Mar 18, 2019

Working

fluorescence aggregation imaging

Version 2

Serena Ding[1]

[1]Imperial College London

dx.doi.org/10.17504/protocols.io.vzje74n

Serena Ding

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.

EXTERNAL LINK

https://www.biorxiv.org/content/early/2018/11/01/398370

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

SAFETY WARNINGS

BEFORE STARTING

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.

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.

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.

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 supernatent as possible after the last wash.

(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.