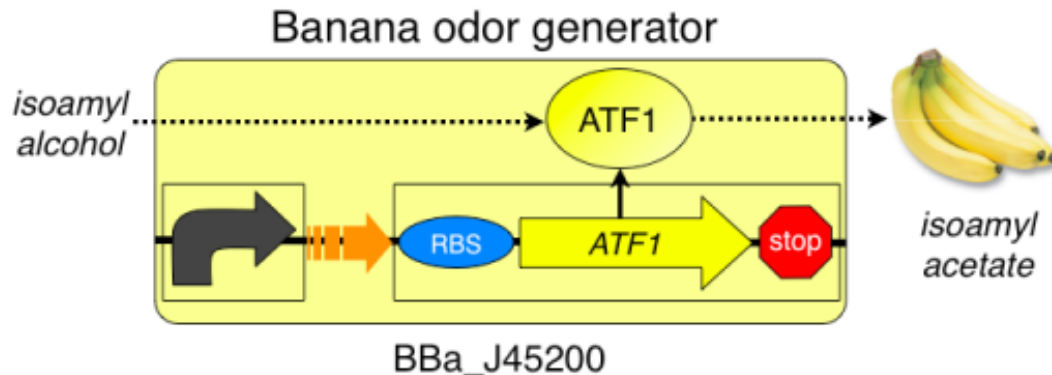


BioBuilding: Synthetic Biology for Students: Eau that Smell

LAB 1: Eau that smell --Protocol A

- Compare 2 competing designs to optimize system performance



Protocol A

You teacher will inform you if you need to conduct the day 1, 2 or 3 procedures.

Day 1

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**.

- 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.
- Repeat with the remaining stab samples, streaking out each onto a different petri dish.
- Place these petri dishes media side up in a 37°C incubator overnight.

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

Day 2:

- 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 (LB) + ampicillin. This volume is more than enough for each strain that each student or team of students must grow.
- Repeat for each strain you will inoculate.
- 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. Alternatively, tubes can be placed on a platform shaker to grow the cells with aeration overnight.

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

BioBuilding: Synthetic Biology for Students: Eau that Smell

Day 3:

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

1. Prepare a stock growth solution with
 - 300 ml Luria broth
 - 300 μ l Ampicillin (final concentration is 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.
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:

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

BioBuilding: Synthetic Biology for Students: Eau that Smell

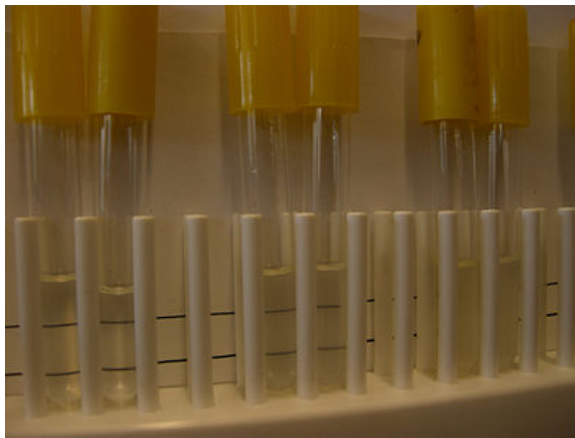
cultures before sniffing. Record your data.

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.

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

BioBuilding: Synthetic Biology for Students: Eau that Smell

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.

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

Data Table

In your lab notebook, you will need to construct a data table as shown below 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 ~ 1 x 10⁹ cells/ml