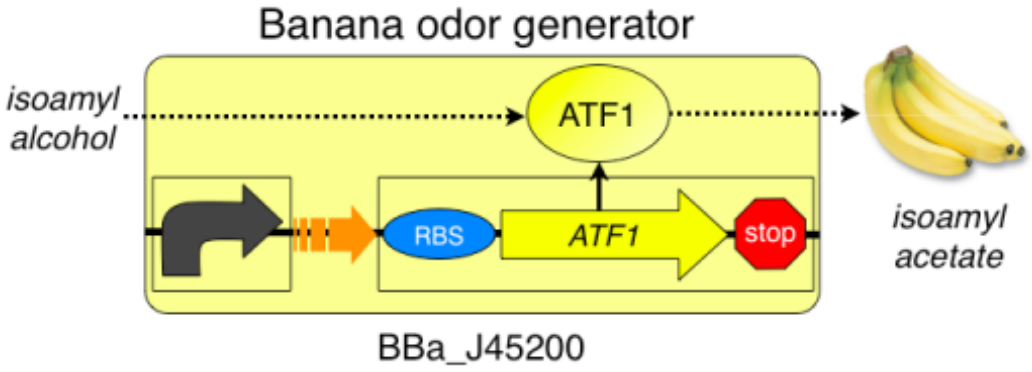
# Lab 1: Eau that smell




**Note: This lab is also described in this Methods in Enzymology chapter**

## 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. Go to Protocol A for the detailed protocol.

Protocol B--Growth curve data collection: This version provides a greater emphasis on collection of growth curve data. In this version, the students subculture from the overnight samples and then assess the banana smell and turbidity (population) of the subcultures every twenty minutes. To increase the number of data points, different classes can measure the same subcultures throughout the day. Alternatively, the subcultures can be refrigerated and warmed back to room temp for 30 minutes prior to the next data point collection. You should expect to collect data over three days to get data points from all phases of the growth curve. Go to Protocol B for the detailed protocol.

Both procedures include 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. Instructions for this measurement are also included with each protocol.

# Needed Materials

## Teacher Provides

- Innoculating loop or sterile toothpicks and bunsen burner
- Sterile tubes for growing liquid cultures of cells
- Cuvettes to measure absorbances if spectrophotometer is not fitted for glass tubes
- 4 x 100 ml erlenmeyer flasks per lab group
- Stir plates and stir bars
- Pipetmen and tips (P1000, P200, P20)
- Pipets (10 ml and 5 ml) and bulbs
- Timers or stopwatches
- Sharpies
- Nitrile or Latex gloves
- If available, rollerwheel at 37° for growing overnight cultures of bacteria (if available)
- If available, vortex for mixing cells prior to additions
- If available, fume hood for measuring isoamyl alcohol (aka isopentyl alcohol) if available

## Kit Provides

**4 strains (see table below)**

- Store stabs at room temp
- Store plates and liquid cultures at room temp or 4° (= fridge) for longer times.

| Strain # | Plasmid | plasmid description | Cells | cells description |
|---|---|---|---|---|
| 1-1 (NB376) | BBa_ J45250 | sigma 54 directing transcription of ATF1, AmpR | BBa_J45999 (NB370) | indole- chassis, CamR |
| 1-2 (NB377) | BBa_ J45990 | sigma 54 plus tetR-4 part inverter directing transcription of ATF1, AmpR | BBa_J45999 (NB370) | indole- chassis, CamR |
| 1-3 (NB378) | BBa_ J45200 | sigma 70 directing transcription of ATF1, AmpR | BBa_J45999 (NB370) | indole- chassis, CamR |
| 1-4 (NB379) | pUC18 | no promoter, no ATF1 gene, AmpR | BBa_J45999 (NB370) | indole- chassis, CamR |

**Chemicals**


**Room Temperature**

- 500 ml LB (= 10 g Tryptone, 5 g Yeast Extract, 10 g NaCl per liter, plus 20g of Agar for plates). **Keep sterile.**
- Banana smell standards
- Turbidity standards

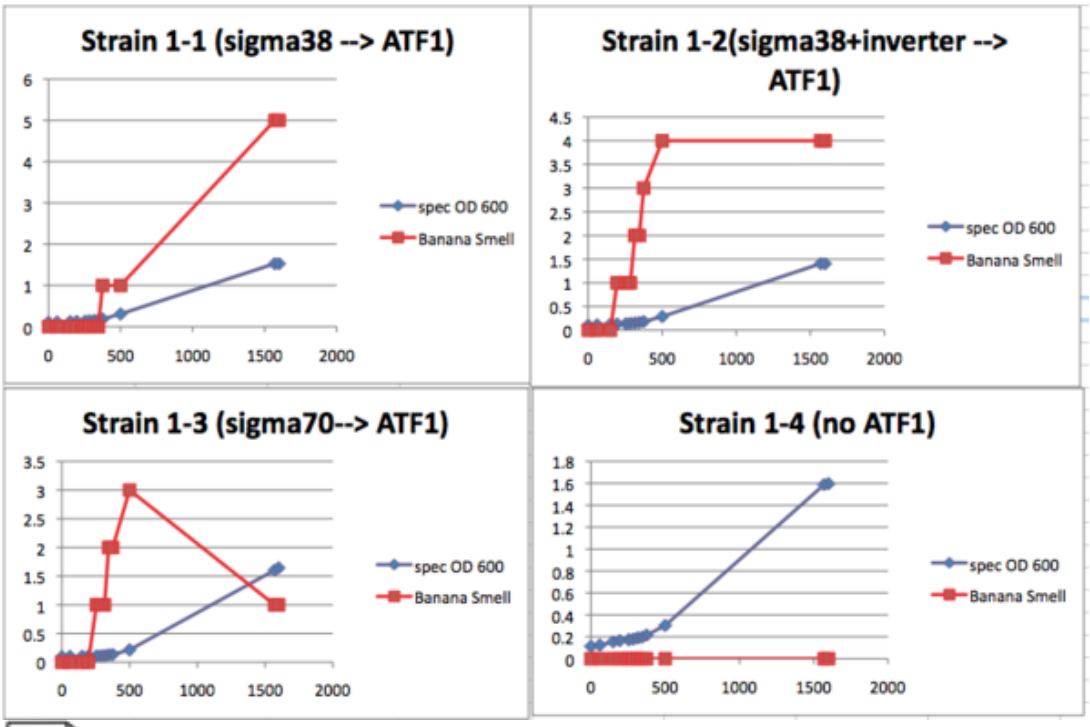
**4° (fridge)**

- 2 ml Amp (100 mg/ml in H2O, filter sterilized)
- 4 LB + Amp plates

**Chemical Hood**

- 1 ml isoamyl alcohol

# Sample Data Set




*TEACHERS:* Note that the original strain is supposed to smell like bananas only during stationary phase but we have found that it actually smells throughout the measurements we've made...perhaps because stationary phase activity starts earlier than we think. Similarly, we have seen the strain 1-2, which has the stationary phase promoter and the inverter, is more active throughout the growth curve and generates a stronger banana smell than strain 1-3, which has the log phase promoter. In our hands, Strain 1-3 is the most "log-phase specific" strain.

# Assessment

## Lab Report Rubric

Download doc or pdf

## Lab Report ScoreSheet

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## Survey

To help us improve the labs, you can

1. send the students here where they can offer anonymous feedback.
2. "join a discussion" from the BioBuilder homepage
3. email us: "info AT biobuilder DOT org"

Thanks!

## Variations to try

- Next version of this series will have BBa_J45400, a 3-methylbutanal generator to allow the strain to convert its own leucine into the ATF1 precursor.
- Try growing the cells at different temperatures?
- If you are using the McFarland standard, would more precise or subtle standards be useful?

## Feedback

We're always looking to hear back from you if you've thought about this unit, tried it, or stumbled across it and want to know more. Please email us through BioBuilder, info AT biobuilder DOT org.