SCIENCE OF GOLDEN BREAD

IN ADVANCE

- Melt YPD in microwave and pour plates
- Restreak "Golden Yeast" onto YPD to isolate single colonies of different colors**

DAY OF LAB

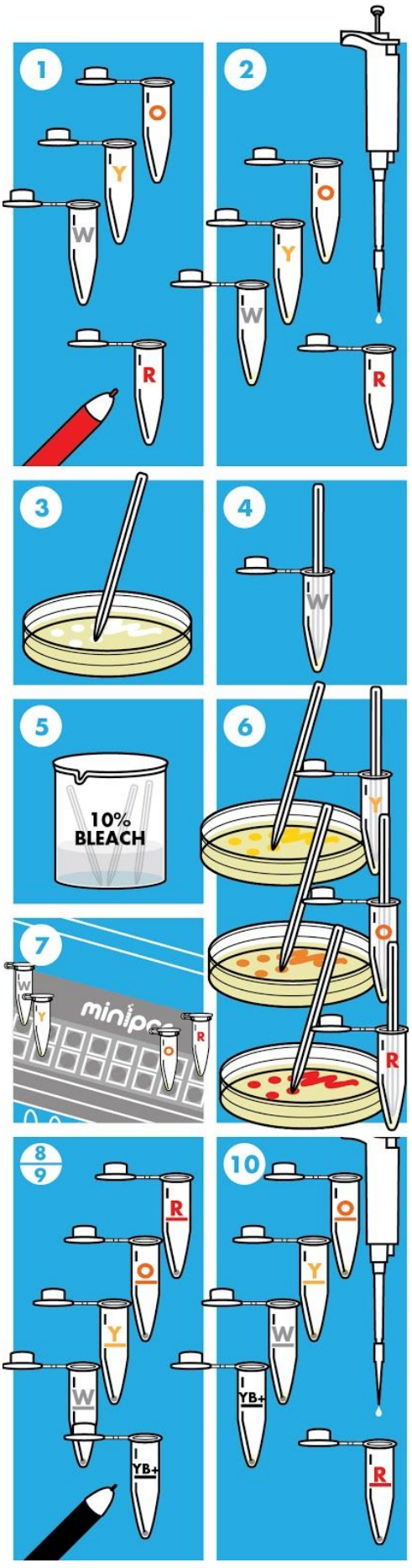
PART 1: DNA EXTRACTION

1. Label four 0.2 mL PCR tubes: W (for white), Y (for yellow), O (for orange), and R (for red)
2. Add 50 µL of miniPCR X-tract buffer to each PCR tube
3. Using a sterile pipet tip, toothpick or inoculating loop, collect a small white colony.
4. Swirl the colony into the X-tract buffer in the PCR tube labeled "W" until there is a uniform suspension of cells.
5. Discard the pipet tip, toothpick or inoculating loop into the waste receptacle to be decontaminated.
6. Repeat steps 3-5 for the yellow, orange and red colonies, choosing a colony of the appropriate color each time.
7. Place the PCR tubes in the thermal cycler and heat them to 95° for 10 minutes.

PART 2: PCR

8. While the DNA is extracting, collect five Illumina PCR beads in 0.2 mL tubes.
9. Label the tubes: "YB+" "W" "Y" "O" "R"
10. To each tube, add 18 µL of PRIMER MIX that includes forward and reverse primers for both the crtYB and actin1 genes.
11. Add 2 µL of the crtYB+ DNA to the tube labelled YB+
12. Add 2 µL of DNA extract from Part 1 to the corresponding tubes, e.g. DNA extracted from a white colony to the "W" tube.
13. Add 5 µL of PCR-grade water to each tube, bringing the volume of all tubes to 25 µL.

We recommend crossing off items on the following reaction checklist as the reagents get added.



BioBuilder Educational Foundation

minipcr bio

SCIENCE OF GOLDEN BREAD

Reaction checklist for each tube

- ⏭ *PCR bead*
- ⏭ *Primer Mix*
- ⏭ *Template DNA*
  - ⏭ *crtYB+ positive control*
  - ⏭ *from White yeast colony*
  - ⏭ *from Yellow yeast colony*
  - ⏭ *from Orange yeast colony*
  - ⏭ *from Red yeast colony*
- ⏭ *PCR-grade water*

14. Place the tubes in the thermal cycler.

15. Run PCR

Initial denaturation	*95 degrees C, 1min*
Denaturation	*95 degrees C, 10sec*
Annealing	*50 degrees C, 10sec*
Extension	*72 degrees C, 15sec*
Number of cycles	*35*
Final extension	*72 degrees C, 1min*

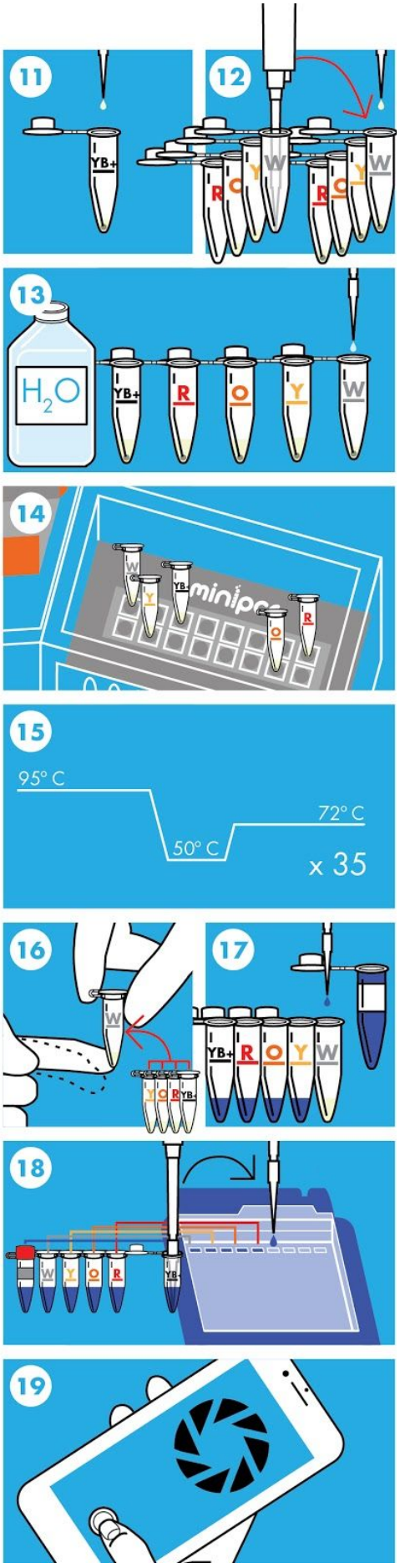
PART 3: ANALYSIS

16. Remove PCR tubes from the machine and flick the contents to the bottom of each tube.

17. Add 5 µL of 6X loading dye to each PCR tube.

18. Load a 2% agarose gel with:
    - 12 µL of DNA Ladder
    - 12 µL of YB+ product
    - 12 µL of "W" PCR product
    - 12 µL of "Y" PCR product
    - 12 µLl of "O" PCR product
    - 12 µL of "R" PCR product

19. Run gel for 10 minutes and photograph your result.



BioBuilder Educational Foundation

minipcr bio

** VIDEO OF PROCEDURE AVAILABLE ONLINE