QUICK GUIDE: ITUNE DEVICES

IN ADVANCE

Grow liquid overnight cultures of the 10 strains to be tested** Don't forget to add ampicillin and IPTG to growth media!

DAY OF LAB

Cell Density Measurements:

1. Label 10 glass tubes "**1**" through "**9**" and "**R**" for reference. Make a 1:10 dilution of each overnight culture by mixing 300 µl of cells with 2.7 mL of bicarbonate buffer.

2. Transfer each sample to a cuvette, filling the cuvettes about three-quarters full.

3. Read and record the absorbance value for each sample. Start by zero-ing the spectrophotometer set at **600** nm using bicarbonate buffer or water.

4. Record the value x 10 in your data table under the **OD600** heading.

5. Discard all dilutions in 10% bleach.

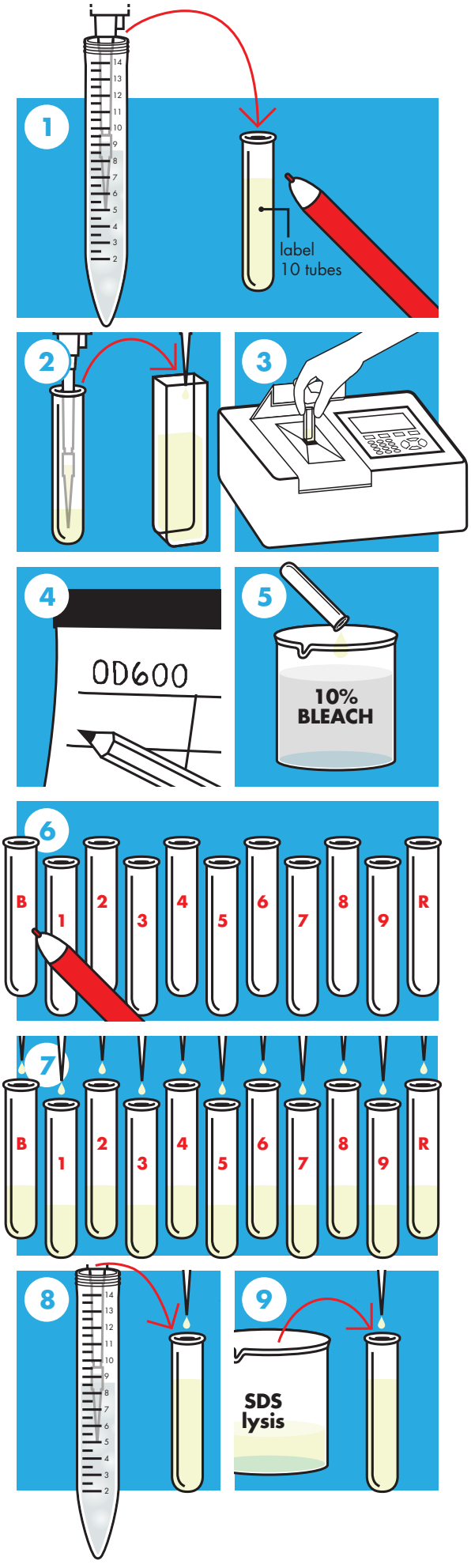
Enzyme Reactions:

6. Label 11 glass tubes "**1**" through "**9**" as well as "**B**" for blank and "**R**" for the reference.

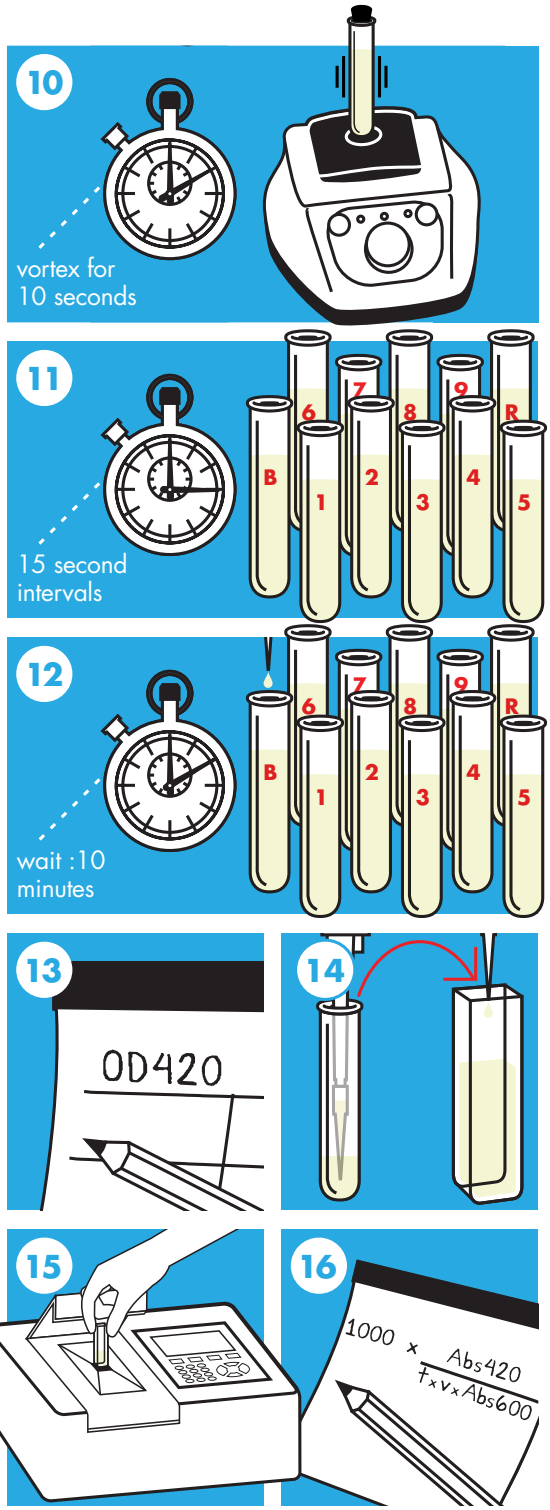
7. Add 1.0 mL bicarbonate buffer to each tube.

8. Transfer 100 µl of cells from the UNDILUTED overnight cultures to the appropriate tube. Transfer 100 µl of bicarbonate to the blank.

9. Add 100 µl of SDS lysis solution to each tube, including the blank.



BioBuilder
Educational Foundation



10. Vortex each tube, including the blank, for EXACTLY 10 seconds.

11. Start the timer when you add 100 µl of ONPG solution to the first tube. Then at 15 second intervals, add 100 µl of ONPG solution to each tube, including the blank.

12. When 10 minutes have elapsed from the time you started the reaction in the first tube, you should stop that reaction by adding 1 mL of the soda ash solution. Add the quench solution at 15 second intervals to all the tubes in the order that you started the reactions, including the blank.

13. Reactions can be refrigerated for later analysis or absorbance data can be collected immediately.

14. Transfer each sample to a cuvette, filling the cuvettes about three-quarters full. (OPTIONAL: Spinning down samples in a centrifuge to pellet cell debris will increase the reproducibility of your absorbance measurements.)

15. Read and record the absorbance value for each sample. Start by zero-ing the spectrophotometer set at **420** nm using the blank reaction.

16. Calculate Miller units for all of your sample data using the following formula:

$$\text{β-gal production in Miller units} = 1000 \times \frac{\text{Abs } 420}{t \times v \times \text{Abs } 600}$$

t is the time in minutes
v is the volume in mLs

** VIDEO OF PROCEDURE AVAILABLE ONLINE