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Bleach synchronisation of *C. elegans*

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol is for obtaining synchronised populations of *C. elegans*. Eggs are harvested from gravid hermaphrodites and allow to grow to L1 arrest for 12-120 hours (0.5-5days). L1 larvae are then fed and left to develop to the appropriate developmental stage

Materials

MATERIALS

 Sodium hypochlorite, 5% Chlorine **Fisher Scientific Catalog #419550010**

 1 M Sodium Hydroxide **Merck Millipore (EMD Millipore) Catalog #1091371000**

Safety warnings

- ! Sodium hypochlorite and Sodium hydroxide are skin irritants and can cause severe burns. Wear gloves and goggles, and wash of spillages immediately

Washing

- 1 Wash hermaphrodites off plate with several ml of M9 solution and transfer to 15ml falcon tube (Fisher Scientific-Falcon 352096)
- 2 Fill falcon tube up to 15ml with M9 solution
- 3 Centrifuge for 2 minutes at 1500 rpm (RCF:210, ascending 9; descending 7) – program 1

Program 1 retains the worms as pellets and the bacteria is suspended as the supernatant

The descending is slow as the worm pellet is loose at this stage which we don't want to break
- 4 Remove supernatant using a plastic Pasteur pipette taking care not to disturb pellet
Leave atleast 0.5ml M9 to avoid disturbing the pellet
- 5 Fill the tube with M9 upto 15ml
- 6 Spin program 1
- 7 *Repeat steps 4-6*
- 8 On final wash remove as much supernatant as possible and add M9 upto 4ml

Bleaching

- 9 Add 4ml 2X Bleach solution (From here onwards try to work as quickly as possible to avoid over-exposure of the worms to the bleach)

USE FRESHLY PREPARED BLEACH EVERYTIME

**Note****2X Bleach solution:**

5% Sodium hypochlorite solution - 4ml
Sterile water - 3.5 ml
1M NaOH solution - 2.5 ml
TOTAL - 10 ml

- 10 Vortex on maximum setting for 4 min (no more as this will damage the eggs)

Make sure the vortex forms

After vortexing, top up the tube with M9 till 15ml
- 11 Centrifuge for 2 mins at 2500rpm (RCF:590, ascending 9; descending 7) – program 2

(Always check the program on the centrifuge before using it)
- 12 Remove supernatant by pouring into waste bottle – pellet should be compact and yellow in colour at bottom of falcon, but be careful not to lose
- 13 Add 15ml M9
- 14 Centrifuge at program 2
- 15 Repeat steps 12-14 four more times

The number of washes is crucial here as we need to get rid of all the bleach
- 16 After final wash add 15ml M9 and store eggs/larvae in the falcon on the rotator that is constantly spinning at 20°C, until feeding

Note

L1 arrested larvae can be starved for up to 5 days before refeeding

Feeding

- 17 Centrifuge larvae on program 2 to pellet



18 Remove supernatant with plastic Pasteur pipette

The pellet is loose here so make sure not to disturb it

19 Add 15ml M9, spin to wash

20 On final wash leave 0.5ml M9 in falcon

21 Resuspend the pellet by gently tapping the tube/flicking it

22 Place droplet containing larvae onto seeded plate and allow to grow to desired developmental state (ie. 2 days for L4s, 2.5 days for young adults)

Use glass pipette to place the droplet onto seeded plate, avoid using plastic pipette as larvae will stick to it



Note

Development times at 20°C:

- 2 days for L4s
- 2.5 days for young adults

Note:

- If you feed larvae within 12hrs of bleaching then they develop faster than the longer arrested ones
- It is a good practice to bleach in two tubes in parallel
- If you drop the tube at any point of the process, make sure to transfer the contents into a new tube as the dropped tube may get cracked resulting in loss of worms during centrifugation/vortexing
- Any unused larvae can be topped up with M9 and stored spinning in the rotator to be re-used
- Use clean autoclaved rubber bulbs for the refeeding everytime to avoid contamination
- Put the used bulb in the box labelled 'Used Teets'

Stages	Grown at 20 C from L1	Grown at 25 C from L1
L1 division	11.7hrs	9hrs
Mid L1	16.9hrs	13hrs
First L2 division	22.1hrs	17hrs
Between L2 divisions	23.4hrs	18hrs
Second L2 divisions	24.3hrs	19hrs
Mid L2	29.9hrs	23hrs
L3 division	32.5hrs	25hrs
Mid L3	37.7hrs	29hrs
L4 division	42.9hrs	33hrs
Mid L4	49.4hrs	38hrs
Early adult	55.9hrs	43hrs
Adult	62.4hrs	48hrs

Table of Development times for different temperatures