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

NAME	CATALOG #	VENDOR
M9 solution for nematode culture	View	
bleaching solution for C. elegans	View	
NGM plate of gravid (pregnant) adults or with many unhatched eggs	View	
Leica L2 binocular microscope	View	

SAFETY WARNINGS

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

1 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).