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Soil bacteria

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Behavioural Genomics

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

The behaviour of C. elegans is dependent on its microbial environment. This protocol describes how to utilise the Tierpsy Tracking software to extract the unique behaviours of C. elegans upon being subjected to 96 different soil bacteria.

We expect varied behaviours driven by the soil bacteria identity.

Materials

Microbes

Soil bacteria
E. coli (OP50)

Materials

100 mm petri dishes
150 mm petri dishes
500 mL bottle
100 mL bottle
15 mL falcon tubes
Pasteur pipettes
Glass pipettes
Round-bottom 96-well plates
Square, flat-bottom 96-well plates
96-peg replicator
Breathe-Easy sealing membrane for 96-well plates
Aluminium plate sealing film for 96-well plates
Ice
Glass slides

Reagents

Pre- & post autoclave reagents for making Nematode Growth Medium (NGM).
M9 medium
5% Sodium hypochlorite
Sterile water
1M NaOH solution
LB broth
LB agar
99% glycerol

Equipment

Integra ViaFill dispenser
Integra ViaFill dispenser tubes (Large)
Integra ViaFlo 96
Integra ViaFlo 96 12.5 µL GRIPTIP pipette tips
15 mL falcon tube benchtop spinner
Scale
Bunsen burner (or Class II Microbiological Safety cabinet)
Autoclave
Tecan Spark Multimode Microplate Reader
LoopBio Motif - Video Recording System (6×12 megapixel camera array setup)

Before start

Ensure you grow enough worms to fill the volume required in the bottle to dispense the worms using the Integra ViaFill.

Day -10. Prepare worm population

20m

1 Pick 10 L4 worms onto 5× 100 mm normal NGM petri dishes seeded with 1500 mL of OP50 (OD = 1.0).
Put the petri dishes in the 20°C incubator to grow the worm population for bleaching (Day -6). 20m

Day -08. Prepare 96-well plates with normal NGM

4h

2 Prepare cholesterol for use in making normal Nematode Growth Medium (NGM) following the protocol below: 40m

Protocol

NAME

Preparing Cholesterol for use in NGM

CREATED BY

Bonnie Evans

Preview

3 Prepare 500 mL of normal NGM following the protocol below: 3h

Protocol

NAME

Making normal NGM

CREATED BY

Bonnie Evans

Preview

4 Dispense 200 µL of autoclaved normal NGM into 24× 96-well plates using the Integra ViaFill according to the protocol below: 20m

Protocol

NAME

Dispensing agar into multiwell plates

CREATED BY

Bonnie Evans

Preview

5 Store the 96-well plates upside-down at 4°C until day of use (Day -01).

Day -06. Bleach worms

2h 30m

6 Bleach the adult worm population according to the following protocol:

2h 30m

Protocol

NAME

Bleach synchronisation of C. elegans

CREATED BY

Ida Barlow

Preview

7 Leave the worms spinning in M9 medium for two days, synchronising the worms to L1.

Day -05. Seed 150 mm plates with OP50

1h

8 Add 2 mL of OP50 (OD=1.0) to 5× 150 mm normal NGM petri dishes. Leave plates without lid in safety cabinet until bacterial culture has dried.

1h

Day -04. Re-feed the L1-synchronised larvae

30m

9 Using a glass pipette, add 4-5 drops of larvae to each of the 150 mm petri dishes. Leave plates in 20°C incubator until adding worms to 96-well plates (Day -1). 30m

Day -02. Dry 96-well plates 2h 5m

10 Measure the weight of 3 plates (with lids on) and record average plate weight on day of pouring. 5m

11 Dry the plates under a hood (or drying cabinet) until the plates lose between 3-5% of their original plate weight (with lids on) 2h

Day -02. Make O/N cultures 2h 30m

12 Dispense 200 µL of LB broth into four round bottom 96-well plates. 15m

- *Work near a bunsen burner or work in a Class II Microbiological safety cabinet.*

13 Take the 96-well plate of soil bacteria out of the -80°C freezer and place the plate in a bucket of ice.

STEP CASE

O/N cultures for experiment 22 steps

14 Let the plate of soil bacteria thaw in the bucket of ice. 15m

15 Dip a 96-peg replicator in the thawed soil bacteria plate, swirl around in the wells, and put the pegs in LB-filled 96-well plate and swirl around. Carefully note the orientation of the soil bacteria plate and the LB-filled plate to ensure the well position is the same when adding the bacteria. 10m

Repeat for three of the LB-filled round-bottom 96-well plates.

- *Work near a bunsen burner or work in a Class II Microbiological safety cabinet.*

16 Dispense 50 µL of OP50 (OD=1.0 in LB) into half of 96-well plate. Dip a 96-peg replicator in the OP50-filled 96-well plate followed by putting the pegs in the last of the four LB-filled round bottom 96-well plate. 10m

17 Add Breathe-Easy sealing membrane over each plate. 5m

18 Place the plates in a shaking incubator at 200 rpm at 28°C Overnight .

Day -01. Add bacteria to square, normal NGM 96-well plates 3h 55m

19 Take the square, normal NGM 96-well plates out of the 4°C storage and spread them out on the bench, upside-down, to adapt them to room temperature. 1h 30m

20 Use the Tecan Spark Multimode Microplate Reader to read the optical density of the soil bacteria plates and the OP50 plate. Ensure all wells have bacterial growth, comparing the OD of the part of the plate with no bacterial innoculation. 20m

- 20s shaking (orbital, 180 rpm) prior to reading the optical density.

21 Replace 100 µL of the bacterial cultures in all plates with 100 µL, diluting the cultures 1:1. 30m

22 Read the optical density of the plates after diluting the bacterial cultures. 20m

- 20s shaking (orbital, 180 rpm) prior to reading the optical density.

23 Use the 96-tip Integra ViaFlo to pipette 10 µl of each inividual bacterial culture into their corresponding wells in the square, normal NGM 96-well plates. 30m

24 Place the bacteria-added square 96-well plates in a Class II Microbiological Safety Cabinet without lids for ~30-45 min to dry the wells. 45m

Day -01. Add worms to square, normal NGM 96-well plates with bacteria 7h 10m

25 Check that worms are egg-laying (D1 adults). Wash the worms off the plates with M9 using pasteur pipettes into 15 mL Falcon tubes. 10m

26 Centrifuge for 2 min at 1500 rpm (RCF: 210, ascending 9; descending 7) - program 1. 2m

27 Replace the bacteria supernatant with new M9. 1m

28 Repeat step 27 and 28 two more times. 6m

29 After the final centrifugation, remove the supernatant and replace with new 8 mL of supernatant. Pour the worms into a 100 mL autoclaved bottle. 3m

30 Add another 8 mL of M9 to the tubes and transfer to the 100 mL bottle. 3m

31 Check the number of worms in 10 µL drop on a small glass slide. Adjust the volume of M9 through additional spinning and adding of new M9 to reach an average of between 10-20 worms per 10 µL drop. 30m

- *The volume of M9 in the bottle where a drop of 10 µL has between 10-20 worms will determine the number of plates that can be done in the screen.*

- *Volume required to prime the ViaFill tubes: 5 mL*
- *Min. volume required to not intriduce bubbles in the tubes: 20 mL*
- *Volume required per plate: 10 µL x 96 wells x 1 plate + 5 mL + 20 mL min. volume= 25.96 mL*
- *Volume required for 20 plates: 10 µL x 96 wells x 20 plates + 5 mL + 20 mL min. volume= 44.2 mL*

32 While stirring Use the Integra ViaFill dispenser with *LARGE* tubing to dispense 10 µL drops of worms to each well of the square, bacteria-added 96-well plates once the bacterial culture has dried. 15m

- *Check that worms have been added to the wells. Blockage can easily occur.*

33 Leave the worm-filled plates in a Class II Microbiological Safety Cabinet until wells are dry (~6h) 6h

- *Wells closer to the edge dry quicker.*
- *Check every 2 hours for dry wells and cover the wells along the edges with masking tape if needed to avoid agar cracking and reducing in size.*

34 Once dry, leave the paltes in the 20°C incubator Overnight

Day 0. Record the plates at 24h after addition of worms 1h

35 At 24h after dispensing the worms into the wells of the square, bacteria-added 96-well plates, record the plates at 25 frames/second with a script that records: 1h

1. 5 min of pre-stimulation.
2. 6 min of blue-light stimulation (three 10s bluelight stimulations spaced 100s apart).

3. 5 min of post-stimulation.