Mating assay V.1

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1 Works for me
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Behavioural Genomics

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ABSTRACT

For imaging drug-treated young adult C. elegans in liquids using the Multiworm tracker. Worms are synchronised by picking L4s, and then the young adults are exposed to drugs for 4 hours prior to imaging for 15 mins in a liquid droplet on a coverslip mounted on a 3.5cm plate.

ATTACHMENTS

liquid imaging protocol_Ida.docx

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PROTOCOL CITATION

Serena Ding 2020. Mating assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.sq3edyn

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ABSTRACT

For imaging drug-treated young adult C. elegans in liquids using the Multiworm tracker. Worms are synchronised by picking L4s, and then the young adults are exposed to drugs for 4 hours prior to imaging for 15 mins in a liquid droplet on a coverslip mounted on a 3.5cm plate.

Generating male stock (if needed) (~ -7 days) eg Monday AM

1 Set the incubator to equilibrate at 30°C. Pick 6 L4 hermaphrodites onto a 55 mm plate, and do this 5 more times to have

a total of 6 plates of 6 L4's. Incubate the L4's at 30°C for 6 hours and let recover at 20°C.

06:00:00 heat shock at 30°C

2 Seed a few 55 mm male stock plates with a honey moon lawn of OP50 (small patch of bacteria to encourage mating).

It's not essential when this step is performed.

Generating male stock (if needed) (~ -3 days) eg Friday

3 Pick males from the heat-shocked plates onto the male stock plates, set up crosses with L4 hermaphrodites. If there are enough males, then set up 3 plates of 5 males x 2 hermaphrodites.

Keep repeating this step every 3-4 days to maintain male stocks throughout the experiment.

Pre-pick L4 animals (-1 day PM)

4 For each strain, pick ~150 L4 hermaphrodites onto 3 separate 55 mm plates (i.e. 50 animals per plate), and ~ 20 L4 males onto a single 55 mm plate.

Imaging (-1 day PM)

5 Seed each 35 mm imaging plates with 20 µL of undiluted OP50 in the centre of the plate. Let inoculate at room temperature over night.

Imaging plates should be about 1 week old and stored in the cold room prior to use

It's advisable to pre-aliquot the OP50 prior to the start of the experiment, to ensure batch consistency across different replicates.

Imaging (Day 0 AM)

6 First identify and label plates in accordance with the experimental Excel template.

The plates will now be labelled with the set number and the rig position as well as half having a black dot annotation.

7 Initialise the experimental set up on the computers with init_exp, open Gecko and adjust settings, etc.

init_exp

Starts the experiment by generating folders on each of the PCs

Windows 10

8 Use a wire or hair pick, transfer 25 adult hermaphrodites from Step 4 onto an imaging plate. Let animals acclimatise to their new environment for 25-30 min.

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Place the animals away from the honeymoon lawn.

9 Use a wire or hair pick, transfer 1 adult male from Step 4 onto the imaging plate containing hermaphrodites from Step 7. Image immediately (ie no acclimation).

Place the animal away from the honeymoon lawn.

10 Image for 15 minutes at 25fps with the .hdf5 file format.

11 Hermaphrodite plates can be re-used if necessary, by removing the male (and the hermaphrodite it has mated with if she's possible to identify) and then adding a fresh male. There is no need to wait between male removal and addition, and image immediately after adding the new male.

12 Transfer the files to the server.

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