Bio-Rad Explorer™

C. elegans Behavior Kit

Quick Guide

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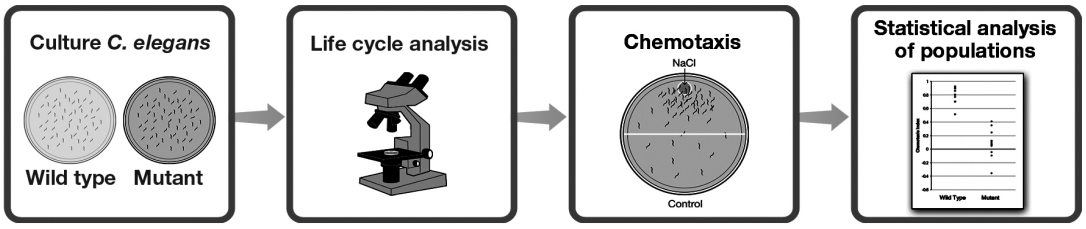
Catalog #1665120EDU



Note: This document is for planning purposes only and may vary from the final product specifications.

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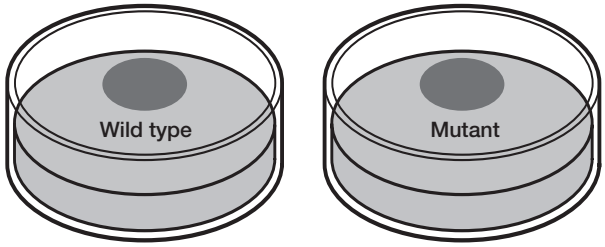
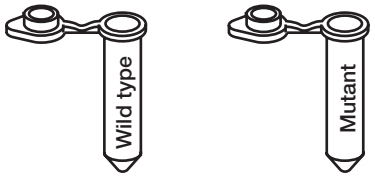
Quick Guide

Lesson 1: *C. elegans* subculture

1. Label one NGM Lite agar subculture plate with *E. coli* OP50-pBAD lawn "Wild type" and the other "Mutant."



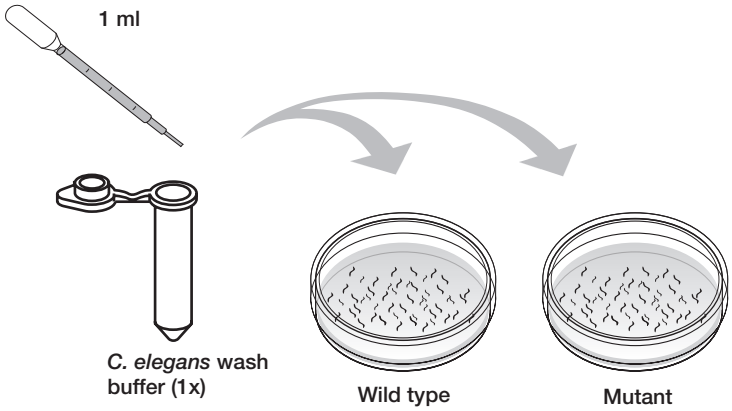
2. Label one microcentrifuge tube "Wild type" and the other "Mutant."



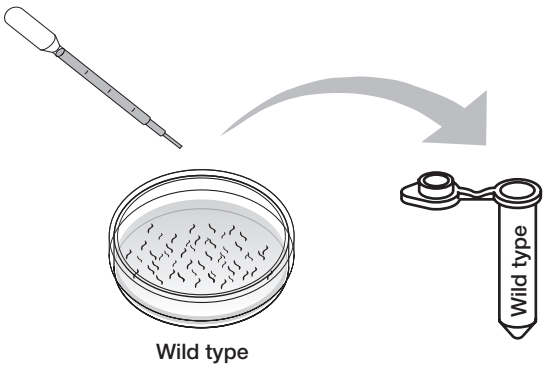
3. Record your observations of what the *E. coli* OP50-pBAD lawns look like.
   - Continuous?
   - Color?
   - Transparent/translucent?
   - Plate coverage?

4. Using a dissection microscope, look at the plates containing your wild-type and mutant *C. elegans* strains. Record your observations.
   - Number of worms on each plate
   - Amount of *E. coli* remaining on each plate
   - Larval stage of worms on each plate
   - Relative percentage of different larval stages on each plate
   - Are eggs present on either plate?
   - Do the mutant and wild-type worms look the same?

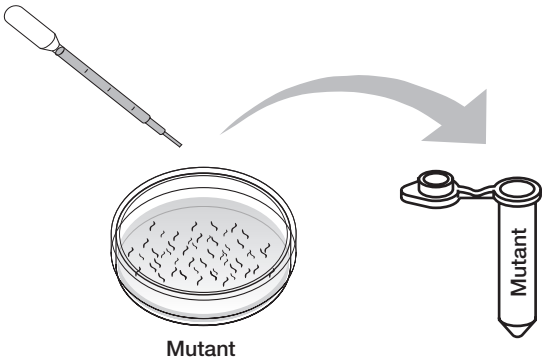
5. Using a sterile DPTP, transfer 1 ml of *C. elegans* wash buffer (1x) to each of the NGM Lite agar plates containing wild-type and mutant *C. elegans*. Rotate the plate to cover the entire surface with *C. elegans* wash buffer. Incubate at room temperature for 30 sec. Save the DPTP in its original wrapper for step 6.



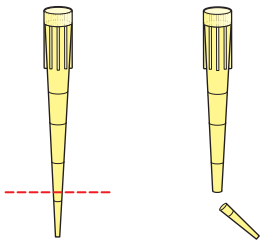
6. Tilt the plate containing the wild-type *C. elegans* at a 45° angle to allow the worms in solution to collect on one side of the plate. Using the DPTP from Step 5, transfer the wild-type *C. elegans* in solution into the microcentrifuge tube labeled "Wild type."



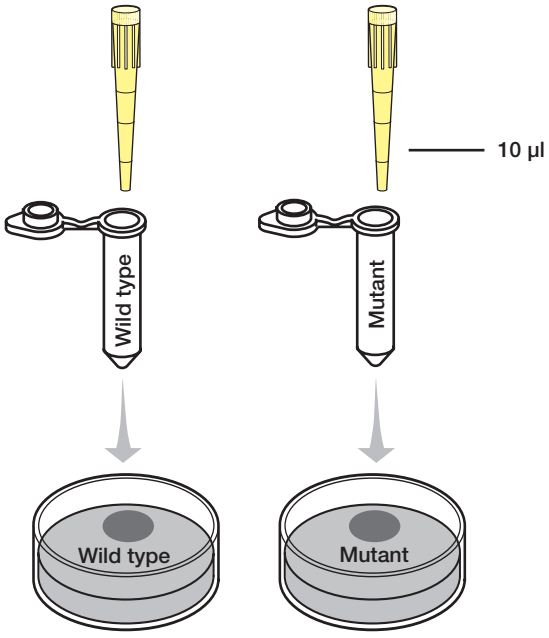
7. Tilt the plate containing the mutant *C. elegans* at a 45° angle to allow the worms in solution to collect on one side of the plate. Using a fresh sterile DPTP, transfer the mutant *C. elegans* in solution into the microcentrifuge tube labeled "Mutant."



8. Allow *C. elegans* in the microcentrifuge tubes to settle for 2 min to form a pellet. While the *C. elegans* settle, cut the ends off two 20 µl tips to make a larger opening.



9. Transfer 10 µl of each *C. elegans* pellet to the appropriately labeled plate containing a lawn of *E. coli* OP50-pBAD bacteria for food.

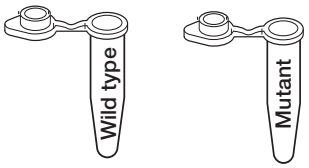


10. Cover plates and incubate at room temperature.

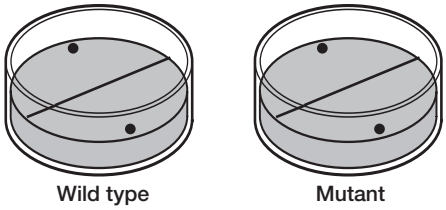
Quick Guide

Lesson 2: Chemotaxis

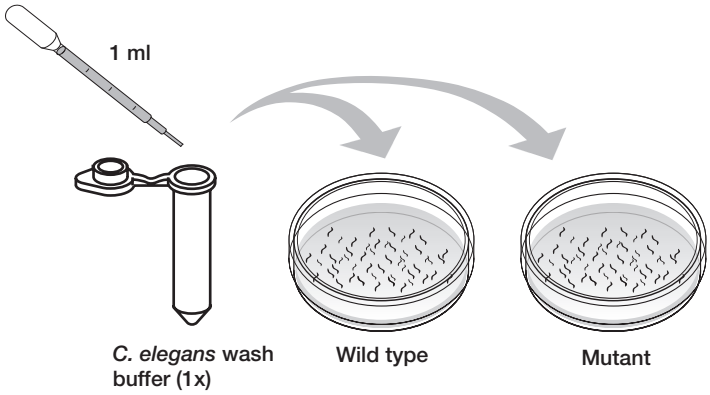
1. Label one 1.5 ml microcentrifuge tube “Wild type” and another “Mutant.”



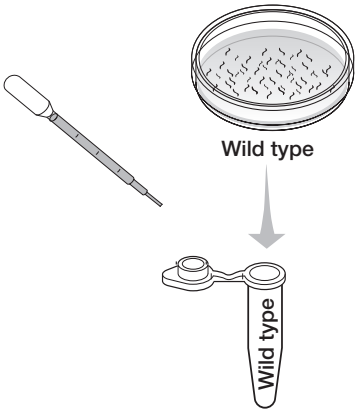
2. Label the bottom of one assay agar plate “Wild type” and the other “Mutant.”



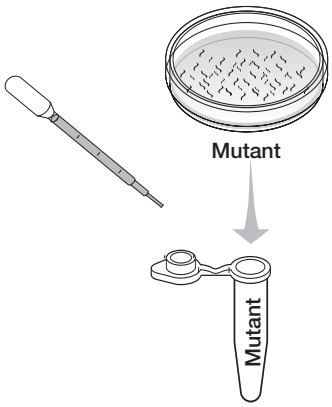
3. Using a sterile DPTP, transfer 1 ml of *C. elegans* wash buffer to each of the two NGM Lite agar plates containing wild-type and mutant C. elegans (from Lesson 1). Rotate the plate to cover the entire surface with *C. elegans* wash buffer. Incubate at room temp for 30 sec–1 min. Save the DPTP in its original wrapper for use in Step 4.



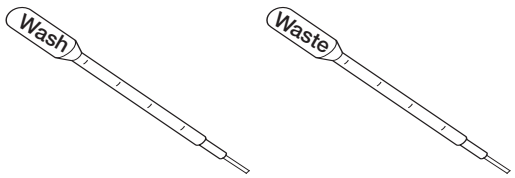
4. Tip the NGM Lite agar plate labeled “Wild type” at a 45º angle and allow the solution to collect in the bottom of the plate. Using the DPTP from Step 3, collect the solution containing the wild-type *C. elegans* and transfer the *C. elegans* in solution into the microcentrifuge tube labeled “Wild type.”



5. Tip the NGM Lite agar plate labeled “Mutant” at a 45º angle and allow the solution to collect in the bottom of the plate. Using a new sterile DPTP, collect the solution containing the mutant *C. elegans* and transfer the *C. elegans* in solution into the microcentrifuge tube labeled “Mutant.”

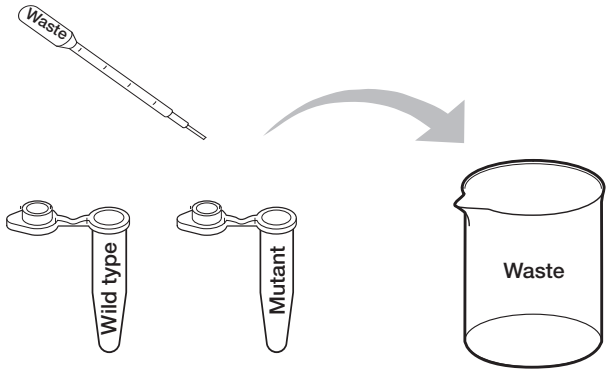


6. Label a new DPTP “Wash” and another “Waste.”

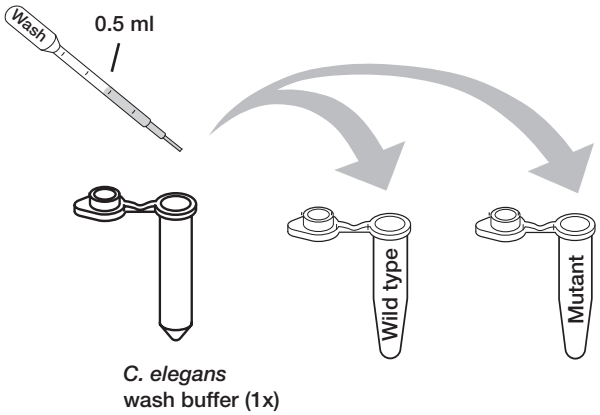


7. Let the *C. elegans* settle to the bottom of the tubes for 2 min to form a pellet.

8. Being careful not to disturb the *C. elegans* pellet, use the DPTP labeled “Waste” to remove the supernatant from each microcentrifuge tube and transfer it to the waste container.

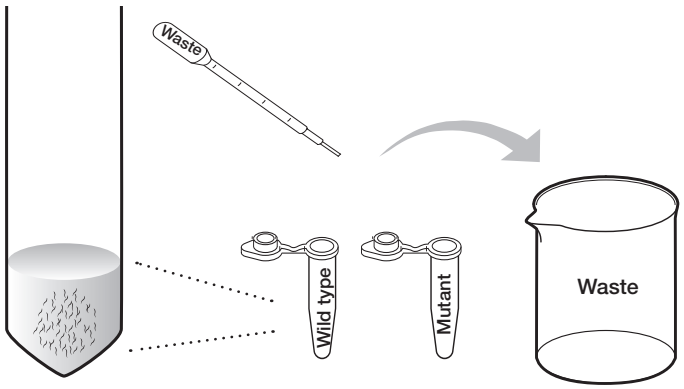


9. Using the DPTP labeled “Wash,” transfer 0.5 ml of *C. elegans* wash buffer to the wild-type and mutant microcentrifuge tubes containing the *C. elegans.*



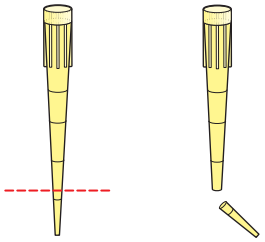
10. Cap and invert both microcentrifuge tubes containing the *C. elegans* 5x to mix. Make sure that the entire pellet has been resuspended.

11. Repeat Steps 8–10 one more time for a total of 2 washes. During the last wash, visually examine the clarity of the *C. elegans* wash buffer in which the *C. elegans* are resuspended. If the *C. elegans* are not dispersed in a clear liquid, obtain additional wash buffer from the instructor and continue washing until the liquid is completely clear.



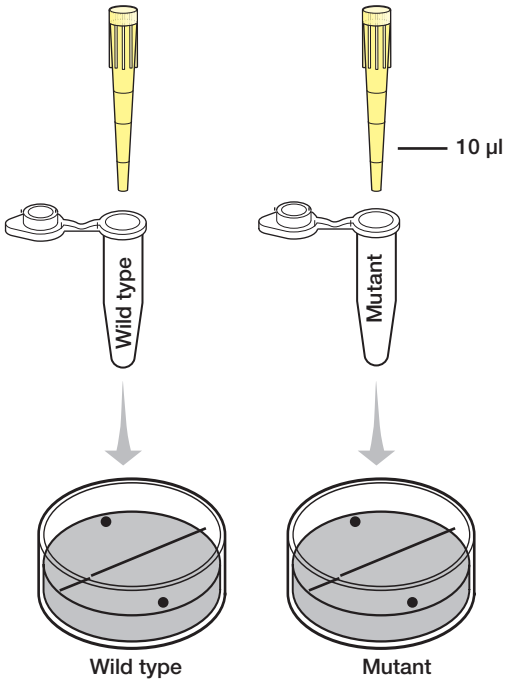
12. After the last wash, discard the supernatants, leaving approximately 50–100 µl of liquid above the pellet.

13. Cut the end of a 20 µl pipet tip to make a larger opening.

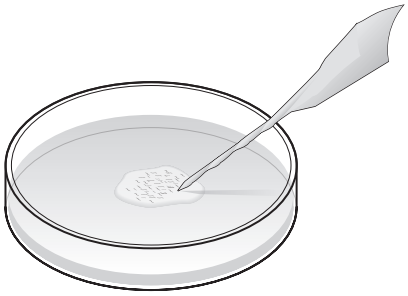


14. Transfer 10 µl of concentrated *C. elegans* pellet to the center of the appropriately labeled assay agar plate.

15. Repeat steps 13–14 with the second *C. elegans* strain.



16. Twist the end of a Kimwipe to a fine tip. Gently insert the twisted Kimwipe into the *C. elegans* to wick away excess fluid. Hold the Kimwipe in place for approximately 5 sec to fully absorb the liquid.



17. Allow the *C. elegans* to migrate across the plate for 30 min.

18. At the end of 30 min, invert the plate and, using a dissection microscope and a fine tip marking pen, mark the bottom of the plate with the locations of *C. elegans* across the plate. Do not mark *C. elegans* that have not moved from the center of the plate as these may have been damaged during the washes.

19. For the wild-type *C. elegans*, record the number of worms on the NaCl side. Record the number of worms on the control side. Repeat for the mutant *C. elegans*.

Wild-type *C. elegans*

NaCl side _____

Control side _____

Mutant *C. elegans*

NaCl side _____

Control side _____

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