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Pharyngeal Pumping Assay

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Behavioural Genomics

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The pharynx is a is a neuromuscular pump found at the anterior end of the alimentary tract, consisting of 20 muscles and 20 neurons. A proper feeding rate in worms is coordinated by the precise timing of pharyngeal movements, with one complete cycle of synchronous contraction and relaxation of the corpus and terminal bulb termed a “pump”. A simple way to measure *C. elegans* feeding is to count how many times worms pump in a minute (pumps per minute). Movement of the grinder (in the terminal bulb) can easily be observed using a stereomicroscope, and because cycles of contraction/relaxation are synchronised along the pharynx, pumps per minute can be measured simply by counting grinder movements.

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Consumables per worm strain:

- 4 x 90 mm petri dish
- 5 x 60 mm petri dish

Reagents for bleaching:

- Sodium hypochlorite, 5% Chlorine (Fisher Scientific, Catalog #419550010)
- 1 M Sodium Hydroxide (Merck Millipore, Catalog #1091371000)

Reagents for preparing NGM agar:

A	B	C
Pre-autoclave	For 1L:	For 500mL:
Sodium chloride	3g	1.5g
BioAgar	17g	8.5g
Bacto Peptone	2.5g	1.25g
Sterile water	975mL	487.5mL

A	B	C
Post-autoclave	Fo 1L:	For: 500mL
5mg/mL cholesterol (store at 4°C and away from light)	1mL	500uL
1M CaCl2	1mL	500uL
1M MgSO4	1mL	500uL
1M KPO4	25mL	12.5mL

Pick L4 worms for bleaching (Day 1)

1 Using an eyelash pick, pick 10 x L4s of each worm strain to be assayed onto 2 x 90 mm plates (pre-seeded with OP50) and incubate at **20 °C**

Pour 60 mm NGM agar plates (Day 2)

2 Prepare and pour the following number of NGM plates per worm strain to be studied:

- 5 x 60 mm (**15 mL** agar per plate)
- 2 x 90 mm (**35 mL** agar per plate)

Therefore make **150 mL** NGM per strain

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To ensure accuracy when weighing out media components it is recommended that you do not prepare agar in volumes less than 250 mL at a time!

2.1 Prepare NGM agar and autoclave

Protocol for preparing NGM: dx.doi.org/10.17504/protocols.io.bvh7n39n

2.2 In the laminar flow hood, pour 5 x 60 mm and 2 x 90 mm plates per worm strain

Plate Pouring
by Priota Islam,
Imperial College London
PREVIEW RUN

2.3 Once plates have dried, store agar-side up in an airtight container in the cold room

Dry/Seed 90 mm plates and bleach worms (Day 5)

3 Dry 2 x 90 mm plates per worm strain in the cabinet dryer (setting 2) for 1.5 hours

3.1 Seed the 90 mm plates with OP50 and leave to dry overnight at room temperature

4 Bleach synchronise worms (picked in Step 1) and store eggs/larvae in a 15 mL falcon tube on the rotator that is constantly spinning at **20 °C** until feeding

Bleach synchronisation of C. elegans
by Ida Barlow
PREVIEW RUN

Refeed L1s (Day 8)

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5 Refeed bleach synchronised L1s

5.1 At 16:50, spin L1s bleached in step 4 using centrifuge program 1 (2500 rpm, 2 mins)

5.2 Using a plastic pipette remove the supernatent, taking care to not disturb the worm pellet, and leave ~1 mL of M9 in the tube

5.3 Gently flick the falcon tube to resuspend the L1s

5.4 Using a glass pipette, drop 1 small droplet around the edges (off food) of the pre-seeded 90 mm plates (prepared in Step 3)

It is recommended that you check the density of worms in the droplet down a microscope and add another if necessary

5.5 Place plates agar-side down in the **20 °C** incubator to allow droplets to dry (~5-10 mins), then invert plates and allow to grow for 2.5 days

Dry and seed 60 mm plates (Day 10)

6 In the morning, take the 60 mm plates from the cold room, and weigh three random plates without their lids

7 Dry plates in in cabinet dryer (setting 2) with lids off until they have lost 3-5% of their weight (approx 2 hours)

8 Aseptically prepare 1:10 dilution of OP50 in M9 in a 15 mL falcon tube:

1 mL OP50

9 mL M9

9 Working by a flame, dispense **30 µL** of the dilute OP50 into the centre of each 60 mm plate

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and allow droplets to dry at room temp

Ensure that you do not touch the agar with the pipette tip and that plates are kept on a flat surface so you get a nice, even circle of food once dried

10 Once the food has dried, leave plates agar-side up at room temp overnight

Assaying rate of pharyngeal pumping (Day 11)

11 Label 5 x 60 mm plates seeded with **30 µL** OP50 (around the edge, so not to obscure the bacterial lawn) per worm strain

12 At 09:00, use an eyelash-pick to transfer 10 x young adult worms (prepared in Step 5) onto the edge of each 60 mm plate (away from food)

This yields a total of 50 worms per strain

13 Incubate plates, agar-side up, at **20 °C** for 1 hour

This allows worms to crawl to the bacterial lawn and start foraging again

14 Working one plate at a time, place a 60 mm plate upon a dissecting stereo microscope at the highest magnification possible and bring the worms/bacterial lawn to focus

14.1 Working on a single worm at a time, identify the grinder (in the terminal bulb of the head) of each worm and ensure this is clearly in focus

See: http://www.wormbook.org/chapters/www_measurepharyngeal/measurepharyngeal.html for detailed images/diagrams of pharynx and terminal bulb

14.2 Use a tally counter to count the grinder movements of a worm over a 20 second period

Repeat this 3 times per worm and record the result as an average of the three counts

14.3 Go clockwise around the bacterial lawn, and repeat the steps above for each worm on the 60 mm plate. Then repeat for each 60 mm plate remaining in the incubator

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15 Calculate pharyngeal pumps per minute of each strain:

pumps per minute = pumps per 20 seconds * 3

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