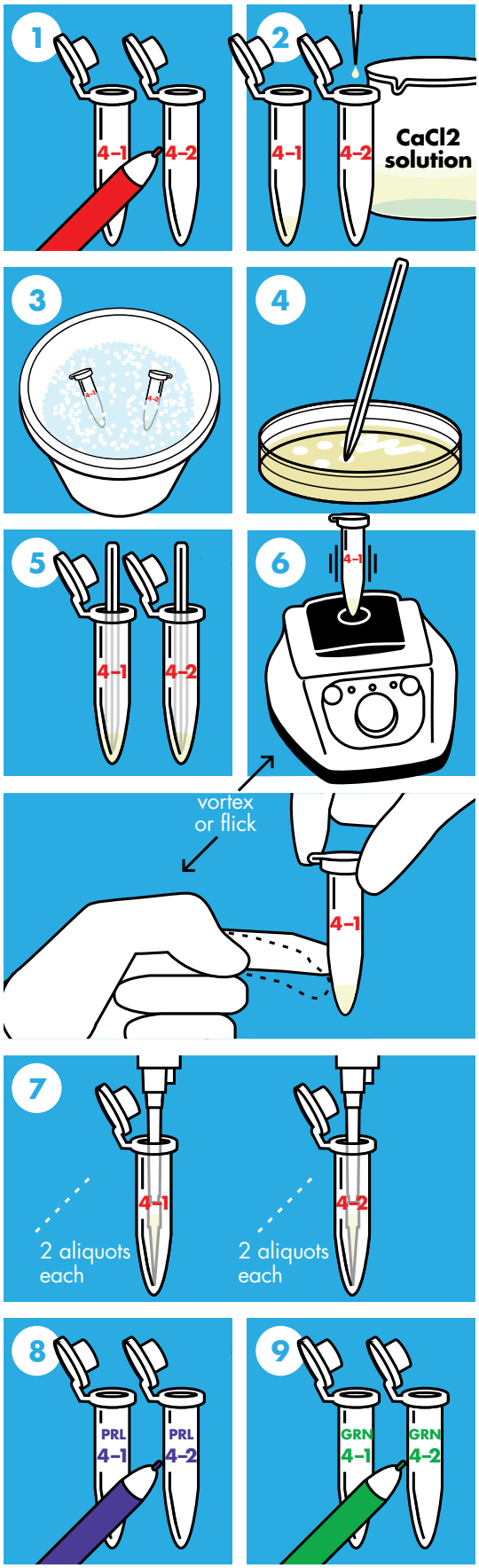
QUICK GUIDE: WHAT A COLORFUL WORLD

IN ADVANCE

Patch **4–1** and **4–2** bacteria on LB petri dishes**

DAY OF LAB

1. Label 2 small microfuge tubes either "**4–1**" or "**4–2**."
2. Pipet 200 µl of CaCl2 solution into each microfuge tube.
3. Place the tubes on crushed ice.
4. Using a sterile pipet tip, toothpick or inoculating loop, scrape a patch of cells off the **4–1** or **4–2** petri dish**. Avoid scraping up the agar.
5. Swirl the cells in the appropriate tube with cold CaCl2 then repeat for the other patch of bacteria.
6. Gently vortex the cells to resuspend them. If no vortex is available, gently flick and invert the microfuge tubes, then return them to your icebucket.
7. Retrieve 2 aliquots of each plasmid for a total of 4 samples (2x **pPRL**, 2x **pGRN**).
8. Label one of the **pPRL** tubes "**4–1**" and label the other **pPRL** tube "**4–2**."
9. Label one of the **pGRN** tubes "**4–1**" and label the other **pGRN** tube "**4–2**."



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10. Flick the tube with the competent "**4–1**" strain and then pipet 100 µl of the bacteria into the tube labeled "**pPRL**, **4–1**." and an additional 100 µl into the tube labeled "**pGRN**, **4–1**."

11. Flick the tube with the competent "**4–2**" strain and then pipet 100 µl into the tube labeled "**pPRL**, **4–2**" and an additional 100 µl into the tube labeled "**pGRN**, **4–1**"

12. Incubate the tubes on ice for ~5 minutes.

13. Heat shock the transformation reactions at 42°C for 90 seconds exactly.

14. Move the tubes to a rack at room temperature and add 0.5 ml LB to each. Close the caps, and invert the tubes to mix the contents.

15. Label the media-side of the LB + amp petri dishes to indicate the strain you've used ("**4–1**" or "**4–2**") and the DNA you've transformed them with ("**pPRL**," "**pGRN**")

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, overnight at 37°C.

After the petri dishes have incubated overnight, count the colonies in each dish.

** VIDEO OF PROCEDURE AVAILABLE ONLINE