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fluorescence aggregation imaging V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

For imaging aggregation behaviour of 40 young adult *C. elegans* on agar using the Twinnie TwinCam system. Worms are synchronised by bleaching and refeeding for 72 hours, and then 40 young adult hermaphrodites are transferred by glass pipette onto a 35 mm regular NGM plate for imaging for 1 hour at 9 fps.

Troubleshooting

Before start

Prior to collecting the full dataset, a single batch of OP50 was grown overnight, diluted to OD600 = 0.75, aliquoted for use on each imaging day, and stored at 4°C until use.

Imaging plate preparation (Day -7)

- 1 A separate batch of imaging plates is poured exactly seven days before each imaging day and stored at 4°C.

Note

Imaging plates are 35 mm Petri dishes containing 3.5 mL low peptone (0.013% Difco Bacto) NGM agar (2% Bio/Agar, BioGene) to limit bacteria growth.

Bleach synchronising worms (Day -7 to -4)

- 2 Bleach synchronise gravid hermaphrodites. Leave on rotator at 20 °C until use.

Re-feed worms (Day -3, PM)

- 3 Re-feed starved L1 worms onto 3-4 plates using a glass pipette. Incubate at 20 °C.

Note

Culture plates are 55 mm Petri dishes containing 15 mL low peptone 0.013% Difco Bacto) NGM agar (2% Bio/Agar, BioGene), and seeded with OP50.

Imaging plate preparation (Day -1)

- 4 Imaging plates are dried at 37°C overnight with the agar side down.

Seeding imaging plate (Day 0, AM)

- 5 The center of an imaging plate is seeded with a single 20 µL spot of cold diluted OP50 (OD=0.75) one to three hours before imaging.

Note

The overnight plate drying step allowed the bacteria to quickly dry atop the media in order to achieve a more uniform lawn by minimizing the “coffee ring” effect that would thicken the circular edge of the bacteria lawn.

Imaging (Day 0)

- 6 Wash animals off of culture plates with M9 and collect in a 15 mL Falcon tube, wash in M9 twice, and aspire as much supernatant as possible after the last wash.

Note

(Optional):
For two-colour imaging, animals with different fluorescent markers were mixed in desired proportion (1-3 red animals out of 40 per experiment) during the washing stage.

- 7 Forty animals are transferred by a glass pipette onto the imaging plate in a small drop of M9, away from the bacteria lawn.
- 8 After M9 is absorbed into the media following worm transfer in liquid, imaging plates containing the animals are subjected to a gentle vibration at 600 rpm for 10 s on a Vortex Genie 2 shaker (Scientific Industries) to disburse animals and synchronize aggregation start across replicates.
- 9 Imaging commences 20 s after the vibration finish. Image acquisition is performed on a DMI6000 inverted microscope (Leica) equipped with a 1.25x PL Fluotar objective (Leica), a TwinCam LS image splitter (Cairn) with a dichroic cube (Cairn), and two Zyla 5.5 cameras (Andor) to enable simultaneous green-red imaging with maximal field of view. An one-hour recording is taken with constant blue (470 nm, 0.8A) and green (cool white, 1.4A) OptoLED illumination (Cairn), and images are acquired with 100 ms exposure at 9 Hz using Andor Solis software (v4.29.30005.0) as TIFF stacks. The microscopy room is maintained at 21°C throughout the recording durations.

Image data processing

- 10 TIFF image data are compressed and analysed using Tierpsy Tracker software.