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# Worm Synchronization Protocol (Bleaching)

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ABSTRACT

A protocol for synchronizing a large number of C. elegans worms. Worm bodies are decomposed using a mixture of bleach and sodium hydroxide, for a short enough time, so that eggs are not damaged. These eggs are then left to hatch in liquid, and the resulting L1's all have approximately the same age.

Source of the original protocol:
Wormbook Methods: http://wormbook.org/toc_wormmethods.html
Synchronization protocol: Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A., Cerón, J. Basic *Caenorhabditis elegans* Methods: Synchronization and Observation. *J. Vis. Exp.* (64), e4019, doi:10.3791/4019 (2012).

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MATERIALS TEXT

Compounds you will need to perform this protocol:
- Bleach (we use supermarket bleach)
- NaOH (8M)
- milli-Q water (the protocol also works with double-distilled water)
- M9 worm buffer (our recipe: https://www.protocols.io/view/preparation-of-m9-worm-buffer-x54v9yo6pg3e/v2)

1 **Wash one[1] or several[2] worm plates[3] with milli-Q water[4]. Put the worms into a 1.7 mL Eppendorf tube[5].**

[1] To wash a plate: Remove a small chunk of agar near the plate border (we use a metal spatula). Using the 1 mL pipette, add 1-2 mL of milli-Q water to the plate. Tilt the plate so that all the water is on the missing chunk side. Pipetting in the hole, take about 1 mL of water, and spray it on the plate to dislodge the worms. When spraying the water, point diagonally towards the plate, to prevent splashes. Do this several times, so that most worms accumulate at the bottom. Then take the water full of worms with the pipette, and put it in an autoclaved 1.7 mL Eppendorf tube. The agar absorbs some water, so if you initially put 2 mL you will get around 1.5 mL in the tube. You can repeat this procedure several times to get as many worms as possible, but unless you really need to get all of them one wash is usually enough.

[2] We typically do this with as many as 8 plates at the same time with no issues (if you have many plates you will have a big worm pellet, and the bleaching may take slightly longer, but we have not found problems with it). To wash more than one plate, you can pellet the worms in the Eppendorf as indicated later, remove the supernatant and add the worms from the next plate to the same Eppendorf.

[3] The plates should contain many hermaphrodites with eggs inside. With N2 worms and



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NGM+OP50 plates, this usually happens about 2 days after starting the plate. It's very important that the plate has bacteria left. Once the worms deplete the bacteria, they lay their last eggs in a few hours, and don't have eggs inside any more (and only the eggs that are inside worms at the beginning of the process survive the synchronization).

[4] Sterile (autoclaved) milli-Q water.

[5] Eppendorfs should be autoclaved before using them. Use clear (i.e. non-colored) Eppendorf tubes, so that you can see the worms inside.

2 **Once in the Eppendorf, wash[1] the worms twice[2] with milli-Q water. After the second wash, remove supernatant leaving ~0.1 mL of liquid (or the volume of the worm pellet if it's bigger than that).**

[1] To wash the worms in the Eppendorf, first pellet them by centrifugation. Then pipet out the supernatant (very carefully, because the worm pellet is very unstable), and add more water. Here and everywhere else in this protocol, use a table-top small centrifuge (which usually work at around 2000 g). Always balance the centrifuge carefully by placing another 1.7 mL Eppendorf opposite to yours, with the exact same amount of distilled water. To pellet adult worms, it's enough to start the centrifuge, wait until it reaches maximum speeds (which takes <5 seconds), and stop it.

[2] Two washes mean that you add fresh milli-Q water twice.

3 **Add 1 mL of milli-Q water (so to a volume of ~1.1 mL).**

4 **Add, in fast succession, 130 µL of NaOH 8M[1] and 130 µL of bleach[2,3]. Close the Eppendorf.**

[1] You can have a small stock of NaOH in a 15 mL Falcon, but be careful with it--it's a strong base! Some people recommend using filtered tips to pipet the NaOH, because the vapors of strong acids and bases can damage the pipets (but apparently this is a bigger issue with acids than with bases).

[2] We keep the NaOH and bleach in dark 5-mL eppendorfs. Bleach must be protected from light, or it will lose its properties (no need for NaOH, but we use dark tubes for both for simplicity).

[3] Some protocols prepare a bleaching solution beforehand. The problem with this is that NaOH and bleach start reacting as soon as you mix them, so the bleaching solution loses strength over time. For this reason, we prefer to add them separately directly to the Eppendorf.

5 **Vortex[1] the Eppendorf, watching from time to time to check the state of the worms. Keep vortexing until most of the worms have dissolved.[2] While the worms dissolve,**



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**place a counterbalance with the right volume[3] in the centrifuge.**

[1] Some people vortex continuously, and only stop to look at the worms. Some people vortex at short bursts, or even just leave the Eppendorf in a shaker for a fixed amount of time.

[2] "Most worms dissolved" basically means that you don't see worms any more when you look at the Eppendorf. This usually takes around 5 minutes, but it will depend on the worm strain and other factors.

[3] ~1.4 mL if all volumes are as described.

6 **As soon as you feel that the worms have dissolved, centrifuge the tube for 30 seconds.**[1]

[1] The liquid usually looks yellow now.

7 **Remove the supernatant leaving ~0.1 mL**[1]**, and add ~1.3 mL**[2] **of M9 worm buffer**[3]**. Centrifuge for 30 seconds.**

[1] Do this carefully to prevent resuspending the now-dissolved worms and their now-free eggs. It's OK if you don't see any visible pellet at this point.

[2] To do this fast, you can use the 1000 mL pipette, but using it whole volume: Press the piston fully (until the second stop), fill it with M9, and then press again the piston to the second stop to release all the M9 into the Eppendorf. The exact volume depends on the brand of the pipette, but it's adequate with the brands we have tested.

[3] Our recipe for M9 can be found here:
https://www.protocols.io/view/preparation-of-m9-worm-buffer-x54v9yo6pg3e/v2

8 **Repeat previous step 3 more times (so a total of 4 washes).**[1]

[1] Be careful with the timing at least for the first two washes, not exceeding the 30 seconds in the centrifuge and not waiting to remove the supernatant and so on (the bleach is still there, killing your future experiment!). The liquid will look less yellow as you proceed with the washes.

9 **After last wash, remove supernatant and transfer the pellet**[1] **to a 50 mL Falcon with 10 mL of M9 worm buffer.**

[1] Leave ~0.1 mL after removing the supernatant. Mix a bit with the pipette to resuspend the eggs, and then transfer to the 50 mL Falcon.

10 **Leave the shaker at room temperature for a time between 16 and 48 hours.**[1]

[1] During this time, the eggs that survived the bleaching will hatch. For N2 worms, 16 hours are



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usually enough for practically all of the eggs to hatch (this may depend on the strain). The eggs hatch at different times, but the L1 larvae will arrest development due to the lack of food. They survive happily in M9 for up to 48 hours (some of them will survive much longer, but after 48 hours mortality starts to be significant). Again, this may depend on the strain.

11 **After this time, estimate the density of worms in the Falcon tube.**[1]

[1] To estimate the density: Take the 20 µL pipette, coat its tip with M9+0.1% Triton by pipetting in and out (the triton prevents the worms from sticking to the pipette tip; it's not essential but recommended). Then take a few 20-µL drops and look at them in a dissectoscope (we usually put the drops on the lid of a Petri dish for this). From this, calculate how many worms per µL there are approximately in the Falcon tube.

12 **Using a triton-coated tip**[1]**, transfer the needed volume**[2] **into a 1.7 mL Eppendorf**[3]**.**

[1] Alternatively, you can add ~1 mL of M9+0.1% triton to the Falcon tube (but then you will have to wash the worms three times instead of one).

[2] We usually seed plates at a density of around 500 larvae per plate (in plates seeded with 200 µL of saturated OP50), or around 1000 larvae per plate (in plates seeded with 800 µL of saturated OP50). This guarantees that they will not deplete the food in 48 hours.

[3] Or several tubes if you need a larger volume. Or a 15 mL Falcon tube.

13 **Centrifuge for 30 seconds**[1]**. Remove supernatant.**

[1] This is for the 1.7 mL Eppendorf tube, in a table-top centrifuge. If you use a 15 mL Falcon tube, centrifuge for 3 minutes at 1500 g.

14 **Add ~1.3 mL of M9. Centrifuge for 30 seconds, and remove supernatant leaving ~0.1 mL.**

15 **Using a triton-coated tip, resuspend the worms and transfer them to NGM+OP50 plates**[1]**.**

[1] Place the worms outside the bacterial lawn. This is important because the worm suspension also contains debris (dead eggs and remains from the mothers). Worms will not eat this voluntarily, but if it gets mixed with the bacteria they will have no choice. Worms that eat debris usually develop abnormally (they usually look underdeveloped).



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