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 We use this protocol and it's working

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Bleaching a population of *C. elegans* nematode worm in a tube V.2

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ABSTRACT

This is the *C. elegans* population bleaching protocol.

MATERIALS

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M9 solution for nematode culture **Contributed by users**

bleaching solution for *C. elegans* **Contributed by users**

NGM plate of gravid (pregnant) adults or with many unhatched eggs **Contributed by users**

Leica L2 binocular microscope **Contributed by users**

SAFETY WARNINGS

Wear a lab coat, gloves and goggles when you handle bleach. Avoid wearing gloves next to a flame.

1. Use *C. elegans* plates that have many gravid hermaphrodites (only the eggs will resist to the bleach treatment). Wash the plates with M9 solution: pipet M9 solution across the plate several

times to loosen worms and eggs that are stuck in the bacteria. Collect the M9+worms solution in a 15 ml Falcon tube. Top up the volume to 14 ml M9.

Tip: Adult hermaphrodites have a tendency to stick to the walls of plastic pipettes. To avoid this issue when harvesting the worms, you can: (i) add a small amount of detergent (NP40 0.005% or tween) to the M9 used to harvest the worms; or (ii) pre-wet the plastic pipette in M9+detergent; or (iii) use glass pipettes.

Tip: Pipette gently to avoid collecting bits of agar together with the worms.

- 2 2. Wash the animals three times (or until the worm suspension is clear of bacteria) with 14 ml M9. To do so, centrifuge the animals at 2,000 rpm for 2 minutes, discard the supernatant with the vacuum pump, re-suspend the worm pellet in 14 ml M9. After the last wash, do not re-suspend the worm pellet in M9 and proceed to step 3. ☒

- 3 3. Add 5 mL of bleaching solution to the worm pellet and immediately proceed to the next step.

Optional: depending on the number for worms/level of contamination, you can increase the volume of bleach solution from 5 ml to 6 ml. You can also use stronger bleaching solution (3 mL NaOH 10M, 7.5 mL Bleach, 40mL H₂O). The standard conditions should work most of the time, however.

- 4 4. Vortex the tube for 6 minutes. Then quickly check the mixture under a dissecting microscope (through the flacon tube). The worms should be dissolved and only eggs should be visible. If so, immediately proceed to the next step.

Note: If necessary, you can vortex 2 additional minutes. Do not over bleach though! If the eggs stay too long in the bleach solution, they will die

- 5 5. Add some **sterile** M9 to the mixture of dissolved worms/eggs in bleaching solution (up to 14 ml final volume). Spin the tube for 1 minute at 2,000 rpm to pellet the eggs. Discard the supernatant with the vacuum pump and immediately proceed to the next step.☒

- 6 6. Resuspend the egg pellet in 14mL sterile M9. Vortex the mixture for five seconds, holding the tube horizontally. Spin the tube for 1 minute at 2,000 rpm to pellet the eggs. Discard the supernatant with the vacuum pump. Repeat this step three times.☒

- 7 7. After the last wash, resuspend the egg pellet in the desired volume. You can either pipette the eggs onto an NGM plate (use a glass pipette) or leave the eggs in liquid suspension (4 ml of sterile M9 recommended) and place them on the rotating wheel (20°C room) overnight (you will have synchronised L1 on the next day).☒