QUICK GUIDE: EAU THAT SMELL

IN ADVANCE

Grow liquid overnight cultures of the 4 strains to be tested**
Mix banana smell standards

DAYS OF LAB

Day 1:

1. In a sterile bottle or flask, prepare growth media (LB + ampicillin + isoamyl alcohol).

2. Remove 1 mL of media and store in the refrigerator. This will be used to blank the spectrophotometer.

3. 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.

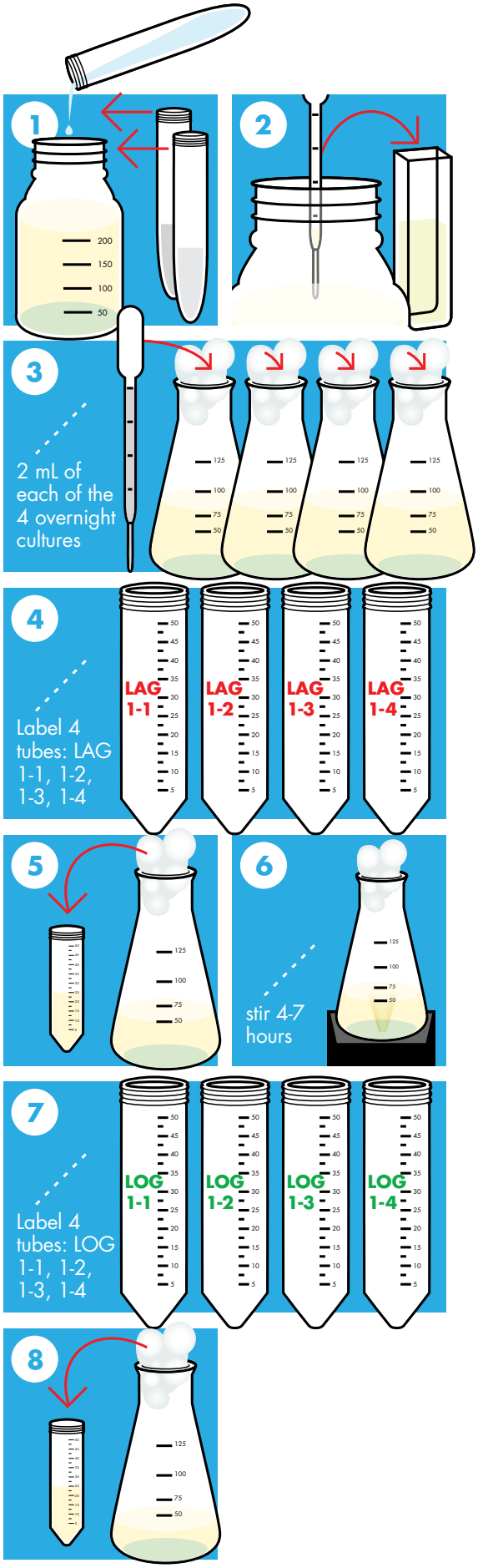
4. Label 4 x 50 mL conical tubes with the word "LAG" and the strain name, 1-1 or 1-2 or 1-3 or 1-4.

5. 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.

8. 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.



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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.

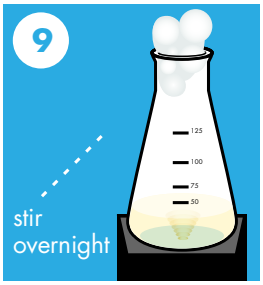
12. Transfer 1 mL from each "LAG" sample to cuvettes.

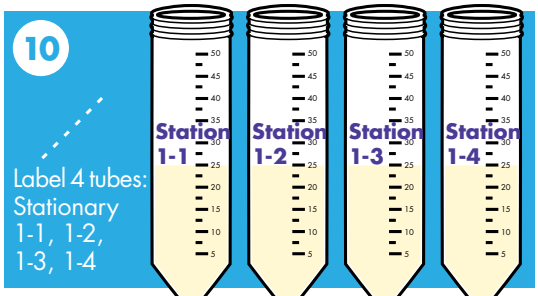
13. Read and record the O600 of each sample. Start by zero-ing the spectrophotometer set at 600 nm using the uninoculated media you saved on Day 1.

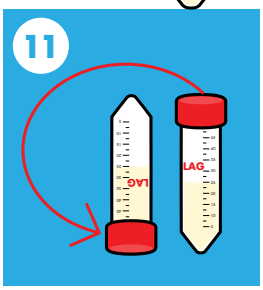
14. Waft the air above the conical tubes towards your nose to test for any evidence of banana smell. Compare the intensity of the banana smell to the banana smell standards.

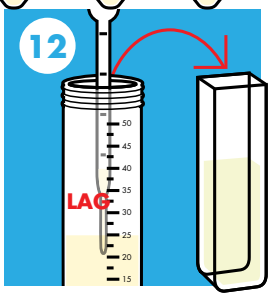
15. Repeat steps **11-14** with the "LOG" and the "STATIONARY" phase cultures.

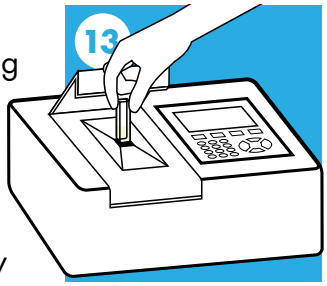
16. Discard all biological materials after decontaminating with 10% bleach

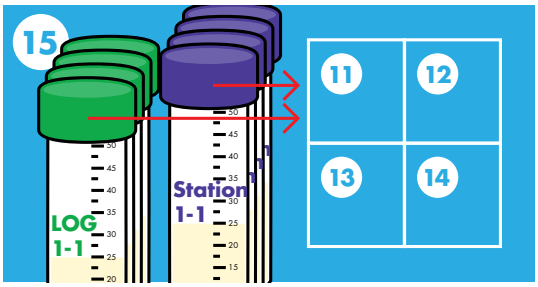


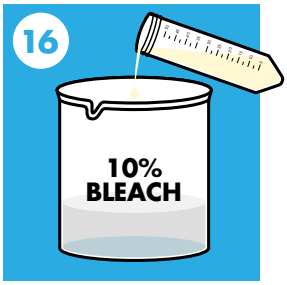












** VIDEO OF PROCEDURE AVAILABLE ONLINE