Synchronized Growth of Worms in Liquid Culture

(large scale for whole cell extract preparation)

Reagents Needed:

S-basal medium complete	0.1M NaCl
M9 (common stock)	NaOH/bleach solution
60% sucrose solution	ddH_2O

Procedure:

I. Starting an unsynchronized liquid culture

1. Combine 250 mL S-basal *complete* medium with OP50 from a 750 mL culture (spun down and resuspended in 20 mL S-basal medium- can be stored at 4°C).
2. Rinse 6 large plates of starved worms (mainly L1s) with 10 mL of M9.
3. Collect the worms in a 50 mL falcon tube, spin at 600xg for 3 minutes and wash once with M9.
4. Resuspend in 10 mL of M9 and add to the prepared 250 mL culture of complete S-basal medium and OP50.
5. Shake the inoculated cultures at 20°C at 200 rpm.
6. Follow the growth of the worms:
    a. Take a 1 mL sample everyday.
    b. Spin down at 600 rpm.
    c. Resuspend the pellet in 50 mL of M9.
    d. Spot a sample on a microscope glass slide and check under a dissecting microscope.

II. Harvesting worms to isolate embryos for starting a synchronized culture

When the majority of the worms are adults with embryos (approximately day 3).

1. Put the flask on ice for one hour and let the worms settle.
2. Aspirate off the media.
3. Transfer the brown slurry to a 50 mL falcon tube.
4. Add ice cold M9 to 50 mL.
5. Spin down at 600xg for 3 minutes.
6. Aspirate off the M9 and repeat steps 4 and 5.
7. Resuspend the worms in 25 mL of ice cold M9 and add 25 mL of ice cold 60% (w/v) sucrose.
8. Mix and spin immediately at 1500xg for 5 minutes. The adult worms should form a brown film on top of the tube.
9. Collect the worms with a 25 mL pipet and transfer them to a new 50 mL tube.
10. Add ice cold M9 to bring it to a volume of 50 mL.
11. Pellet the worms and aspirate off the supernatant.
12. Add 25 mL ice cold 0.1M NaCl.
13. Let worms settle for 5 minutes.
14. Aspirate off the supernatant.

15. Add ice cold 0.1M NaCl up to a volume of 30 mL.
16. Mix 5 mL 5M NaOH with 10 mL bleach in a 15 mL tube.
17. Immediately add the NaOH/bleach solution to the 30 mL worm suspension.
18. Vortex for 5 seconds, let it stand at room temperature for 2 minutes and vortex again.
19. Repeat step 18 four times for a total bleaching time of 10-15 minutes. Follow the progress by examining samples under the dissecting microscope. Stop bleaching when only embryos remain.
20. Immediately centrifuge at 800xg for 1 minute.
21. Aspirate off the supernatant.
22. Add sterile water to a total volume of 50 mL.
23. Centrifuge at 700xg for 2 minutes.
24. Repeat step 23.
25. Add 10 mL of M9 to resuspend the worm pellet and transfer the worm solution to a 500 mL flask.
26. Shake the flask at 22 °C for 18-20 hours to allow the embryos to hatch.

III. Seeding second round with synchronized L1 worms

1. Prepare S-basal medium and OP50 as in part I, step 1.
2. Transfer the worm solution to a 50 mL falcon tube and chill them on ice for 5 minutes.
3. Spin at 600xg for 3 minutes.
4. Aspirate off the supernatant.
5. Repeat steps 2-4.
6. Add 5 mL of sterile M9 to resuspend worms.
7. Add them to the 250 mL S-basal medium/OP50 culture.
8. Let the worms grow at 20°C at 200 rpm until desired growth stage (examine growth every day under the dissecting microscope as in part I, step 6).
9. Harvest worms as described in part II, steps 1-11.
10. Flash-freeze worm pellet with liquid nitrogen and store it at -80°C.

Recipes:

M9 (1L)
* Common lab stock in worm room.
5.8g $Na_2HPO_4 \cdot 7H_2O$
3.0g KH_2PO_4
5.0g NaCl
0.25g $MgSO_4 \cdot 7H_2O$
ddH_2O to 1L
• Filter (0.22µm) and bottle.

S-Basal Medium Complete (~100mL)
To 100mL of S-Basal Medium add:
300µL 1M $MgSO_4$
300µL 1M $CaCl_2$
1mL 100X trace metal solution
1mL 1M potassium citrate (pH 6)
• Use sterile technique. Do not autoclave.

S-Basal Medium (1L)
5.9g NaCl
50mL 1M KPO_4 (pH 6.0)
1.0mL cholesterol (5mg/mL in ethanol)
ddH_2O to 1L
• Bottle and autoclave.

Trace Metals Solution (500mL)
0.346g $FeSO_4$•$7H_2O$
0.93g Na_2EDTA
0.098g $MnCl_2$•$4H_2O$
0.012g $CuSO_4$•$5H_2O$
ddH_2O to 500mL
• Bottle and autoclave.
• Store in the dark

NaOH/Bleach Solution (15mL)
5mL 5M NaOH
10mL bleach (non-germicidal)

Reference:

Based on the protocol from Cold Spring Harbor Laboratories *C. elegans* Course.
Compiled by Janine Kirstein, 2008.