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Egg Prep for Bleach Synchronization (Cabreiro Lab)

Forked from Synchronization via Bleaching (Spot Bleaching) protocol

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In Development This protocol is published without a DOI.

Behavioural Genomics

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SUBMIT TO PLOS ONE

ABSTRACT

Egg prep protocol for bleach synchronisation of *C. elegans.*

PROTOCOL CITATION

Saul Moore 2021. Egg Prep for Bleach Synchronization (Cabreiro Lab). **protocols.io** https://protocols.io/view/egg-prep-for-bleach-synchronization-cabreiro-lab-bsgfnbtn

FORK NOTE

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PARENT PROTOCOLS

In steps of

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MATERIALS TEXT

For Bleach Mix:

350 µl Sodium Hypochlorite [Acros Organics, 10/15% active chlorine, 7681-52-9]

400 µl NaOH [4M]

Prepare worms

1 Wash worms off the plates with a few mL of M9 buffer into a 15mL Falcon tube.

2 Leave the Falcon tube to stand for a while until the worm settle to the bottom in a loose pellet.

3 Remove the supernatant leaving 2mL M9 solution in the tube (with the worms pelleted)

Prepare Bleach Mix

4 Mix together in an Eppendorf tube:

400 µl NaOH [4M]

350 µl Sodium Hypochlorite [Acros Organics, 10/15% active chlorine, 7681-52-9]

Egg Prep - Bleaching

5 Add 350µL bleach mix to the 2mL solution of worms in M9

6 Vortex for 5 min (checking every 30 seconds under a microscope to see if the worms are broken apart and eggs have been released).

7 Once the majority of eggs have been released, quench the solution by topping up the Falcon tube to 14mL with M9.

8 

Centrifuge at 6700 rpm for 2 minutes to pellet the eggs to the bottom.

9 Carefully remove the supernatant using a plastic Pasteur pipette.

10 Top up the Falcon tube to 14mL with M9.

11 

Repeat Steps 8 - 10 three more times, to thoroughly quench the bleach solution.

12 After the final wash, top up to 10mL with M9.

13 Pipette the 10mL solution onto an empty 60mm plate using a glass Pasteur pipette, and incubate at 20°C.

Egg Prep - Pooling L1s

14 The next morning, transfer the newly hatched L1 larvae in 10mL M9 solution to a sterile conical Eppendorf tube using a

glass Pasteur pipette.

15 Centrifuge for 2 minutes at maximum speed (14,000 rpm).

16 Using a glass Pasteur pipette, remove as much supernatant as possible, and then pool all L1 worms together into a single Eppendorf tube.

17 Dispense 3µL of L1 solution on the lid of a 60mm Petri plate, and count under microscope how many worms are in the droplet. Use this to estimate the total worm concentration of the Eppendorf.

18 Using a DISTRIMAN Gilson repetitive pipette, dispense approximately 500 worms onto each plate.
NB: The concentration may be adjusted by re-centrifuging and removing less supernatant with the Pasteur pipette (to dilute the solution if needed), and re-counting the number of worms in 3µL.