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Swarming assay (high number)

Forked from a private protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.uagesbw

Behavioural Genomics

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ABSTRACT

For imaging swarming behaviour of a very high number (thousands+) of young adult C. elegans on agar using the Cyclops single worm tracker. Worms are synchronised by bleaching and refeeding for 72 hours, and then 300 uL of young adult hermaphrodites are transferred by glass pipette onto a 90 mm regular NGM plate for imaging for 15 hours at 0.1Hz.

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ABSTRACT

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Bleach synchronising worms (-7 to -4 day)

1 Bleach synchronise 3 large plates (90mm) worth of animals, making sure lots of gravid hermaphrodites are present. Leave on rotator at 20 °C until use.

Re-feeding worms (-3 day PM)

2 Re-feed worms onto 3-4 large plates (90mm) using a glass pipette. Incubate at 20 °C.

90 mm plates are pre-seeded with undiluted OP50. Lawn does not have to be any specific shape - uniform, with gaps, all is ok, depending on experimental needs.

Imaging (Day 0 PM)

3 Set appropriate frame rate using CommonVision control box (0.1 Hz). Open Gecko and initialise experiments: fixed duration (60 minute recording, repeat 15 times), MJPG format.

4 Pool animals together from 3 large plates (90mm), wash in M9 twice, and aspire as much supernatent as possible after the last wash.

5 Transfer ~160 uL of worm onto an imaging plate (90 mm plate pre-seeded with undiluted OP50 at least one day prior) using a glass pipette, making sure to carry as little liquid as possible as this will disperse bacteria on the imaging plate.

Some liquid will inevitably spill onto the lawn, which is ok. This is where the worm aggregate/front initially forms.

6 Place the plate inside the tracker with an appropriate field of view (i.e. worm front at the edge of the illuminated circular field of field, so that they are expected to sweep over the field of view). Image overnight.

Imaging (Day 1 AM)

7 Close Gecko. Transfer the files to the server.

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