QUICK GUIDE: GOLDEN BREAD

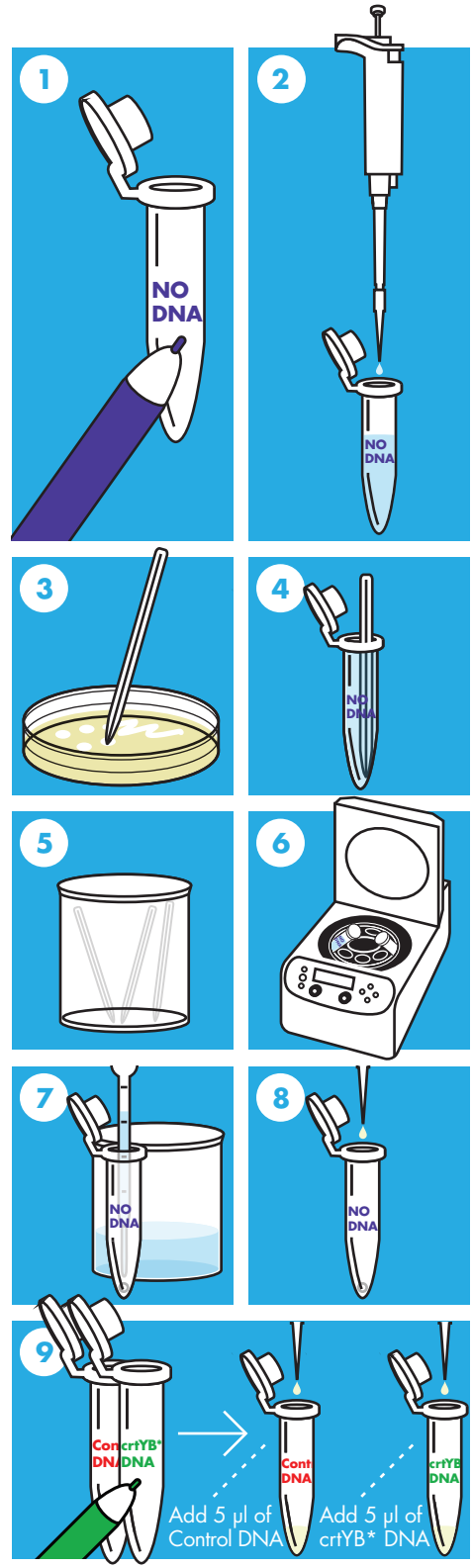
IN ADVANCE

Melt YPD in microwave and pour plates.

Restreak "Golden Yeast" onto YPD to isolate single white colonies**

DAY OF LAB

1. Label 1 microfuge tubes: **NO DNA**.
2. Add 500 µl of **EZ - Solution I** to the **NO DNA** tube.
3. Use a sterile pipet tip, toothpick or inoculating loop, scrape an isolated white colony of yeast off the petri dish.
4. Swirl the colony into the **EZ - Solution I** in the **NO DNA** tube.
5. Discard the pipet tip, toothpick or inoculating loop into a waste receptacle to be decontaminated.
6. Microfuge the tube you have prepared, coordinating with another group or setting up a "blank" microfuge tube with 500 µl of water. Spin the tubes for 30 seconds at full speed.
7. After microfuging the tubes, the cells will have collected as a white pellet in the bottom of the tube. Remove as much of the supernatant as you can, using a pipet and discarding the liquid into a waste receptacle to be decontaminated.
8. Resuspend the pellet in 150 µl of **EZ - Solution II**, pipetting up and down to make a homogeneous solution.
9. Label two more microfuge tubes: **Control DNA**, **crtYB* DNA**. Add 5 µl of the appropriate DNA to the tubes, changing tips between aliquots. Skip this step if the DNA has already been aliquoted for you.



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10. Add 50 µl of cells from the **NO DNA** tube to the **Control DNA** tube. Pipet up and down to mix.

11. Add 50 µl of cells from the **NO DNA** tube to the **crtYB* DNA** tube. Pipet up and down to mix.

12. Add 500 µl of **EZ - Solution III** to each microfuge tube. Solution III will be goopy, but the amount you pipet does not need to be precise. Pipet up and down to mix, changing tips between tubes.

13. Incubate the tubes at 30° Celcius (C) for one hour. Periodically flick the tubes to mix during the incubation.

14. Label the media side of three SC-trp petri dishes as **NO DNA/white colony**, **Control DNA/white colony**, and **crtYB* DNA/white colony**. Add your initials and today's date to each.

15. Pipet 200 µl of each sample onto the media of the appropriate petri dish. Spread evenly across the dish with a sterile spreader.** Discard spreader and microfuge tubes into the waste receptacle to be decontaminated.

16. 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