

·



Version 1 ▼

Oct 20, 2020

Mating assay V.1

Serena Ding¹

¹Imperial College London

Works for me

dx.doi.org/10.17504/protocols.io.sq3edyn

Behavioural Genomics

Serena Ding

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

DOI

dx.doi.org/10.17504/protocols.io.sq3edyn

PROTOCOL CITATION

Serena Ding 2020. Mating assay. protocols.io

https://dx.doi.org/10.17504/protocols.io.sq3edyn

KEYWORDS

behaviour, C. elegans, liquids

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Aug 20, 2018

LAST MODIFIED

Oct 20, 2020

PROTOCOL INTEGER ID

14843

ATTACHMENTS

liquid imaging protocol_Ida.docx

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

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

mprotocols.io 10/20/2020

Citation: Serena Ding (10/20/2020). Mating assay. https://dx.doi.org/10.17504/protocols.io.sq3edyn

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).



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

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.



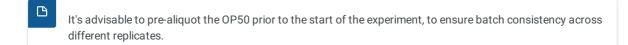
Pre-pick L4 animals (-1 day PM)

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)

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





Imaging (Day 0 AM)

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



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



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.

© protocols.io 2 10/20/2020

- 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.
- 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.