

## **C. elegans synchronization via bleaching**

### **Overview**

A synchronous population of worms may be needed for some experiments in order to eliminate variation in results due to age differences. The synchronization protocol consists of treating worms with a bleach solution. Eggs are resistant to bleach and after several washes they are collected and transferred to new NGM plates, generating the new synchronous population.

### **Materials**

M9 buffer

Worm Bleach Solution

Plates with *C. elegans* gravid adults

### **Procedure**

Note: the volumes suggested in this protocol are calculated for two 6 cm plates of worms.

1. Using a P1000, wash worms off of plates with M9 buffer and transfer them into a 15-mL Falcon tube. The exact volume of M9 buffer used isn't critical, as long as you add enough to sufficiently wash all the worms/eggs off the plate –generally two or three mL M9 per plate is enough.

*Note: Worms from multiple plates can all go into the same tube as long as they're the same strain.*

2. Spin the tubes in the table-top centrifuge for 1 minute at 1500 rpm to pellet the worms.

3. Remove and discard the M9 supernatant, taking care not to disturb the white worm pellet.

*Note: The worm pellets are very loose, so in general, when removing supernatants, it's better to err on the side of caution and leave a little buffer on the worms rather than risk accidentally sucking up and losing some of your worms.*

4. Add 5 mL of Worm Bleach Solution to the worm pellet. Immediately cap the tubes tightly and shake vigorously for three minutes straight (continue shaking the whole time without stopping) to ensure the worms will be sufficiently killed/dissolved.

5. At the end of the three-minute bleach incubation, immediately add 10 mL of M9 to stop the bleaching. Spin the tubes at 1500 rpm for 1 minute.

6. Remove and discard the bleach/M9 supernatant, leaving behind the eggs and any worm debris (the pellet should now look yellowish and will probably be noticeably smaller than your starting pellet).

*Note: Prolonged exposure to bleach can eventually be harmful to eggs so it is important to remove the bleach as soon as possible after the 3-minute incubation – ideally the eggs should not remain in bleach for longer than 6 minutes total, including the shaking time.*

7. Wash eggs by adding 10 ml of M9 buffer and inverting the tube to resuspend the egg pellet. Spin and remove supernatant.

8. Repeat wash step.

9. Resuspend egg pellet in 1mL M9 buffer.

10. Optional: Pipet 5  $\mu$ L drops of 1:10 dilution of your egg suspension onto a glass slide and count the number of eggs per drop on the scope to get an average. Count all visible eggs, including any eggs still stuck inside any worm carcasses.

*Note: Eggs/worms can be too big to fit through P20 tips, so when pipetting small volumes of eggs/worms, you'll need to cut the end off of the P20 tip with scissors/razor blade before using it.*

11. Place tubes in rotor wheel at room temperature for 20-24 h. Let the eggs hatch overnight with gentle rocking. Since there is no food the larvae should be halted at the L1 stage.

12. Pellet L1 worms by centrifugation (1500 rpm for 1 minute). Discard supernatant, leaving  $\sim$ 100  $\mu$ L. Resuspend the worms in the remaining M9 volume.

12. Distribute the liquid onto seeded NGM plates.

*Note: too many worms may exhaust the food faster than expected. Ideally, seed  $\sim$  500 L1 worms per plate. You can count the number of worms in a 5  $\mu$ L drop to calculate worm concentration.*

13. Let liquid dry before inverting plates. Incubate plates at 20°C for two days for young adult, pre-egg-laying worms.

*Note: C. elegans develops at a different rate depending on the temperature. While it takes about 90 hours from the moment the egg is laid until the new worm starts to lay its own eggs at 15 °C, 45 hours are enough when grown at 25 °C. The incubation times and temperatures can be adjusted as necessary.*

## Solutions

Worm Bleach Solution (100 mL):	40 mL H <sub>2</sub> O
	40 mL bleach (5% hypochlorite)
	20 mL 5N KOH

*Note: bleach loses potency after it has been open for a while, in part due to its photosensitivity. Cover bottle with aluminum foil and use within a month of making it.*

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