Experimental Protocols

Last update on 10/23/2013

- **Small scale bleaching protocol**
  - Objective

    To synchronize worms at L1 stage using a 1.5 mL centrifuge tube
  - Procedure
    1. Before beginning, check plates for gravid adults and eggs
       * must be at this stage to bleach
    2. Add 1 mL M9 buffer to each well in a plate
    3. Wash plate with Pasteur pipette
    4. Transfer worm solution to 1.5 mL Ependorf centrifuge tube
    5. Centrifuge at 3000 rpm for 30 seconds
    6. Aspirate to 0.3 ml
    7. Add 500 μL of 1M NaOH
    8. Add 200 μL of bleach directly from bottle
    9. Vortex immediately
       * Vortex every minute until worms crack open and begin to dissolve
    10. Centrifuge at 300 rpm for 30 seconds
    11. Aspirate above pellet
    12. QS to 1 mL
    13. Vortex
    14. Centrifuge at 3000 rpm for 30 seconds
    15. Repeat steps 10-13 three times
    16. Aspirate to 0.2 mL
    17. Add 2 mL of M9 buffer to one well of the sterile 6-well plate
    18. Add 2 μL of 5 mg/mL cholesterol to well
        * Do not flame cholesterol
    19. Use New Pasteur pipette to mix and transfer worm solution to well
        * Do not allow worm solution to go above neck of Pasteur pipette
    20. Incubate at 20°C overnight

◆ FUdR Dosing

- Objective

  FUdR dosing prevents eggs from hatching, allowing the synchronization of the life cycle of the worm population to be maintained.

- Procedure

1. Calculate the amount of FUdR and deionized water needed for the experiment.
   - Desired concentration is 25 μmol FUdR/L agar
   - If using a 10 g/L FUdR stock solution, this corresponds to 3.69 μL for 6 mL total volume (the volume of a well in a 6-well plate)
   - For reference, this calculation is how the 3.69 μL is obtained:
     (25 μmol/L)(6 mL)(1 L/10^3 mL)(1 mol/10^6 μmol)(246.2 g/mol)(1 L/10 g) (10^6 μL/L) = 3.69 μL 10 g/L FUdR per 6 mL
   - To prepare diluted FUdR solution:
     3.69 μL of 10 g/L FUdR + 96.31 μL water → 100 μL FUdR solution for a single dosage in a well.
2. Calculate the number of wells needed for the experiment with some extra
3. Prepare diluted FUdR solution for dosing multiple wells
4. Dose 100 μL onto each well in a 6-well plate at L4

◆ Lifespan assay

Day 0
- Conduct the small scale bleaching protocol to isolate eggs from gravid worms
- Incubate the eggs overnight at 20°C in M9 solution containing cholesterol

Day 1
- Concentrate hatched L1 using a centrifuge
- Transfer ~70 worms into each well in a 6-well NGM plated seeded with OP50
- Incubate the worms at 20°C

Day 3 (worms at L4)
- Dosed the plate with FUdR at 25 μmol/L agar to prevent eggs from hatching

Day 4 ~
- Scan plates every day

◆ Locomotion assay with food

This assay was conducted in conjunction with the lifespan assay.

Day 0

- Conduct the small scale bleaching protocol to isolate eggs from gravid worms
- Incubate the eggs overnight at 20°C in M9 solution containing cholesterol

Day 1

- Concentrate hatched L1 using a centrifuge
- Transfer ~70 worms into each well in a 6-well NGM plated seeded with OP50
- Incubate the worms at 20°C

Day 3 (worms at L4)

- Dosed the plate with FUdR at 25 μmol/L agar to prevent eggs from hatching

Day 4 (worms at 1 day of adulthood)

- Take videos for 30 seconds for each well

◆ **Locomotion assay without food**

Day 0

- Conduct the small scale bleaching protocol to isolate eggs from gravid worms
- Incubate the eggs overnight at 20°C in M9 solution containing cholesterol

Day 1

- Concentrate hatched L1 using a centrifuge
- Transfer 100 worms into each well in a 6-well NGM plated seeded with OP50
- Incubate the worms at 20°C

Day 4 (worms at 1 day of adulthood)

- Rinse the worms with cold S-basal solution three times and then transfer to unseeded 6-well NGM plates
- Wait approximately 20 minutes
- Take videos for 30 seconds for each well

◆ **Body size assay**

Day 0

- Conduct the small scale bleaching protocol to isolate eggs from gravid worms
- Incubate the eggs overnight at 20°C in M9 solution containing cholesterol

Day 1

- Concentrate hatched L1 using a centrifuge
- Transfer 100 worms into each well in a 6-well NGM plated seeded with OP50

- Incubate the worms at 20°C

Day 4 (worms at 1 day of adulthood)

- Collect worms from each well with M9 solution and transfer to unseeded 6-well plates
- Kill the worms by adding 20 μL of 1 M sodium azide into each well
- Scan the plates

◆ **Egg laying experiment**

Day 0

- Conduct the small scale bleaching protocol to isolate eggs from gravid worms
- Incubate the eggs overnight at 20°C in M9 solution containing cholesterol

Day 1

- Concentrate hatched L1 using a centrifuge
- Transfer 100 worms into each well in a 6-well NGM plated seeded with OP50
- Incubate the worms at 20°C

Day 3

- Incubate *E. coli* OP50 bacteria overnight

Day 4 (worms at 1 day of adulthood)

- Prepare seeded 24-well plates prior to the egg laying experiment
  1. Dry 24-well NGM plates for 10 min in a fume hood
  2. Drop 5 μL OP50 on each well
  3. Dry the plate for 5 min in a fume hood
- Conduct the egg laying experiment using worms at 28 hr from L4 larval stage
  1. Drop 700 μL M9 onto a well in a 6-well plate and transfer worms into a centrifuge tube
  2. Leave the tube for a while in an ice box until worms are precipitated
  3. Aspirate up to 100 μL
  4. Vortex the tube and take 10 μL worm solution from the tube and drop ~10 worms onto a well in a 24-well plate seeded with OP50
  5. Incubate at 20°C for 90 min
  6. Drop 15 μL sodium azide on a well to kill worms and dry it for a while. Eight multiple wells were used for each strain.
  7. Store the plate at 4°C if you want to scan the plate later
  8. Scan the plate