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Tracking *C. elegans* behaviour on *Agrobacterium*

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e.warren Warren[1]

[1]LMS

Behavioural Genomics

e.warren Warren
LMS

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We use this protocol and it's working

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Abstract

Tracking of behavioural phenotypes and morphology of *C. elegans* fed *Agrobacterium*

Materials

Low peptone NGM:
2% BioAgar, 0.013% Bacto Peptone, 0.3% NaCl, 1mM $CaCl_2$,
1mM $MgSO_4$, 1mM KPO_4, 5 µg/mL Cholesterol

Yeast Extract Peptone (YEP) agar:
1% Bacto peptone, 1% Yeast extract 0.5% NaCl, 1.5% BioAgar

Yeast Extract Peptone (YEP) media:
1% Bacto peptone, 1% Yeast extract 0.5% NaCl

Induction Media:
1% NH4Cl, 0.145% MgSO4, 0.15% KCl, 0.01% CaCl2, and 0.0025% FeSO4-7H2O,
2 mM NaPO4 (pH 5.6), 50 mM 2-(4-morpholino)-ethane sulfonic acid (MES), 0.5%
glucose + 100 µM Acetosyringone

LB-Nematode Grown Media (NGM) Agar:
1% tryptone, 0.5% yeast extract, 1% NaCl, 1mM CaCl2, 1mM MgSO4, 1mM KPO4
dx.doi.org/10.17504/protocols.io.bnh2mb8e, 1.7% BioAgar, 5 µg/mL Cholesterol

Plate preparation

1 Low peptone (0.013%) NGM https://dx.doi.org/10.17504/protocols.io.2rcgd2w) was dispensed into each well of a 96-square well plate (Whatman UNIPLATE: WHAT-77011651) using an Integra VIAFILL https://dx.doi.org/10.17504/protocols.io.bmxbk7in).
The plate was placed in a drying cabinet to lose 3-5% of weight by volume. Wells were then seeded with 5 µL of *E. coli* (OP50, OD600 0.1).

Agrobacterium preparation

2 8 days before starting the tracking, *Agrobacterium* transformed with T-binary vectors containing T-DNA designed to express *flp-1* (dna1959), Amyloid-β (dna1977), MmTX1 (dna1948), MmTX1 with signal peptide sequences *clec-1* (dna1947), or *Spp1*(dna1946), controls of wrmScarlet (WS) (dna1401), BFP (dna1621) or no plasmid (Control), was streaked to Yeast Extract Peptone (YEP) agar plates (containing appropriate antibiotics) and grown for 3 days at 28°C.

3 5 days before starting the tracking, 20 ml YEP media (containing appropriate antibiotics) was inoculated with a single colony of *Agrobacterium*, and incubated overnight at 28°C and 250 rpm.

4 4 days before starting the tracking, overnight cultures were diluted 1:100 with YEP media (containing appropriate antibiotics), and cultured at 28°C at 250 rpm until the OD600 reached 0.3.

5 *Agrobacterium* was pelleted by centrifugation (5 min 4000g), before resuspending in 1 ml Induction Medium containing 100 µM Acetosyringone.
Samples were shaken at 50 rpm at room temperature for 23 hours.

Coincubation of *C. elegans* and *Agrobacterium*

6 4 days before tracking N2 *C. elegans* were bleach synchronized.
dx.doi.org/10.17504/protocols.io.2bzgap6

7 3 days before starting the tracking, LB-NGM plates (containing appropriate antibiotics) were seeded with 1 ml induced *Agrobacterium* culture and allowed to dry.

8 Approximately 50 synchronized L1 worms were dispensed per plate, and kept at 20°C for 3 days

9 On the day of the tracking, 3 worms from each *Agrobacterium* plate were picked to a well of the prepared 96 well low peptone NGM agar plate.

Tracking

10 Videos were acquired and processed as described in https://doi.org/10.1038/s42003-022-03206-1

10.1 Videos were acquired at 25 frames per second using a shutter time of 25 ms and a resolution of 12.4 µm/px. Three videos were taken sequentially: a 5 min pre-stimulus video, a 6 min blue light recording with three 10 s blue light pulses starting at 60, 160, and 260 s, and a 5 min post-stimulus recording. Videos were segmented and tracked using Tierpsy Tracker (**https://doi.org/10.1098/rstb.2017.0375**).

10.2 Following tracking, features were extracted describing morphology, “length_50th” (median worm length), and movement, “speed_50th” (median worm speed), “motion_mode_forward_frequency” (frequency of forward motion in events/second) and “motion_mode_pasued_frequency” (frequency of paused motion in events/second).