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Synchronisation of Nematodes Growing on NGM Plates (*various options*)

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We use this protocol and it's working

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Abstract

This protocol describes different methods to achieve a synchronised nematode culture.

Depending on your species, one strategy might work better than the other.

I describe the "classical" bleaching method, a filtering method using cell strainers, and a starvation method.

Guidelines

Your nematode species might need different growth temperatures and you might need to adjust the incubation and growth times given here according to your species.

Some nematode species do not do well with bleaching. You can test lower bleaching concentrations. If bleaching is not possible, you need to change to synchronisation by filtering or starvation.

Depending on your species you might need different mesh size for your cell strainers.

Materials

- NGM agar plates 90 mm diameter seeded with 1.5 to 2 ml OP50 bacteria liquid culture.

Note: Plates must be dry to accommodate the volume of OP50. If they are too wet the OP50 will flow all over the plate and it will take very long to dry.

- E. coli strain for feeding for example OP50; grown over night in LB @ 37°C
- Amcor ParafilmTM M Laboratory Film
- Liquid OP50 culture or other food bacteria for seeding the plates
- L-shaped cell spreaders e.g. Cole-Parmer L-Shaped Cell Spreaders, Sterile, Polypropylene, Individually Wrapped; 100/Cs, Item: UY-04396-52
- Transfer pipets e.g. VWR[®], Transfer Pipettes, Graduated; VWR Catalog Number: 612-6803
- 5M NaOH
- 5% Bleach solution e.g. Reagecon Sodium Hypochlorite 5% w/v, SLS, Cat#: SH5WV1
- Distilled water
- Shaking incubator
- 15 ml conical tubes e.g. CELLSTAR[®], BLUE SCREW CAP, NATURAL, GRADUATED, WRITING AREA, STERILE, 50 PCS./BAG; Item No.: 188271-N
- Vortexer
- Centrifuge for 15 ml tubes e.g. Eppendorf Centrifuge 5910R
- M9 buffer
- 50 ml conical tube e.g. CELLSTAR[®], BLUE SCREW CAP, NATURAL, GRADUATED, WRITING AREA, STERILE, 25 PCS./BAG; Item No.: 227261-N
- Tween 20
- M9 buffer with 0.01% (v/v) Tween 20 (fridge temperature)
- Stereoscope to check the stage of nematodes and the progression of bleaching
- Cell strainers (e.g. 37 µm; size you need might vary for different species) e.g. reversible strainers, STEMCELL Technologies, Catalog # 27215 37 µm Small, 50/Box.
- Cell strainers (e.g. 20 µm for L2 Larvae) e.g. pluriStrainer Mini Cell Strainer, pore size 20 µm, Cat#: 43-10020-50

Safety warnings

- ! ▪ This protocol requires you to wear a lab coat and nitrile gloves.
- Please collect the waste in a suitable container and dispose of the waste in accordance with your local regulations.

Before start

Initial worm plates need to be clean!

If any contamination is seen in the plates perform bleaching or another cleaning method (if clean → **growth plates** can be set up).



Growing nematode plates for synchronisation

1w 3d

- 1 Put an equal number of adult females or hermaphrodites containing embryos on 9 cm NGM plates seeded with food bacteria. I usually put 20 worms on each plate. Prepare the needed number of plates to meet your target worm number. Prepare at least 10 plates or more. If certain plates start showing contamination those plates need to be discarded. Do not use contaminated plates for synchronisation!
- 2 Seal the plates with Parafilm.
Put the plates in a box and incubate the box at the respective growing temperature for the nematode species.
- 3 Let the nematodes grow to the appropriate stage for synchronisation:
Bleaching: The plates have a lot of eggs.
Filtering: You can filter for eggs. Plates should have a lot of eggs.
Starvation: All bacteria is gone and the starved plates show L2 only. It is similar to the filtering method but with L2.

Option 1: Synchronisation by Bleaching

3d 20h 34m

- 4 Chill a centrifuge which can accommodate 50 ml and 15 ml tubes down to 4°C
- 5 Add a small amount of **fridge-chilled** M9 to each plate. Gently swirl to release the worms, and carefully pour into a 50 ml Falcon tube (if you have many plates, you can add M9 to the first plate, pour it into the second plate, and so on. So you do not end up with a large volume.)
Keep the falcon tube on ice to keep the solution cool.
Eggs usually stick to the plate. You can use a sterile plastic plate spreader for removing the eggs and bring them in solution.
- 6 The plate washing can be repeated if a lot of eggs remain on the plate.
- 7 Fill the falcon tube(s) up to 40 ml with additional M9 and invert the falcon tube(s) several times.
- 8 Spin tubes at 2500 rcf for 8 mins at 4°C.
(**Important:** Different species might settle at different g-forces. You might need to go higher with the g-force if you see still a lot of worms floating after the centrifugation.)
- 9 Remove the supernatant with a transfer pipet. Take care not to disturb the worm pellet.

8m



- 10 Fill the falcon tube(s) back up with chilled M9 supplemented with 0.01% Tween.
- 11 Spin tubes @ 2500 rcf for 8 mins @ 4°C. 8m
- 12 Take off the supernatant.
If the volume of worms show more than 2 ml divide the worms in several 15 ml tubes.
- 13 Add a bit of distilled water and transfer to a 15 ml conical tube.
Transfer max. 2 ml worms in one 15 ml tube.
- 14 Add distilled water to a total volume of 5 ml.
- 15 Prepare 1 x Bleaching Mix solution (for 1 × 15 ml tube):
500 µl 5M NaOH
1 ml 5% Bleach
fill up with distilled water to 5 ml
- 16 Add the freshly prepared Bleaching Mix to the 5 ml nematode solution.
- 17 Constantly mix the solution by vortexing. 10m
Check the solution under the stereoscope after 6 min every minute.
The bleaching can be stopped when all the worms have dissolved and only eggs are present.
The maximum time for bleaching is 12 minutes. After this the embryos survival is affected.
- 18 When only eggs are seen, fill up the 15 ml tube with M9 Tween.
- 19 Spin down for 2 min @ 800 rcf. 2m
- 20 Take off supernatant with transfer pipette and wash twice in 14 ml M9 Tween. Centrifuge 2 min @ 800g. 2m
Optional: You can use a 40 µm cell strainer to remove leftover worm carcasses and leftover agar clumps from the plates. Only the eggs will go through.



- 21 Remove the supernatant and add a bit of M9. Then transfer the eggs to a 50 ml conical tube which was pre-washed with M9 Tween.
- 22 Fill the tube up to 15 ml M9 and put the tube in a 25 °C incubator with light shaking. I tape the tube horizontally.
- 23 Leave the tube at least 1.5 days in the incubator @ 25 °C. Do not leave them longer than 2.5 days. 2d
- 24 After all embryos are hatched pour the solution with the worms in a 15 ml conical tube.
- 25 Spin down the arrested L2 larvae for 2 min @ 800 rcf. 2m
- 26 Fill the tube with 14 ml M9 Tween and spin again 2 min @ 800 rcf. Then remove all but 2 ml supernatant and transfer the worms to a 2.0 ml tube. 2m
- 27 To count the worms for setting up the plates make a 100x dilution:
Take 50 µl of the worm solution and mix with 450 µl M9 Tween.
From this 10x dilution take again 50 µl and add 450 µl M9 Tween.
Vortex well and immediately put 3 × 5 µl drops on a slide. Count the worms and calculate the number of worms per µl in the original solution.
Example worm count: 10 / 15 / 12
Average is 12.3 worms
12.3 : 5 (per µl) *100 (dilution) → 246 worms per µl
- 28 Add about 4000 worms on one 9 cm plate seeded with food bacteria.
Depending on the species you can add more worms. The plate should not run out of food until reaching the desired stage.
- 29 Put the plates in a temperature controlled incubator for the appropriate time. 1d 20h
For example for *C. elegans* the worms need 1 day and 20 hours @ 25 °C to reach young adulthood.
You can check the the required time for your strain/species with a small scaled test experiment.

Option 2: Synchronisation by Filtering

3d 20h 22m

- 30 **Filtering eggs:**
The plates must contain a lot of eggs.



- 31 Chill a centrifuge which can accommodate 50 ml and 15 ml tubes down to 4°C.
- 32 Add a small amount of **fridge-chilled** M9 to each plate. Gently swirl to release the worms, and carefully pour into a 50 ml Falcon tube (if you have many plates, you can add M9 to the first plate, pour it into the second plate, and so on. So you do not end up with a large volume.)
Keep the falcon tube on ice to keep the solution cool.
Eggs usually stick to the plate. You can use a sterile plastic plate spreader for removing the eggs and bring them in solution.
- 33 The plate washing can be repeated if a lot of eggs remain on the plate.
- 34 Fill the falcon tube(s) up to 40 ml with additional M9 and invert the falcon tube(s) several times.
- 35 Spin tubes at 2500 rcf for 8 mins at 4°C.
(**Important:** Different species might settle at different g-forces. You might need to go higher with the g-force if you see still a lot of worms floating after the centrifugation.)
- 36 Remove the supernatant with a transfer pipet. Take care not to disturb the worm pellet.
- 37 Fill the falcon tube(s) back up with chilled M9 supplemented with 0.01% Tween.
- 38 Spin tubes @ 2500 rcf for 8 mins @ 4°C. Take off the supernatant.
- 39 Add a bit of M9 with Tween and transfer to a 15 ml conical tube.
- 40 Fill the tube with M9 Tween and invert several times.
- 41 Take a fresh 15 ml tube and add a 37 µm cell strainer. Add a bit of M9 Tween through the strainer.
- 42 Filter the washed worms with eggs through the strainer. If the strainer gets blocked reverse it and wash it out in a different tube.

8m

8m



- 43 The resulting liquid should only contain eggs and smaller larvae. The L4 and adults should be gone.
If you see remaining big worms the filtering should be repeated.
- 44 Take a new 15 ml tube and take a 20 μ l cell strainer. Wash it with M9 tween.
- 45 Filter the egg and small worm suspension through the filter.
The eggs are in the filter for harvest you have to take another 15 ml tube and reverse the filter. Wash it out with M9 Tween.
- 46 Repeat the 20 μ m filtering.
The egg harvest should contain only eggs now. A minor contamination with small larvae is possible with this protocol. Fill the tube with M9 Tween and check under the stereoscope.
- 47 Spin the egg solution for 2 min 800 rcf. 2m
- 48 Remove supernatant and fill the 15 ml tube with M9.
- 49 Transfer the egg solution to a 50 ml conical tube which was pre-washed with M9 Tween.
- 50 Put the tube in a 25 °C incubator with light shaking. I tape the tube horizontally.
- 51 Leave the tube at least 1.5 days in the incubator @ 25 degrees. Do not leave them longer than 2.5 days. 2d
- 52 After all embryos are hatched and growth arrested pour the solution with the worms in a 15 ml conical tube.
- 53 Spin down the arrested L2 larvae for 2 min @ 800 rcf. Remove supernatant. 2m
- 54 Fill the tube with 14 ml M9 Tween and spin again 2 min @ 800 rcf. 2m
Then remove all but 2 ml supernatant and transfer the worms to a 2.0 ml tube.
- 55 To count the worms for setting up the plates make a 100x dilution:
Take 50 μ l of the worm solution and mix with 450 μ l M9 Tween.



From this 10x dilution take again 50 µl and add 450 µl M9 Tween.
Vortex well and immediately put 3 × 5 µl drops on a slide. Count the worms and calculate the number of worms per µl in the original solution.

Example worm count: 10 / 15 / 12

Average is 12.3 worms

12.3 : 5 (per µl) *100 (dilution) → 246 worms per µl

- 56 Add about 4000 worms on one 9 cm plate seeded with food bacteria.
Depending on the species you can add more worms. The plate should not run out of food until reaching the desired stage.

- 57 Put the plates in a temperature controlled incubator for the appropriate time.
For example for *C. elegans* the worms need 1 day and 20 hours @ 25 °C to reach young adulthood.

1d 20h

You can check the the required time for your strain/species with a small scaled test experiment.

Note: The synchronisation might not be perfect using this filtering protocol.

Option 3: Synchronisation by Starvation

2d 0h 14m

- 58 The plates are starved (bacteria is gone) and the plates contain mostly L2 larvae.

- 59 Chill a centrifuge which can accommodate 50 ml and 15 ml tubes down to 4°C.

- 60 Add a small amount of **fridge-chilled** M9 to each plate. Gently swirl to release the worms, and carefully pour into a 50 ml Falcon tube (if you have many plates, you can add M9 to the first plate, pour it into the second plate, and so on. So you do not end up with a large volume.)

Keep the falcon tube on ice to keep the solution cool.

- 61 Fill the falcon tube(s) up to 40 ml with additional M9 and invert the falcon tube(s) several times.

- 62 Spin tubes at 2500 rcf for 8 mins at 4°C.
(**Important:** Different species might settle at different g-forces. You might need to go higher with the g-force if you see still a lot of worms floating after the centrifugation.)

8m

- 63 Remove the supernatant with a transfer pipet. Take care not to disturb the worm pellet.

- 64 Add a bit of M9 with Tween and transfer to a 15 ml conical tube.



- 65 Fill the tube with M9 Tween and invert several times.
- 66 Take a fresh 15 ml tube and add a 20 μ m cell strainer. Add a bit of M9 Tween through the strainer.
- 67 Filter the small worm suspension through the filter.
- 68 Repeat the filtering step.
The solution should contain only L2 larvae now.
- 69 Spin the worm solution for 2 min 800 rcf. Remove supernatant. 2m
- 70 Fill the tube with M9 Tween and centrifuge again. Remove supernatant.
- 71 Add a small volume of M9 and transfer the worms in a 50 ml conical tube. Fill the tube up to 15 ml total volume with M9 buffer.
- 72 Put the tube in a 25 °C incubator with light shaking. I tape the tube horizontally.
- 73 Leave the tube at least 1 day in the incubator @ 25 degrees. Do not leave them longer than 2 days. 1d
- 74 After L2 worms are arrested in growth pour the solution with the worms in a 15 ml conical tube.
- 75 Spin down the arrested L2 larvae for 2 min @ 800 rcf. Remove supernatant. 2m
- 76 Fill the tube with 14 ml M9 Tween and spin again 2 min @ 800 rcf.
Then remove all but 2 ml supernatant and transfer the worms to a 2.0 ml tube. 2m
- 77 To count the worms for setting up the plates make a 100x dilution:
Take 50 μ l of the worm solution and mix with 450 μ l M9 Tween.
From this 10x dilution take again 50 μ l and add 450 μ l M9 Tween.



Vortex well and immediately put $3 \times 5 \mu\text{l}$ drops on a slide. Count the worms and calculate the number of worms per μl in the original solution.

Example worm count: 10 / 15 / 12

Average is 12.3 worms

$12.3 : 5 \text{ (per } \mu\text{l}) * 100 \text{ (dilution)} \rightarrow 246 \text{ worms per } \mu\text{l}$

- 78 Add about 4000 worms on one 9 cm plate seeded with food bacteria. Depending on the species you can add more worms. The plate should not run out of food until reaching the desired stage.
- 79 Put the plates in a temperature controlled incubator for the appropriate time. This needs to be tested before. You can check the the required time for your strain/species with a small scaled test experiment.

1d