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Synchronized C. elegans culture on NGM plates for FACS isolation of single cells

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We use this protocol and it's working

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Abstract

This protocol details the steps necessary to scale-up and synchronize *C. elegans* cultures for FACS isolation of embryonic cells, expressing germ layer nuclear markers. We cultured worms with agar-based NGM plates to reduce any confounding effects that may be introduced by large scale liquid culture. This protocol utilizes one round of mixed-stage culture growth followed by one round of synchronized growth. After scale-up and synchronization, this protocol provides details for culture conditions necessary for FACS of embryonic cells.

Materials

Strains:
- OP50 E. coli
- FACS control *C. elegans* strain, i.e. N2
- FACS sorting *C. elegans* strain, i.e. OD1719

Reagents:
- LB Broth Mix (Genesee 11-120)
- M9 buffer
  - 3 g KH_2PO_4 (Sigma-Aldrich P0662)
  - 6 g Na_2HPO_4 (Thermo Fisher S373)
  - 5 g NaCl (Sigma-Aldrich S9888)
  - 1 ml 1 M $MgSO_4$ (Sigma-Aldrich 208094)
  - H_2O to 1 liter
- NGM plates
  - (complete protocol: http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html#d0e214)
  - 3g NaCl (Sigma-Aldrich S9888)
  - 17g agar (Genesee 20-249)
  - 2.5g peptone (VWR 89406-350)
  - 975g H_2O (sterile and deionized)
  - 1ml 1M $CaCl_2$ (Sigma-Aldrich C3306)
  - 1ml 5mg/ml cholesterol (Fisher 501848291)
  - 1ml 1M $MgSO_4$ (Sigma-Aldrich 208094)
  - 25ml 1M KPO_4 Buffer pH 6.0 (108.3 g KH_2PO_4, 35.6 g K_2HPO_4, H_2O to 1 litre) (Sigma-Aldrich P0662, P3786
- Bleaching solution
  - Sodium Hypochlorite Solution, 6% available chlorine (Ricca Chemical, 7495.7-32)
  - 5N NaOH (Fisher S318-100)

Consumables:
- 100 mm petri dishes "large plates"
- 15 ml centrifuge tubes (Peak PS-695)
- 5 ml centrifuge tubes

Equipment:
- Swinging bucket rotor refrigerated centrifuge (Eppendorf 5810R)
- Pipet-Aid (VWR 89166-464)
- 20°C incubator (Caron 7001-28-1)

Prepare OP50 seeded NGM plates

1 Using sterile technique, pick an OP50 colony and inoculate a 250 ml bottle of sterile LB.

2 Incubate OP50 liquid culture at 37°C overnight

3 Make Nematode Growth Media (NGM) with 100 mm petri dish (15 plates per sample, 1 liter = 40 plates) **(hereafter referred to as "large plates")**. See the following WormBook page for **NGM protocol**.

4 Allow plates to dry 72h

5 Seed each NGM plate with 1 ml OP50 liquid culture.

6 Cover as much agar surface as possible by moving the plate in first a circular pattern, then a figure 8 pattern.

7 Dry the OP50 seeded plates at room temperature with the lids on for three days until there is no more excess liquid

Grow mixed stage cultures of cell sorting strain

8 Identify a 60 mm petri plate culture of the sorting strain that has recently exhausted the E. coli lawn

9 Chunk the plate into 6 equal pieces

10 Transfer 3-4 chunks to 3-4 fresh large NGM OP50 plates with the worm-covered surface facing down

11 Place sorting strain cultures in 20°C incubator for 48 hours, until the E. coli lawn is exhausted 4d

First embryo synchronization with hypochlorite solution

12 Harvest mixed-stage sorting strain worms from all 3-4 plates by washing each plate individually with ~5 ml of M9 — 1h

12.1 Transfer mixed stage worm suspension to a 15 ml conical centrifuge tube

12.2 Pellet the worms by centrifuging for 1 minute at 1,000 rcf

12.3 Aspirate the supernatant

12.4 Harvest worms from other plates as outline above

12.5 Resuspend the worm pellet with worm suspension from the newly washed plate

12.6 Repeat this process until worms have been harvested from all 3-4 plates into a single 15ml tube

13 Once all plates have been harvested, wash the worm pellet with fresh M9 to remove excess E. coli by pelleting and resuspending in fresh M9. The final worm pellet yield should be ~0.5 ml.

14 Once the worm suspension is free of E. coli, centrifuge again and remove all M9 supernatant from the worm pellet

15 Resuspend the worm pellet in 8 ml of H2O

16 Add 0.9 ml of Sodium Hypochlorite Solution (Ricca Chemical, 7495.7-32) and 1.44 ml of 5N NaOH to the worm suspension

17 Resuspend the worm pellet with brief vigorous vortexing

18 Incubate at room temperature for 6 to 8 minutes. While incubating shake the tube or place on a nutator. The time to bleach the worms depends on the worm pellet volume,

with larger worm pellets taking longer. Do not incubate for longer than 8 minutes.

19 Monitor the progression of the hypochlorite treatment. Larval worms should dissolve, adult worms will begin to break at the vulva and release embryos. I typically monitor the treatment by looking through the tube under a dissection microscope.

20 Once the worms are sufficiently dissolved, centrifuge the tube for 30 seconds at 2,000 rcf

21 Decant the supernatant and wash the embryo pellet by adding 10 ml of M9 to quench the hypochlorite treatment

22 Wash the bleached embryos a second time. Centrifuge the tube for 30 seconds at 2,000 rcf to pellet the embryo suspension. Decant the supernatant and resuspend the embryo pellet in 10 ml of M9.

23 Wash the bleached embryos a third time. Centrifuge the tube for 30 seconds at 2,000 rcf to pellet the embryo suspension. Decant the supernatant and resuspend the embryo pellet in 3 ml of M9.

24 Measure the approximate concentration of embryos in suspension

24.1 Shake or vortex the tube to ensure the embryos are evenly distributed in the suspension

24.2 Aspirate 2 ul of embryo suspension with a p10 pipette. Pipette the embryo suspension up and down at least four times before moving on.

24.3 Dispense the embryo suspension on a clean microscope slide/plastic plate and add ~5 ul M9

24.4 With a cell counter, count the number of embryos on the slide under a dissection microscope. Dilute the embryo suspension if there are too many to count.

25 Seed 10 large NGM/OP50 plates with 3,000 embryos. Incubate at 20°C for approximately 72 hours until worms are gravid.

26 At the beginning of the next day, chunk one recently starved 60 mm N2 plate to a fresh large NGM OP50 plate. This key step will serve as the negative GFP control for cell sorting.

Second embryo synchronization with hypochlorite solution

27 Harvest both synchronized sorting strain worms and mixed stage N2 worms in parallel by washing individual plates with ~5 ml of M9 and collecting in two separate 15 ml conical centrifuge tubes

28 Pellet the worms by centrifuging for 1 minute at 1,000 rcf

29 Discard the supernatant

30 Resuspend the worm pellet with worm suspension from another large plate

31 Repeat this process until worms have been harvested from all 10 plates for the sorting strain and 1 N2 plate

32 Once all plates have been harvested, wash the worm pellet once with fresh M9 to remove excess E. coli. The final worm pellet yield for the sorting strain should be ~1 ml.

33 Once the worm suspension is free of E. coli, centrifuge again and remove all M9 supernatant from the worm pellet

34 Resuspend the worm pellet in 8 ml of H2O

35 Add 0.9 ml of Sodium Hypochlorite Solution (Ricca Chemical, 7495.7-32) and 1.44 ml of 5N NaOH to the worm suspension

36 Resuspend the worm pellet with brief vigorous vortexing

37 Incubate at room temperature for 6 to 8 minutes. While incubating shake the tube or place on a nutator. The time to bleach the worms depends on the worm pellet volume, with larger worm pellets taking longer. Do not incubate for longer than 8 minutes.

38 Monitor the progression of the hypochlorite treatment. Larval worms should dissolve, adult worms will begin to break at the vulva and release embryos. I typically monitor the treatment by looking through the tube under a dissection microscope.

39 Once the worms are sufficiently dissolved, centrifuge the tube for 30 seconds at 2,000 rcf

40 Decant the supernatant and resuspend the embryo pellet in 10 ml of M9 to quench the hypochlorite treatment

41 Wash the bleached embryos a second time. Centrifuge the tube for 30 seconds at 2,000 rcf to pellet the embryo suspension. Decant the supernatant and resuspend the embryo pellet in 10 ml of M9.

42 Wash the bleached embryos a third time. Centrifuge the tube for 30 seconds at 2,000 rcf to pellet the embryo suspension. Decant the supernatant and resuspend the embryo pellet in 3 ml of M9.

43 The final embryo yield should be approximately 0.01 ml for the wildtype N2 stain and 0.2ml for the fluorescent sorting strain.

44 Incubate on a rotator ~3 hours to allow the mesoderm marker to express. For embryo stage FACS experiments, move on to the **embryo dissociation protocol**