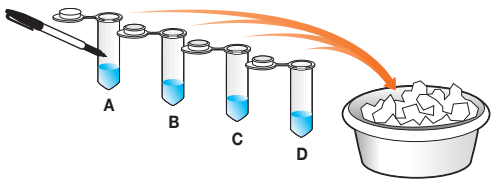
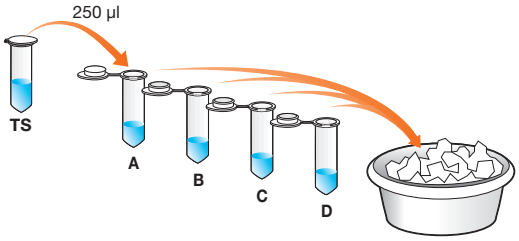
Protocol

1. ***Label four 2.0 ml microcentrifuge tubes A–D and place on ice.***

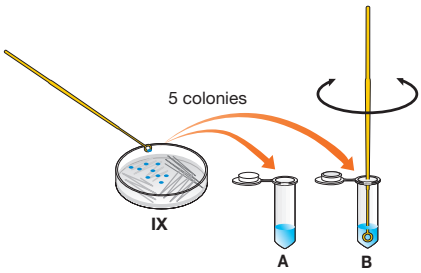


2. ***Add 250 µl ice cold transformation solution (TS) to each tube. Place back on ice.***



3. ***Using a new inoculation loop, pick five colonies from the IPTG/X-gal (IX) plate.***

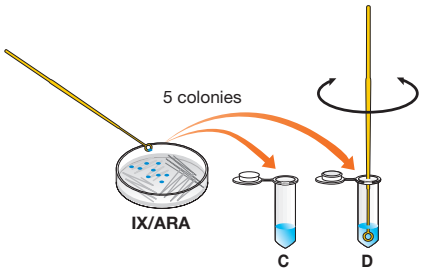
   Swirl the loop in tube A for at least 1 min until all the bacteria are dispersed in the solution. No bacteria should remain on the loop. Immediately place tube back on ice.



4. ***Repeat step 3 for tube B with a new loop.***

5. ***Using a new loop, pick five colonies from the IPTG/X-gal/Ara (IX/ARA) plate.***

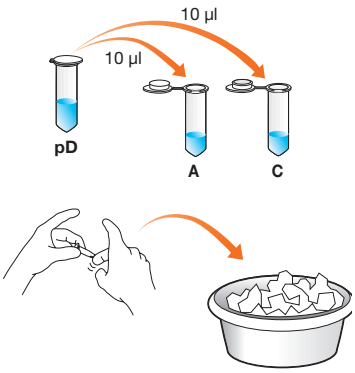
   Swirl the loop in tube C for at least 1 min until all the bacteria are dispersed in the solution. No bacteria should remain on the loop. Immediately place tube back on ice.



6. ***Repeat step 5 for tube D with a new loop.***

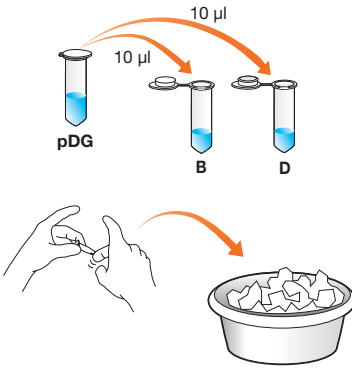
7. ***Using a new pipet tip, add 10 µl pLZDonor (pD) plasmid to tube A. Close the tube, flick three times to mix, and place on ice.***

   Using another new pipet tip, repeat with tube C.



8. ***Using a new pipet tip, add 10 µl pLZDonorGuide (pDG) plasmid to tube B. Close the tube, flick three times to mix, and place on ice.***

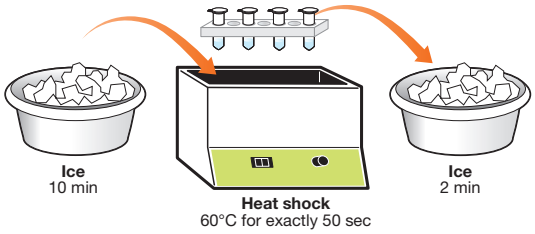
   Using another new pipet tip, repeat with tube D.



9. ***Incubate on ice for at least 10 min.***

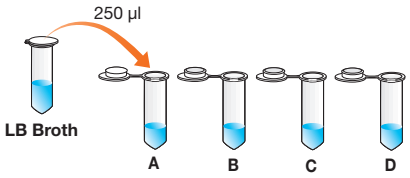
10. ***Bring tubes <u>on ice</u> to the water bath or dry bath.***

    Heat shock at 60˚C for <u>exactly 50 sec.</u> Be sure the bottoms of the tubes contact the water.



11. ***Immediately return the tubes to ice for 2 min. Then transfer to a tube rack.***

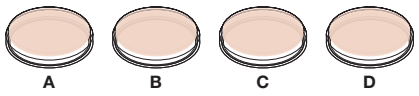
12. ***Using a new pipet tip, add 250 µl LB nutrient broth to each tube. Close each tube and gently flick three times to mix. Leave at room temperature for 20 min to overnight.***



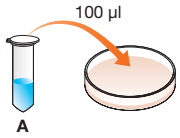
STOP

***Stop.** Ask your instructor whether to proceed now or tomorrow.*

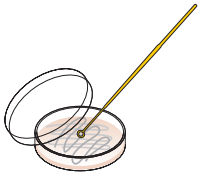
13. ***Near the edges, label the bottoms of four IX/SPT plates A–D. Add your initials and date.***



14. ***Gently flick tube A to resuspend the bacteria. Using a new pipet tip, transfer 100 µl of sample A onto plate A.***

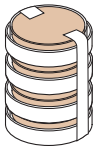


15. ***Using a new inoculation loop, spread the liquid evenly on plate A. Rotate the plate several times in the process. Do not pierce or jab the agar surface.***



16. ***Using a new pipet tip and inoculation loop each time, repeat steps 14 and 15 for samples B–D.***

17. ***Cover, stack, tape, and label your plates. Incubate the plates upside-down at 37°C for 24 hr or at room temperature for 2–3 days.***



18. ***After incubation, check your plates for color development. If blue and white colonies are indistinguishable, refrigerate your plates at 4°C for 1–5 days until the color difference is easily distinguishable.***

Counting colonies and analyzing results

Count the blue and white colonies on your plates and record the numbers in Table 5. Use a permanent marker to mark a dot on the bottom of the plate under each colony as you count it. If there are too many colonies on a plate to count, divide your plate into quadrants and count colonies using steps 19 and 20.

19. ***On the bottom of each plate, use a ruler and a permanent marker to divide the plate into equal quadrants.***