

## 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 <sub>2</sub> O   |

### 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<sub>2</sub>HPO<sub>4</sub>•7H<sub>2</sub>O

3.0g KH<sub>2</sub>PO<sub>4</sub>

5.0g NaCl

0.25g MgSO<sub>4</sub>•7H<sub>2</sub>O

ddH<sub>2</sub>O to 1L

• Filter (0.22µm) and bottle.

#### S-Basal Medium Complete (~100mL)

*To 100mL of S-Basal Medium add:*

300µL 1M MgSO<sub>4</sub>

300µL 1M CaCl<sub>2</sub>

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<sub>4</sub> (pH 6.0)

1.0mL cholesterol (5mg/mL in ethanol)

ddH<sub>2</sub>O to 1L

- Bottle and autoclave.

Trace Metals Solution (500mL)

0.346g FeSO<sub>4</sub>•7H<sub>2</sub>O

0.93g Na<sub>2</sub>EDTA

0.098g MnCl<sub>2</sub>•4H<sub>2</sub>O

0.012g CuSO<sub>4</sub>•5H<sub>2</sub>O

ddH<sub>2</sub>O 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.

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