

## Synchronizing worms by hypochlorite treatment

1. Grow up desired number of 10 cm plates for bleaching. You want there to be maximal number of gravid adults. Each 10 cm plate yields approximately 5000 embryos. Strains vary significantly in this amount. It is easier to chunk more 10 cm plates than you think you might need for synchronization.
2. Wash worms off plates using a sterile glass pipette into a 15 ml conical tube. You can squirt M9 onto one plate of Strain A, transfer the liquid to another plate of strain A, etc. Wash all plates from a single strain into one 15 ml conical tube. It is easy to pour from the final 10 cm plate into the 15 mL conical with a little bit of practice.
3. Spin down worms at 1100 rpm for 1 minute using the table-top clinical centrifuge.
4. Aspirate off the M9, being careful to not suck up any of your worms.
5. Add 6 ml of bleaching solution to each 15 mL conical. Shake the tubes manually, use a nutator, or use the plate vortex if you have many strains.
  - a. After four minutes of shaking, check to see if the adult worms are dissolved every minute until complete.
  - b. Be very careful not to over bleach the worms. Once you see that the carcasses are nearly dissolved, move to the next step. If there are only embryos left, then you have gone too long.
  - c. The freshness of the bleach solution matters. Ineffective bleaching solution (kept at 4°C longer than one month or kept at room temp for longer than eight hours) will require more time to bleach worms and will not dissolve the worms uniformly.
6. Once there are just a few adults remaining, **move quickly** to spin down the embryos at 1100 rpm for 30 seconds. It is best to have the reagents next to the sink and prepared for the next few steps.
  - a. Some table-top centrifuges do not have an option of 30 seconds, so you must stop the centrifuge manually after 30 seconds.
7. **Immediately** decant off the bleach into the sink.
8. Add 10 ml of M9 using a sterile glass pipette to each 15 mL conical tube.
9. Invert three times as you walk back to the clinical centrifuge.
10. Spin down the embryos at 1100 rpm for 30 seconds.
11. Decant off the M9 into the sink.
12. Repeat steps 8-11 two more times.
13. After the third M9 wash, resuspend the embryos in 2 mL of S medium, K medium, or M9 (depending on your application).
14. If desired, determine the titer of the embryos by counting the number of embryos in 5  $\mu$ l. Adjust the resuspension liquid to obtain the desired concentration (*e.g.* 0.5 - 1 embryo per  $\mu$ l).

15. Transfer the embryo solution to a labeled glass culture tube.
16. Place all glass tubes on a roller drum at 20°C to allow embryos to hatch. Allow tubes to remain on roller drum for a minimum of 12 hours to make sure all the embryos hatch.
17. For most applications, re-titer the L1s to determine the concentration that survived the bleach protocol. Plate out onto fresh plates or feed in the culture tubes to restart development. You can pipet L1 larvae with pipet tips.

Bleaching Solution

2 ml NaOCl from Fisher (cat# SS290)

0.5 ml 10 M NaOH

in 10 ml of sterile water