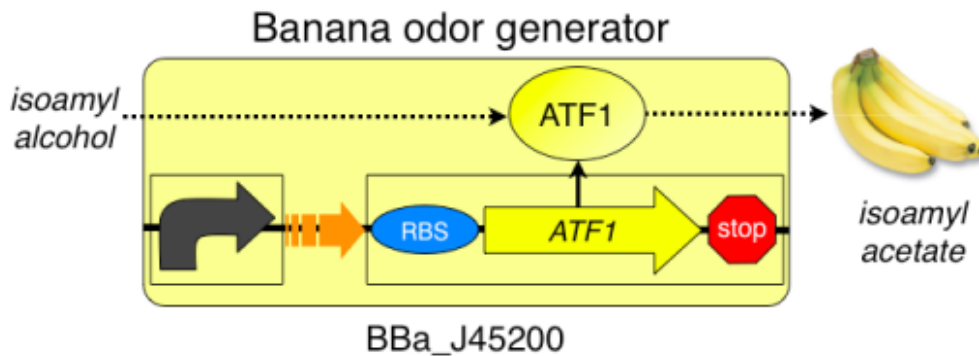


BioBuilding: Synthetic Biology for Teachers: Eau that Smell

Lab 1: Eau that smell --Protocol A



Teacher Considerations

This lab provides a valuable opportunity to teach microbiology techniques, population growth dynamics, molecular genetics and basic synthetic biology concepts in a meaningful, real world way. As can be seen in the discussion questions for the lab report, the analysis of the lab will provide the students with a chance to do meaningful error analysis and examine the difference between quantitative results and qualitative results. This lab offers two different protocols based on the time the teacher wishes to allow. Each of these protocols covers the same concepts but allows for different emphases.

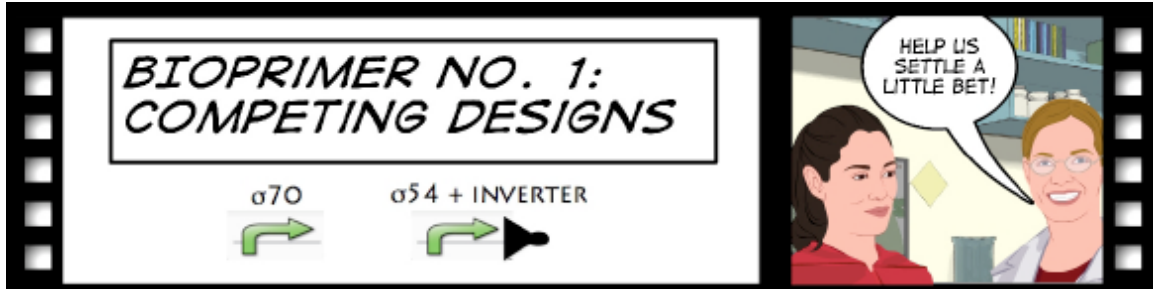
Protocol A--Condensed data collection: This is a shorter procedure for the students. This shorter protocol emphasizes data analysis over data collection. With this protocol the teacher can choose to have the students do the initial bacterial culturing if more microbial techniques are to be emphasized. Essentially, a day prior to any data collection, the large cultures are set up. Part of the starter culture is immediately removed and placed in the refrigerator. This serves as the lag phase sample. After 5-7 hours, a second sample is removed. This serves as the log phase sample. The last third of the culture should be allowed to grow overnight. This serves as the stationary phase sample. The samples can then be provided to the students the following class day, allowing the students to collect data in single lab period.

It will take about 4 class periods in a typical High School AP Biology or Biotechnology class for the students to do all the culturing and data collection. If instead the teacher prepares in advance the samples as outlined in Part 1 of the procedure, the students can conduct the smell tests and population measurements in one class period.

The procedure includes instructions for using a spectrophotometer to measure the population growth. If a spectrophotometer is not available, the population can be easily measured using the McFarland Turbidity methodology, as explained below.

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Workflow Protocol A Classroom Content



- [BioBuilder](#) material that sets up this lesson starts [here](#)
 - **Day 1:** streak strains from stabs onto plates
 - **Day 2:** grow strains from plates as liquid overnights
 - **Day 3:** subculture bacteria in larger volumes, place lag phase samples in refrigerator, incubate log phase samples for 5-7 hours and refrigerate, allow stationary phase samples to incubate overnight
 - **Day 4:** provide students with lag, log and stationary samples for data collection
- When you are done with this lab, ask your students to submit their data [here](#).

Annotated Procedure



TEACHERS: Clean-up instructions. Provide containers at each work stations for student biological waste such as pipet tips, eppendorf tubes, spreaders, inoculating loops, and plates. Be sure to follow hazardous waste procedures as set forth by your school or municipality. Generally, it is safe to soak the material in each container with a 10% bleach solution for 2 hours. Materials can then be discarded into the regular trash. You can find more information about microbiology lab safety [here](#)

Day 1:

TO DO

- Streak out strains from stabs to plates
- Prepare banana extract standards

Streak out strains from stabs to plates

We will be receiving our bacterial strains with the plasmids already inserted. The strains may come in the form of a "stab" or "slant," a test tube with a small amount of bacteria on a slanted media, in which case you will have to streak out the bacteria onto a petri dish to continue the experiment. If the bacteria have arrived on petri dishes, you can proceed to **Day 2**.

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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 petri dishes media side up in a 37°C incubator overnight.

A video of this procedure is [here](#).



TEACHERS: You may wish to conduct this procedure yourself. However, if time allows, the students will enjoy learning these microbiological techniques. If your class will test the whole set, there will be 4 strains to streak out. Strains can also be streaked out on LB+Amp+Cam if you'd like to verify the indole- strain background.

Prepare banana extract standards

The banana extract is provided in the kit. It will be necessary to make up the standards following the table.

Standard	concentration	Extract in H ₂ O (final volume 25 mls)
0	0	0
1	0.1%	25 µl
2	0.25%	62.5 µl
3	0.5%	125 µl
4	1%	250 µl
5	2.5%	625 µl
6	5%	1.25 ml



TEACHERS: 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.

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Day 2:

TO DO:

- Grow liquid overnights of bacterial strains
- Prepare Turbidity standards (if no spectrophotometer is available)

Grow liquid overnights of bacterial strains

1. Using a sterile inoculating loop or toothpick or pipet tip, transfer a bacterial colony from one of the petri dishes to a large sterile culture tube containing 3 ml of Luria Broth and 3 μ l of ampicillin. This volume is more than enough for each strain that each student or team of students must grow.
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.

A video of this procedure is [here](#).



TEACHERS: If you do not have a roller wheel and an incubator, you can increase the volumes of each culture to 10ml and grow them in small erlenmeyer flasks with stir bars stirring them slowly on a stir plate. Cultures are stable and active for a week at least (stored in the fridge) but will take considerably longer to start growing on the day you subculture (~3 hours rather than 1).

If Not Using a Spec: Prepare turbidity standards

As the populations of bacteria increase, the culture media gets increasingly turbid. Using the [McFarland Turbidity Scale](#), it is possible to estimate the changes in turbidity. The results will not be as precise as what you would measure with a spectrophotometer, but the changes over time will be detected and the results can be graphed.

Turbidity Scale	OD 600	1% BaCl ₂ / 1% H ₂ SO ₄ (mL)
0	0	0.0/10
1	0.1	0.05/9.95
2	0.2	0.1/9.9
3	0.4	0.2/9.8
4	0.5	0.3/9.7
5	0.65	0.4/9.6
6	0.85	0.5/9.5
7	1.0	0.6/9.4



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

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Day 3: Creating the stock cultures

TO DO:

- Inoculate large volumes for culturing



TEACHERS: The procedure assumes each lab group will measure all 4 cultures at each of the time points and that large cultures are prepared to be shared by the whole class. The 75 ml cultures should be sufficient for up to 12 lab groups assuming each group takes only one time point. Should you wish to supply more groups, increase the solutions and the amount of bacteria added by a factor equal to the number of lab groups. Should you wish each group to prepare its own stocks, you can scale down the amount for each sample. Ultimately, each group will need only 2 mls of each sample to read on day 4.



TEACHERS: Should you want the students to conduct this procedure, you may want to divide the class into quarters and have each group make up the samples for one treatment.

A video of this procedure is [here](#).

1. Prepare a stock growth solution with
 - 300 ml Luria broth
 - 300 μ l Ampicillin (final concentration of 100 mg/liter)
 - 250 μ l isoamyl alcohol
2. Mix this stock growth solution, by swirling the bottle or vortexing gently.
3. If you will be using a spectrophotometer, set aside 2 ml of this mixture for each student group into a small sterile culture tube. This aliquot will serve as the blank for the spectrophotometer. Store this in the refrigerator.
4. Move 75 ml of the broth solution to 125ml sterile erlenmeyer flask and add 2ml of bacteria from one of the overnight cultures, e.g. strain 1-1.
5. Repeat the addition of 2ml of bacteria to 75 ml of broth in the erlenmeyer flasks for each of the overnight cultures.
6. Label four 50 ml conical tubes: Label each tube T_0 and indicate the bacterial strain (e.g. 1-1).
7. Remove 25 mls from culture 1-1 and place in the conical tube and store in the refrigerator. This will be the lag phase sample you will read on **Day 4**.
8. Repeat the previous step for each culture.
9. Cover the flasks with foil and start them gently stirring on the stir plates for 5-7 hours. This is done at room temperature. Record the time each culture started spinning.
10. Label four 50 ml conical tubes: Label each tube T_{log} and indicate the bacterial strain (e.g. 1-1). Record the number of minutes since the culture started spinning.

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11. Remove 25 mls from culture 1-1 and place in the conical tube and store in the refrigerator. This will be the log phase sample you will read on **Day 4**.
12. Repeat the previous step for each culture.
13. Allow the remaining cultures to incubate overnight on the stir plates at room temperature. These will be the stationary phase samples you will read on Day 4.

Day 4: Student Sampling



TEACHERS: At this point, you can place the 12 labeled cultures (e.g 1-1 lag, 1-1 log, 1-1 stationary and so forth) on a front desk and have each group withdraw a 2 ml aliquot of each sample to read with the Spec 20 or the McFarland standards.



TEACHERS: You may want to have some groups use the Spec 20s while others use the McFarland standards. This will lead to interesting comparisons and discussions.

Procedure if using a spectrophotometer

1. Remove 2 ml from each sample to read lag phase density of each. If you are testing all 4 samples you should now have 5 small test tubes (4 with bacterial samples and one blank (the media you saved on **Day 3**)).
2. Prepare the spectrophotometer by setting it to OD600.
3. Read the blank and adjust the % Absorbance to zero.
4. Read the sample tubes and record the % Absorbance.
5. Sniff the flask for any evidence of a banana smell, comparing the smell with the banana extract standards. Be sure to shake the standards and the cultures before sniffing. Record your data.
6. Repeat the above steps with the log phase samples and then the stationary phase samples.
7. Calculate the bacterial population: 1 OD600 unit = 1×10^9 bacteria.



TEACHERS: Students will note that the banana smell dissipates a bit while the tube is open. They can close the tube for a minute and then re-shake to bring back the smell.



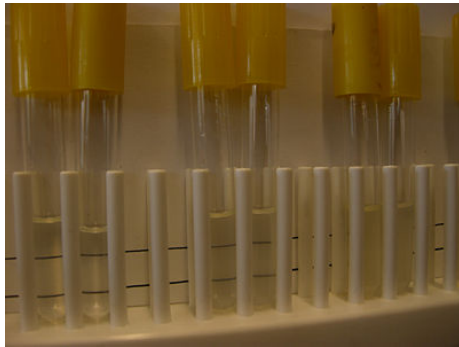
TEACHERS: The students will probably notice the subjective nature of smell as they argue over the values. You may want to encourage each group to select a designated "smeller" in order to increase consistency. However, do encourage all the students to take a whiff. The qualitative nature of the banana assay will lead to interesting discussions about the nature of data.

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Procedure, if no spectrophotometer is available

The turbidity of the bacterial populations can be estimated using the [McFarland Turbidity Scale](#). This method uses suspensions of a 1% BaCl₂ in 1% H₂SO₄ that are visually similar to suspensions of various populations of *E. coli*.

1. Remove 2 ml from each sample to read lag phase density of each. If you are testing all 4 samples you should now have 4 small test tubes.
2. Following your teacher's instructions, obtain small clear test tubes containing the turbidity standards. The tubes should contain enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom. Make sure each tube is properly labeled with its turbidity standard number. If you are filling the tubes from stock bottles of the standards, use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
3. Place the standards in a test tube rack that allows you to view them from the side. Use small tubes and place enough standard in each to fill the tube to a height of about 1 inch (2.5 cm) from the bottom.
4. On a blank index card or paper use a marker to draw two thick black lines. These lines should be within the height of the standards.
5. Place the card with the lines behind the standards.
6. To compare your bacterial cultures to the standards, you will need to place the bacterial sample in a test tube of the same size and equal volume as the standards. Be sure to label these sample tubes.
7. Place the sample tube next to the standard tubes. You should move the sample to compare it



- to the standard tubes with the most similar turbidity. You can make this assessment more precise by looking for a standard that most similarly obscures the black lines on the background card.
8. Use the table below to determine the comparable OD 600.
 9. 1 OD 600 unit equals approximately 1×10^9 cells.
 10. Sniff the flask for any evidence of a banana smell, comparing the smell with the banana extract standards. Be sure to shake the standards and the cultures before sniffing. Record your data.
 11. Repeat the above steps using the McFarland Standards with the log phase samples and then the stationary phase samples.



TEACHERS: It is likely that each stationary phase samples will have a turbidity greater than McFarland standard 7. The students can use 7 as the value in that case. You may advise the students that they can use half values (e.g. 1.5). This will also lead to interesting questions about the nature of data.

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Data Table

Students will need to construct a data table something like the one shown below. They will need a table like this for each of the samples.

Banana Smell Data Table

Phase	Time (minutes from T_0)	OD 600	Cells/ml	Banana smell (0-6)
Lag	T_0			
Log	$T_0 +$			
Stationary	$T_0 +$			

1 OD 600 unit $\sim 1 \times 10^9$ cells/ml