVE

BIOBUILDING: USING BANANA-SCENTED BACTERIA TO TEACH SYNTHETIC BIOLOGY

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Abstract
Student interest in synthetic biology is detectable and growing. Each year teenagers from around the world participate in iGEM, a summer long synthetic biology competition. As part of their iGEM experience, undergraduates design and construct novel living systems using standardized biological parts. One engineering feat was accomplished by the 2006 MIT iGEM team, who modified the normally putrid smell of bacteria so that the cells generated pleasant scents, such as wintergreen and banana. We have taken advantage of their project as well as other iGEM successes to develop a teaching curriculum for high schools and colleges. The curriculum includes four hands-on activities and two classroom assignments. We envision these activities either complementing existing instruction, for example in an advanced placement biology lab, or replacing some outdated, cookbook lab classes that are often used as gateways to undergraduate research opportunities. The activities we have developed also introduce engineering and technology concepts that are often overlooked in the
1. Introduction

Having observed the profoundly successful learning experiences that high school and college students have had through the international Genetically Machines Competition (iGEM; Mitchell et al., 2010), we have turned to synthetic biology to reinvigorate and reengage students in classes at the high school and college level. In particular, we have recast or extended student iGEM projects so as to align them with existing teaching frameworks, or to meet educational needs in biological engineering laboratory classes (Kuldell, 2007). The content we offer includes a lab activity inspired by the iGEM project from the 2006 MIT team, namely Eau d’coli. These bacteria have been genetically engineered to smell like mint during the exponential (“log”) phase of bacterial growth and like bananas during the stationary phase (MIT 2006 iGEM team: http://openwetware.org/wiki/IGEM:MIT/2006).

In transforming the Eau d’coli project and other iGEM successes into hands-on activities at BioBuilder.org that could be widely adopted, we considered generally accepted hallmarks of good curricula as well as more pedestrian logistical questions. The activities had to explicitly address engineering- and technology-teaching standards (National Academies Standards: http://www.nap.edu/catalog.php?record_id=4962, National Education Standards: http://www7.nationalacademies.org/bose/Standards_Framework_Homepage.html) using investigative frameworks (National Research Council, 2000, Wiggins and McTighe, 1998), and had to be provided through an open digital platform (e.g., Khan Academy: http://www.khanacademy.org/). Additionally, since we are both teachers, we are sensitive to implementation issues for these activities. Instructions had to provide real, “rubber meets the road” guidance. For example, the resources provided to teachers had to include guidelines for fitting the activities into short or long lab periods, rubrics for grading the work, and digital forums for posing practical questions and sharing clever solutions.

One illustrative example of the “BioBuilder” curriculum grew from a project called “eChromi” which was the 2009 iGEM Grand Prize winner carried out by students at the University of Cambridge (University of Cambridge 2009 iGEM team: http://www.echromi.com/). As part of their work, students generated a bacterial palette, genetically reprogrammed
strains to appear shades of red, green, and purple. Unexpectedly, the performance of these color-generating genetic programs depended on the genetic background of the bacterial chassis. Though the iGEM team did not see this variable behavior as much more than odd, we took advantage of the observation as the point of departure for an investigative teaching lab that could replace the standard transformation lab commonly taught in high school advanced placement (AP) biology classes (Pearson AP Biology Labs: http://www.phschool.com/science/biology_place/labbench/lab6/concepts1.html). In our “BioBuilder” lab 4, students transform a B-type and a K-type bacterial host with the purple and the green color-generating plasmids. As with the traditional lab series, students are able to observe that DNA can be transferred to confer phenotypes and that selectable markers can be used to identify cells with plasmids. In the context of the BioBuilder lab, however, there is motivation for performing this DNA transformation. Students can directly examine the presumed equivalence of host strains and can ask why DNA might be expressed differently. They can identify future experiments and applications to exploit their observations. In other words, they become practitioners of science and engineering, as opposed to technicians.

In this chapter, we detail a teaching laboratory that extends the 2006 MIT iGEM team’s “Eau d’coli” project (Fig. 12.1). Students grow four strains of genetically engineered cells. One of the strains serves as the zero-smell standard. Another strain is designed to generate a banana-flavored smell when the cells are growing in stationary phase. Students are asked to investigate the performance of the remaining two strains. These strains use distinct genetic programs to generate the banana smell during the exponential (“log”) phase of growth. Observing that equivalent designs can perform differently in real life is an important lesson for budding engineers. The lab activity further offers a chance for students to learn important microbiological techniques and behaviors, in a charismatic and interesting context.

2. Eau d’coli

The banana-flavored smell from the genetically engineered Eau d’coli cells arises from the conversion of isoamyl alcohol to isoamyl acetate. Isoamyl acetate has a banana smell (Fig. 12.2).

Isoamyl alcohol (also called 3-methyl-1-butanol, isopentyl alcohol, or isobutylcarbinol) can be added to the bacterial growth media, where it is efficiently imported into the cells. There, it is converted to isoamyl acetate by the product of ATFI, a gene from Saccharomyces cerevisiae that was cloned between a bacterial ribosome binding site and a transcriptional terminator to make a three part “banana-odor generator.”

To regulate the production of the banana smell, promoters that are active during distinct stages of the bacterial growth cycle were used. These promoters
differ in their affinity for sigma-factors that can associate with the RNA polymerase core. For cells designed to smell like bananas during stationary phase, a sigma-38 regulated promoter, pOsmY (Hengge-Aronis et al., 1993),
was cloned upstream of the device. For cells designed to smell like bananas during log phase growth, either a sigma-70 regulated promoter, pTetR (Lutz and Bujard, 1997), was cloned upstream of banana-odor generator, or a four part genetic inverter was added to the sigma-38 based construct (BBa_J45990: http://partsregistry.org/Part:BBa_J45990). To best detect the banana smell, the cellular chassis bore a mutation in the \textit{tnaA} gene, inhibiting indole production and effectively eliminating the putrid smell that characterizes most \textit{Escherichia coli} (YYC912 strain reference: http://cgsc.biology.yale.edu/Strain.php?ID=64826). The genetic devices associated with this synthetic system were all entered into the Registry of Standard Biological Parts (Registry Homepage: http://partsregistry.org/Main_Page) and are tabulated here (Table 12.1).

A combination of techniques was employed by the MIT iGEM team to characterize the growth and behavior of their system. To assess the effect of the synthetic devices on cellular growth rate, the team measured changes in turbidity of the cultures over time. Growth curves were then correlated with expression from the devices by fusing the promoters to GFP instead of the banana-odor generator. In this way, the fluorescence of the cells over time could be measured and used as a predictor of timed scent production in that system (Fig. 12.3). Finally, gas chromatography and a “sniff” test were used on the complete systems to look for isoamyl acetate generated by the cells as they grew (Fig. 12.4).

### Table 12.1 Registry of standard biological parts for “Eau That Smell” experiment

<table>
<thead>
<tr>
<th>BBa_J45999</th>
<th>BBa_J45199</th>
<th>BBa_J45250</th>
<th>BBa_J45200</th>
<th>BBa_J45990</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole-free chassis</td>
<td>Banana-odor generator</td>
<td>Sigma-38 controlling banana smell</td>
<td>Sigma-70 controlling banana smell</td>
<td>Sigma-38 promoter plus 4 part genetic inverter generating banana smell</td>
</tr>
</tbody>
</table>

3. **“Eau That Smell” Teaching Lab Using the MIT iGEM Team’s Eau d’coli Cells**

The cells that were engineered by the MIT 2006 iGEM team smell distinctly of bananas, almost like a banana smoothie, by the time they reach fully saturated growth. However, the completed system leaves only a few teachable questions and experimental manipulations open to teachers. We extended the existing behavior of the Eau d’coli project so students...
could actively engage with it. Drawing from the National Science Standards (National Academy Standards website http://www.nap.edu/openbook.php?record_id=4962), we first identified a design opportunity, namely

![Diagram of Eau d'coli growth and cell population control]

**Figure 12.3** Characterization of Eau d'coli growth and cell population control. Portions of the Eau d'coli system were characterized by examining the growth rate (panel A) and timing of gene expression (panel B). In both cases, the relevant promoters were fused to GFP and changes in turbidity and fluorescence were measured over time. The data suggest Eau d'coli's output can be controlled by natural changes in cell population. Source: http://openwetware.org/wiki/IGEM:MIT/2006/osmY_Results.
Figure 12.4  Isoamyl acetate output from Eau d’coli. Isoamyl acetate appears as a distinct and detectable peak on a gas chromatography when Eau d’coli cells are grown in the presence of isoamyl acetate (panel A), but only when cells bear the banana-odor generator, BBa_J45200 (panel B). Purified isoamyl acetate was used to confirm the retention rate of the compound (panel C). The pronounced peak for indole reflects the fact that the strains expressing these devices were wild-type for tnaA. Source: http://partsregistry.org/Part:BBa_J45200:Experience.
alternative genetic circuits that both met the stated goal of making log-phase banana smell. Next, we allowed the students to experimentally evaluate and then choose the better performing solution. The teacher’s materials that we provide include some guidance for helpful assessment of the student’s work, rubrics to guide the student’s communication of experimental limitations and questions to motivate future directions.

In our hands, the banana-smell is less intense when directed by a sigma-70 promoter than when controlled by a sigma-38 promoter and a four part genetic inverter. However, the former design does a better job of expressing the device during log-phase only. This experimental result presents an interesting “choice” for the students to weigh (Fig. 12.5).

3.1. Growing starter cultures for the students

To begin this experiment, a high school or college teacher would request a kit from us. We could also send kits to summer iGEM teams who need preliminary training. These kits include four bacterial strains, three that should smell like bananas when grown in the presence of isoamyl alcohol and one negative control strain. The kits will also include, as needed, growth media, banana-scent standards, and turbidity standards. The strains will be sent 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, teachers and their students will have to further culture the bacteria by streaking out the stabs onto LB (Luria Broth) + ampicillin plates, as instructed below, and then further growing liquid starter cultures the following day. Note that since the host strain is chloramphenicol resistant, the selection can equally well be carried out in LB + ampicillin + chloramphenicol. If used, chloramphenicol stock solutions are made as 34 mg/ml ethanol and are used at a 1:1000 dilution in plates and in liquid culture.

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 LB agar (1% tryptone, 0.5% yeast extract, 1% NaCl, 2% agar) + ampicillin (200 μg/ml final concentration).
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.

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 LB (recipe is identical to that for petri dishes but the agar is omitted) and 5 μl of ampicillin (stock solution = 100 mg/ml sterile water).
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.

3.2. Bacterial growth and scent curves

Once the liquid starter cultures for each strain have been grown, students can inoculate larger volumes of cells to be examined for growth and banana-smell over time. Cells are grown in flasks in sufficiently large volumes so the

**Figure 12.5** Behavior of “Eau that Smell” experimental and control strains. Growth and banana-smell generated by each experimental strain was assessed. Growth curves, shown with the blue lines, are measured as changes in turbidity over time. Banana smell, shown with red lines, was calibrated to the smell standards and is plotted for each time point on the growth curve. Growth time, in minutes, is shown on the x-axis. Unexpectedly, the log-phase promoter (strain 1-3) generates a less pronounced banana-flavored smell but is more tightly controlled to express only during the log-phase of growth. Source: data collected by J. Dixon.
smell associated with each strain is pronounced, and the volume can support
the removal of multiple aliquots for turbidity measurements. We have
described the lab with 50 ml of each culture growing in 100 ml Erlenmeyer
flasks on stir plates at room temperature. Instructors will have to scale the
materials according to the number of students and the availability of
equipment.

Depending on the time available for teaching, the experiment can be run
in different ways. A longer time period such as a 3 or 4 h college lab would
allow the students to follow much of the growth curve, ideally from the end
of lag phase through exponential growth and into stationary phase, all in one
day. In cases when students are in the lab for an hour or less, the experiment
can be carried out over several days, with an instructor placing the growing
cells into a refrigerator between days to slow down their growth. In this
case, the instructor overseeing the lab should warm the cells back to room
temperature in advance of the students’ time in lab and should note the time
the cultures spend at room temperature so students can accurately determine
changes in turbidity over time. It is important that the growing cultures
remain covered with foil or plugs while they cool and warm so the smell
associated with each strain can be determined. Additionally, students should
be discouraged from smelling the cultures too often since the smell will
dissipate and, if smelled too often, it can give some students a headache.

Data collected should be plotted with time as the common x-axis, and
with both turbidity and smell sharing the y-axis. Turbidity can be measured
with optical density at 600 nm using a spectrophotometer or by comparison
to the MacFarland Turbidity Scale, described below. The intensity of the
banana smell can be described with the following standards, prepared as
indicated.

### 3.2.1. Banana-scent standard

An arbitrary scale has been established to associate numerical values to the
intensity of the banana smell. This scale is based on the smell of a series of
banana extract dilutions. The banana extract is an oil and will not dissolve in
water. However, the concentrations are low and as long as the standard is
given a shake before smelling, a suspension is sufficient. We have used
banana extract made by “Frontier Natural Flavors” but suspect other brands
would work equally well. The dilutions are prepared as indicated in
Table 12.2, storing the solutions in plastic 50 ml conical tubes at room
temperature until needed.

### 3.2.2. Measuring banana-smell and cell growth

1. Prepare a stock growth solution with 300 ml LB (1% tryptone, 0.5%
yeast extract, 1% NaCl), 300 µl ampicillin (stock solution = 100 mg/
ml sterile water), 250 µl isoamyl alcohol. Note that isoamyl alcohol
stock should be used in a chemical fume hood since the smell can irritate the eyes, nose, and lungs. Once diluted, the isoamyl alcohol gives off a sweet and not pungent smell, though students and teachers who spend too much time smelling even the dilution may develop a headache or a stomach ache.

2. Mix this stock growth solution, by swirling the bottle or vortexing gently.

3. Set aside 2 ml of this mixture for each student group into a cuvette. This aliquot will serve as the blank for the spectrophotometer.

4. Move 50 ml of the broth solution to a 100 ml sterile Erlenmeyer flask and add 2 ml of bacteria from one of the overnight cultures, for example, strain 1-1.

5. Repeat the addition of 2 ml of bacteria to 50 ml of broth in an Erlenmeyer flask for each of the overnight cultures.

6. Cover the flasks with foil or a cotton plug, and swirl them gently.

7. Remove 2 ml from each sample to read the starting density of each. If you are testing all four samples you should now have five cuvettes, four with bacterial dilutions, and one blank.

8. Prepare the spectrophotometer by setting it to OD$_{600}$.

9. Note the time and take an “initial” density reading for the bacterial samples. This time should be noted as “$T_0$.” Discard all samples except the blank.

10. Add a stir bar to each culture flask and place the flasks onto stir plates. Stir slowly. Cover the flasks with foil.

11. After 20 min, remove 1–2 ml from each sample and place in a cuvette.

12. Read the uninnoculated sample (blank) and set the % absorbance of this sample to zero.

13. Read each sample and record the % absorbance.

14. Sniff the Erlenmeyer flasks for any evidence of a banana smell, comparing the smell with the banana extract standards. Be sure to shake the standards and swirl the cultures before sniffing. Record your data.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (%)</th>
<th>Extract in H$_2$O (final volume 25 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
<td>25 µl</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>62.5 µl</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>125 µl</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>250 µl</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>625 µl</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>1.25 ml</td>
</tr>
</tbody>
</table>
15. At 20-min intervals repeat steps 11–14.
16. Between time points, you can calculate the bacterial population using the approximation of $1 \text{OD}_{600} \text{ unit} = 1 \times 10^9$ bacteria.

### 3.2.3. Notes to teachers

A teacher presenting this lab has an opportunity to teach microbiology techniques, population growth dynamics, molecular genetics, and basic synthetic biology concepts in a meaningful, real world way. Given that engineering practice in general and synthetic biology in particular are not commonly taught, we provide lesson materials to introduce the lab activities found on the “BioBuilding” wiki. For example, the “Eau that Smell” lab can be framed with a one page “bioprimer” in which two characters discuss the relevant merits of the designs that will be compared. Terms that are unfamiliar to the teacher or students can be learned through the glossary link on the BioBuilding site, or through short animations that further extend the narrative between the characters. The animations and activities are collected on BioBuilder.org (Fig. 12.6).

### 3.3. Assessment

To show their understanding of the system, students can be asked to discuss:

- How well were they able to measure the population growth?
- How well were they able to smell bananas?
- Did each strain and their associated devices produce the same results?
- Did the genetic systems affect the growth curve of the bacteria?

Students performing this lab also have a chance to do meaningful error analysis and examine the difference between quantitative and qualitative results. When the students analyze their data, they might consider:

- How does each part of the experiment add to the conclusion?
- What errors or experimental parameters might lead to data variability?
- How confidently can they state their results?
- Are they equally confident in both the growth data and the smell data?
- Is using smell to measure the isoamyl acetate valid?
- What methods did they use to try to increase their confidence in the results?
- How might they change this system to better quantify the banana smell?
- Is there another kind input or output they would suggest including?
- If they could construct a different genetic system, what might they construct?

This final question could easily be used as the bridge to other “BioBuilding” activities or to a summer iGEM project, depending on the teaching context.
4. Teaching Labs Modified for Resource-Stretched Settings

For teaching settings that do not have a reliable spectrophotometer or a sufficient number of instruments to use in a class setting, we offer a modification to the standard protocol. To assess turbidity, the bacterial solutions can be compared to the MacFarland Turbidity standards. The turbidity standards are prepared as suspensions of BaCl₂ in H₂SO₄ and are visually similar to suspensions of growing *E. coli*.

**Figure 12.6** BioPrimer #1. To introduce students to the scientific and engineering underpinnings of the “Eau that Smell” lab activity, a one page “BioPrimer” can be circulated and discussed. When accessed from the internet, the BioPrimer has active links to terms that are unfamiliar, allowing the concepts to be learned through animations or additional readings. Multiple BioPrimers could serve as the framework for a semester long biological engineering class or a biotechnology course. Source: Animated Storyboards and BioBuilder.org.
Other activities available through BioBuilder.org offer similar instructions for modifications when equipment is unreliable or limiting. For example, the “iTune Device” lab generates a yellow colored product that the students can compare to a Benjamin Moore Paint Chip to approximate intensity readings.

4.1. Preparation of MacFarland Turbidity standards

Dilutions of 1% BaCl₂ in 1% H₂SO₄ are prepared according to Table 12.3. The standards are stable at room temperature for at least a month, more likely longer, and can be aliquoted by the students themselves or in advance by the instructors.

4.2. Comparison of growing cultures to Turbidity Standards

1. Turbidity standards should be aliquoted into small clear test tubes. The tubes should contain enough of each standard to fill the tube to a height of about 1 in. (2.5 cm). Tubes must be properly labeled with its turbidity standard number.

2. The aliquots of the turbidity standards should be placed in a test tube rack that allows the liquid to be viewed from the side.

3. On a blank index card or piece of folded printer paper, two thick black lines should be drawn with a marker. These lines should be placed on the card or paper to fall within the height of the standards.

4. Place the card with the lines into the test tube rack behind the standards (see Fig. 12.7).

5. To compare the bacterial cultures to the turbidity standards, an aliquot of the growing cultures should be moved into a test tube of the same size as the standards. A volume approximately equal to that of the standards should be used and the identity of the samples should be included on the tube’s label.

<table>
<thead>
<tr>
<th>Turbidity scale</th>
<th>OD₆₀₀</th>
<th>1% BaCl₂/1% H₂SO₄ (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.0/10</td>
</tr>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.05/9.95</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.1/9.9</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>0.2/9.8</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>0.3/9.7</td>
</tr>
<tr>
<td>5</td>
<td>0.65</td>
<td>0.4/9.6</td>
</tr>
<tr>
<td>6</td>
<td>0.85</td>
<td>0.5/9.5</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>0.6/9.4</td>
</tr>
</tbody>
</table>
6. The sample tubes can be placed next to the turbidity standards to be compared side-by-side. The standard that best represents the turbidity of the samples will be the one that obscures to the same extent the black lines drawn on the card.

7. Table 12.3 can be used to determine the OD$_{600}$ value using each turbidity standard.

8. If the number of cells is to be calculated, then 1 OD$_{600}$ unit can be approximated as equal to $1 \times 10^9$ cells/ml.

5. Summary

Most students at the high school and college level today could hardly imagine a world that did not include digital resources. Indeed, when students in Michael Wesch’s “Digital Enthography” class at Kansas State University tackled the question: “what is it like being a student today,” they documented their findings as a video (“Vision of Students Today”: http://www.youtube.com/watch?v=dGCJ46vyR9o) and posted it to YouTube. In their video, the students present the results of their class surveys by holding $8\frac{1}{2} \times 11$ in. signs or by showing their computer screens to the camera. Some of their messages, like “my average class size is 115” and “I buy hundred dollar textbooks that I never open” are not new issues for students at the university level. However, some aspects they present do seem different, for example, “I facebook through most of my classes” and “My neighbor paid for class but never comes.”
How is a teacher to respond to the digital distractions that are a hallmark of this era? One (not unappealing) reply is to say something like, “stop goofing off and study.” Indeed, experience says that students who put more into any class are the ones who get more out of it. So when students offer only a portion of their energy and attention in class, they are only short-changing themselves. Nonetheless, most teachers are hopeful that the subject they teach will excite others, and so most teachers look for ways to engage the students.

Perhaps, a more constructive reply to the issue of digital distractions is to meet the students on their technology playing field. If students really do “spend 3.5 h a day online” then perhaps a winning strategy is to teach through technology and online tools. Digital quiz-lets, discussion forums, and internet games can encourage students to interact more often and more thoughtfully with the class content. But when poorly implemented, cyber-oriented teaching can fall short, seeming more like “chocolate-covered broccoli” than any novel and engaging tool for interaction (Gorsky and Blau, 2009). Additionally, the recent failure of Google’s “Wave” learning management system is a sobering reminder that technological advances must not be too complicated for average users, and must integrate well with the way people actually teach (Young, 2010).

Synthetic biology offers a mechanism for training students in an engaging and novel way. iGEM continues to attract summer students worldwide (iGEM: http://2010.igem.org/Main_Page), and the Adventures in Synthetic Biology comic is “given out like candy” (DIY-bio: http://diybio4beginners.blogspot.com/2009/02/adventures-in-synthetic-biology-comic.html). These two educational successes are leveraged at the BioBuilder website (http://www.biobuilder.org/) which expands iGEM projects like the banana-smelling bacteria, into digitally accessible materials for teaching and learning.

ACKNOWLEDGMENTS

We thank the 2006 MIT iGEM team for their hard and thoughtful work to establish the Eau d’coli system. We further thank Ginkgo Bioworks for DNA construction. These teaching materials were developed over two summers with the support of SynBERC, an NSF-funded Engineering Research Center, and the support of MIT’s Department of Biological Engineering.

REFERENCES


