

Bleaching to synchronize worms

1) Wash the worms into a 15-ml Falcon tube with 1x M9. Centrifuge at 650g for 1 min in a Beckman Coulter Allegra 6KR or similar at RT to pellet the worms.

2) Wash the pellet two times more as in Step 1A(ii) to remove excess bacteria.

3) After the final wash, resuspend the worms in 3.5 ml of 1x M9. Add 0.5 ml of 5 N KOH and 1 ml of 6.15% commercial Clorox bleach. Incubate for 3-7 min at RT and vortex vigorously for 30 s every minute. Proceed to the next step as soon as the worms dissolve and the eggs are released (monitor the Falcon tube under a dissecting microscope).

4) Pellet the eggs by centrifugation at 650g for 3 min. Discard the supernatant.

5) Wash the pellet four times with 10 ml of 1x M9. Centrifuge at 650g for 3 min between washes to pellet the eggs.

6) In the same tube, allow the eggs to hatch overnight at 20°C either rocking or rotating. As the worms cannot continue the life cycle in the absence of food, all the eggs will hatch and arrest at the L1 larval stage.