











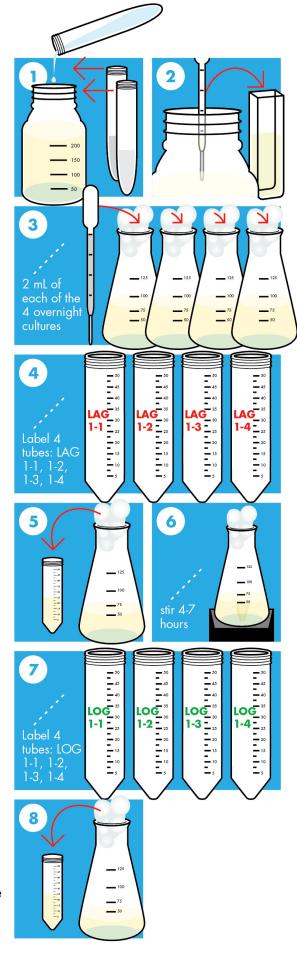
Grow liquid overnight cultures of the 4 strains to

Mix banana smell standards

DAYS OF LAB

Day 1:

- In a sterile bottle or flask, prepare growth media (LB + ampicillin + isoamyl alcohol).
- Remove 1 mL of media and store in the refrigerator. This will be used to blank the spectrophotometer.
- 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.
- Label 4×50 mL conical tubes with the word "LAG" and the strain name, 1-1 or 1-2 or 1-3 or 1-4.
- 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.
- 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.





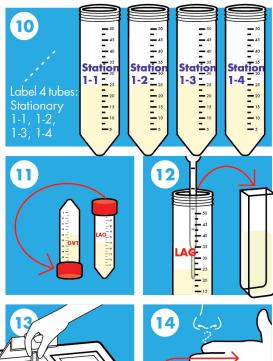


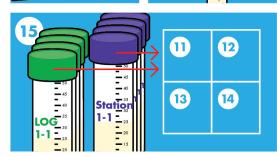
- **9.** Grow remaining volumes of each culture in Erlenmeyer flasks with stirring at room temperature or 37°C overnight. Be sure to record how long the cells grow.
- 10. Label 4 x 50 mL conical tubes with the word "STATIONARY" and the strain name, 1-1 or 1-2 or 1-3 or 1-4. Transfer the grown cultures 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







Waft air

towards your nose

