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!

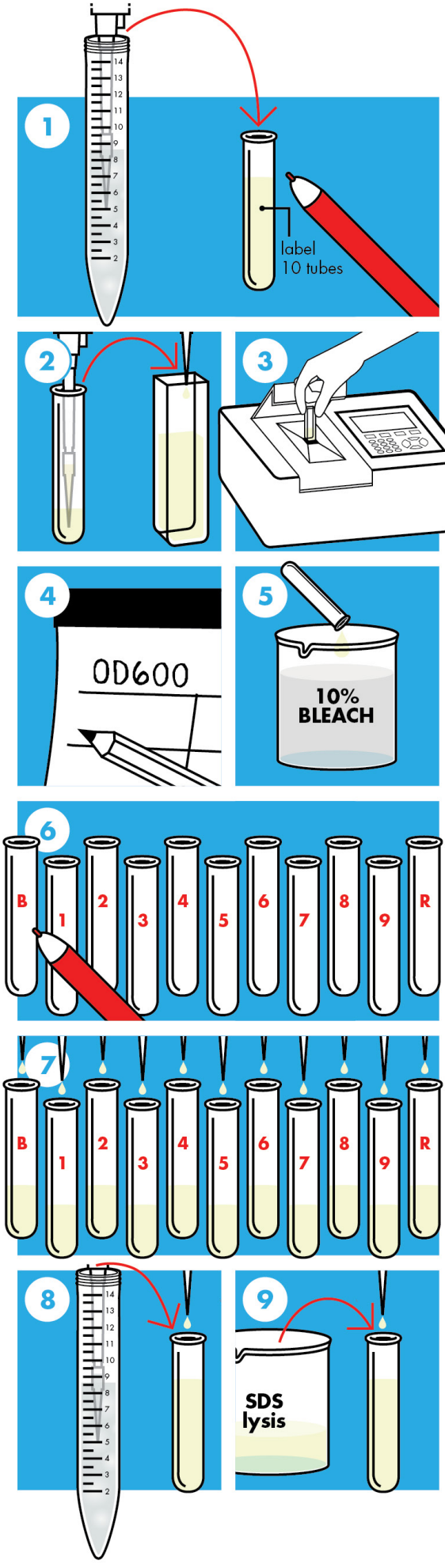
DAY OF LAB

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.



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11. Add 50 µl of competent solution to each tube. Pipet up and down to resuspend the cells. Change pipet tips between tubes.

12. Add 5 µl of sterile water to the tube labelled (**–**). This is your negative control. Add 5 µl of pRS414 DNA to the tube labelled (**+**). This is your positive control. Add 5 µl of pRS414+crtYB' DNA to the tube labelled **Exp**. Flick all tubes to mix contents.

13. Add 500 µl of transformation solution to each microfuge tube. Solution will be goopy. Pipet up and down to mix, changing tips between tubes.

14. Incubate tubes at 30° Celcius (C) for one hour along with 3 SC-trp petri dishes with lids slightly ajar to evaporate the moisture from the media surface. Periodically flick the tubes to mix during the incbation.

15. Label the media-side of the petri dishes as (**–**), (**+**) or **Exp**.

16. Pipet 250 µl of each sample onto the media of the appropriate petri dish. Spread the sample evenly across the dish with a sterile spreader.** Discard spreader and remainder of transformation mix in 10% bleach solution.

17. Incubate petri dishes, media side up, for 2 days at 30°C.

After the petri dishes have incubated for 2 days, count the colonies of each color in every dish.

** VIDEO OF PROCEDURE AVAILABLE ONLINE