Agar plates

Steps:

1. Grow *Pristionchus pacificus* ≥ 3 healthy generations on agar plates spotted with OP50 bacteria.

2. Transfer a single young adult to a new 60 mm agar plate, and grow for 5-6 days, then transfer 20 young adults to n_1 x 10 cm plates (starter plates).

3. Grow for 5-6 days, then transfer 20 young adults to n_2 x 10 cm plates (prep. plates).

4. Five days later, wash worms with M9 into 50 ml conical tubes, record volume and synchronize with 30% NaOH/bleach (0.5 ml NaOH,1 ml bleach/ 3.5 ml washed worms; 20 plates yields ~ 30 ml volume) for 10 minutes. Filter carcasses by applying through a 140 µM nylon net (milipore), then pellet 500 x g, 1 minute, room temperature. Wash eggs-J1 with 5 ml M9, and re-centrifuge. Remove M9 and re-suspend in n_2 x 100 µl M9. Aliquot 100 µl egg-J1 to n_2 new 10 cm plates, and grow 22° C for 20-22 hours for J2, 48 hours for J3, 72 hours for J4, and 86 hours for J4-non gravid adults.

5. Harvest by washing into 50 ml conical tubes, then apply to 5 µM vacuum filtration system to remove bacteria. Wash with 2 x 50 ml applications of M9, then wash worms off of filter into a new 50 ml conical. If collecting J2-3, wash with 1.5 ml and transfer to a 1.5 ml tube, then centrifuge max speed, 4° C, 15 minutes. J4 and young adults can simply be set at room temperature for 5 minutes to settle before removing supernatant. If necessary, one can remove eggs from J4-young adults by repeating this step several times, as the adults are more dense than eggs, and will selectively pellet while eggs remain in the supernatant. Once pure, transfer adults to a 1.5 ml tube and briefly pellet with a table-top centrifuge, and remove supernatant. Record packed-worm volume(s), and flash-freeze in liquid N2 and store -80° C.

Liquid Culture

Notes:

- The following is for preparation of multiple 10 ml liquid cultures. I find their synchronization to be very similar to agar, and the amount of adults per 10 ml culture at the end of 5 days is ~ 5,000.
- You can add Nystatin as an anti-fungal agent at a concentration of 20 µg/ml to each 10 ml culture.

Steps:

1. Prepare 100 ml of OP50 per *n* = number of gravid 10 cm plates, or 3 x 60 mm plates. Dilute 1 ml of OP50 liquid culture (<7 days old) into 4 ml LB and obtain OD_{600}. This (1:5) dilution should be approximately 0.1 (and thus the undiluted is 0.5). If it is, proceed to centrifuge 100 ml x the number of plates. If not, back calculate and dilute bacterial culture with water to obtain 1:5 dilution that = 0.1.

2. Centrifuge with a Sorval SLA-3000 rotor (200 ml per plastic tube) 30 minutes, 4°, 4,250 rpm/3,050 x g.

3. Decant media, and re-suspend bacterial pellets in 9 x *n* ml sterile S-medium. Combine, and aliquot 9 ml each to 250 ml sterile Erlenmeyer flasks.

4. Bleach 3 x 0.6 cm plates, or 1 x 10 cm plates (see method above), wash eggs-J1 in water or M9 (500 x g, 30-60 seconds), and re-suspend in 1 x *n* ml S-medium. It is important not to wash worms with S-medium before or after the bleach because it will start to precipitate.

5. Aliquot 1 ml to each 250 ml Erlenmeyer flask. Incubate 180 rpm, 20-22 ° C.

6. To harvest, apply liquid to 5 µM sterile filter apparatus, and wash 2 x with 50 ml M9. Wash filter with 1.5 ml fresh M9 into a new 50 ml conical. If bacteria are still observed, repeat 5 µM filtration. Proceed with collecting worm pellets as described above in “agar plates” protocol.