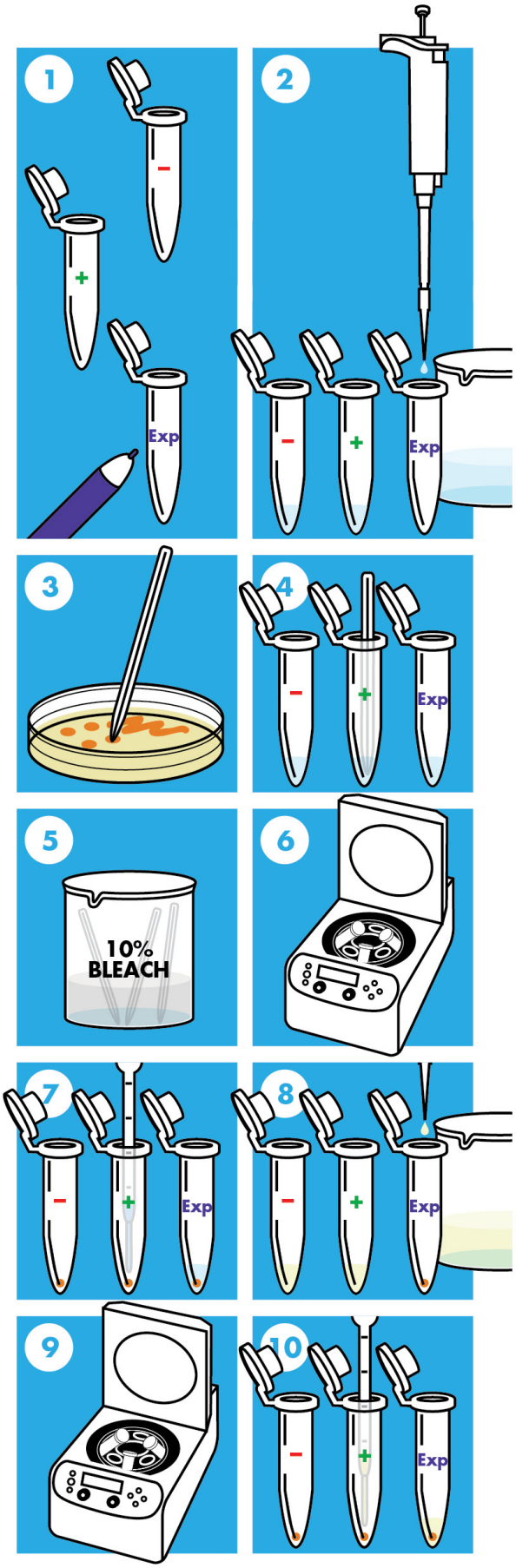
QUICK GUIDE: GOLDEN BREAD

IN ADVANCE

Restreak yeast for single colonies**

DAY OF LAB

1. Label 3 microfuge tubes (–), (+) , and **Exp**.
2. Add 500 µl of sterile water into each labelled tube.
3. Using a sterile pipet tip, toothpick or inoculating loop, scrape a large colony of yeast off the petri dish.
4. Swirl the colony into the tube labeled (–) and repeat for the other tubes, using a new colony each time.
5. Discard the toothpicks into a beaker of 10% bleach.
6. Balance the tubes in the microfuge and spin for 30 seconds at full speed (~14,000 rpm).
7. Remove as much of the supernatant as you can using a pipet. Change pipet tips between microfuge tubes. Discard supernatant into 10% bleach solution.
8. Add 500 µl of wash solution to each microfuge tube. Pipet up and down to resuspend the cells. Change pipet tips between tubes.
9. Balance the tubes in the microfuge and spin for 30 seconds at full speed (~14,000 rpm).
10. Remove as much of the supernatant as you can using a pipet. Change pipet tips between microfuge tubes. Discard supernatant into 10% bleach solution.



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