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Microbiome Assay 96WP

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Protocol status: Working

We use this protocol and it's working

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Preparing worms

- 1 Using an eyebrow hairpick, pick 10 L4-stage N2 worms onto each of 10 OP50-seeded 90mm petri plates 4 days prior to bleaching (e.g. on Monday if bleaching on Friday)
- 2 On day of bleaching (e.g. Friday) follow the protocol *Bleach synchronization of C elegans*
- 3 Keep the tube with bleached N2s on a rotator at 20C incubator until refeed (make sure not to exceed 5 days as the worm behaviour is not consistent post this time frame)
- 4 If tracking is intended to be performed on the following Thursday, then refeed the arrested L1s on the Monday post bleaching at about 3pm following the protocol *Bleach synchronization of C elegans*
- 5 Store the refeed L1 plates at 20C incubator

Dispensing low peptone NGM on 96WP for imaging

- 6 At least 2 days prior to tracking day (e.g. Tuesday if tracking is planned the following Thursday) make about 250ml low peptone NGM following the protocol *Making low peptone NGM for imaging plates*
- 7 Dispense 150ul of agar into each well of the 96WP using the integravia fill following the protocol *Dispensing agar into multiwell plates*
- 8 Let the agar dry and store the plates at 4C (lid side down) until used (plates can be stored for up to a week prior to use)

Making liquid bacterial culture

- 9 Streak the bacterial strain of interest on appropriate LB plate at least a week prior to tracking using the protocol *Streaking bacteria from frozen glycerol stock* (a freshly streaked plate can be stored at 4C and used for up to 1 month)
- 10 Grow an overnight culture of the bacterial strain 3 days prior to tracking (e.g. on a Tuesday afternoon if tracking is to be intended on Thursday) following the protocol *Growing overnight bacterial culture*



- 11 The following post overnight incubation, take the bacterial cultures out of the shaker and measure their optical densities at 600nm wavelength
- 12 Dilute the bacterial cultures with LB broth (if needed) to obtain an OD₆₀₀=1
- 13 Keep the diluted bacterial culture at 4C until used for seeding later that day

Seeding the 96WP with bacterial culture using Opentrons

- 14 Design a Python script for operating the OpenTrons, defining the necessary labware (1 flat-bottom 96wp [source plate], 2 Whatman 96wp [destination plates], 2 pipette tip racks 10ul), as well as pipette parameters (aspirating/dispensing volume), and the series of commands to be executed by the robot. Before using the robot, first make sure that the script will run successfully by calling it using 'opentrons_simulate'.
- 15 Connect OpenTron to laptop/desktop computer via USB, and open the OpenTrons app (Wi-Fi has not been set up yet; to connect to the robot, you must first turn OFF the computer Wi-Fi).
- 16 Click 'ROBOT' sidebar tab, look for robot ID 'OT2P20180526A07' and click the slider button to connect.
- 17 Proceed to 'PROTOCOL' sidebar tab, click 'Open' and select the OpenTron script you wish to execute.
- 18 Proceed to 'CALIBRATE' sidebar tab, and follow the on-screen instructions to calibrate the robot, first placing empty (dummy) labware (1 flat-bottomed 96WPs, 2 Whatman 96WPs, 2 OpenTrons 10ul tip-racks) in the correct slots in the robot deck, and then adjusting the pipette (Left = P10 8-channel multi-pipette) arm's positioning over custom labware, as per on-screen instructions.
- 19 Once the calibration is satisfactory, exchange the empty labware for the actual experiment labware.
- 20 Proceed to 'RUN' sidebar tab, and click 'Start Run' to seed 2 separate 96WP Whatman plates (destination plates) from 2 columns of wells in a single source plate as per the protocol script:
- 21 A1-H1 (1st column) maps to each column in the first destination plate (12 replicates).



- 22 A2-H2 (2nd column) maps to each column in the second destination plate (12 replicates).
- 23 The wells in row A and row E contain OP50 control, all other rows contain test bacteria.
- 24 Allow to dry under a hood for about 1 hour.

Adding L4s to the seeded 96WP using integra

- 25 2 days post refeeding the L1s (i.e. Wednesday, if refeed on Monday) wash the worms off the maintenance plates using few milliliters of M9 and collect in a 15ml falcon tube and fill up till 15ml with M9
- 26 Centrifuge the tube at 1500rpm for 2mins followed by removal of the supernatant using a pasteur pipette and addition of another 15ml of M9
- 27 Repeat the steps 2 more times
- 28 After the final wash remove the supernatant as much as leaving behind 10ml in the falcon tube
- 29 Add 10ul of 1:10 dilution of Tween to the worm solution
- 30 Take 3-4 5ul aliquots of the worm solution on a maintenance plate and then count the number of worms on each droplet
- 31 Calculate the average number of worms on each 5ul of the solution and dilute with M9 if required to obtain the desired number of worms in 5ul of the worm solution
- 32 Dispense 5ul of the worm solution onto each well of the seeded 96WP following the protocol *Dispensing worms onto multi well plates*
- 33 Store the plates at 20C to be tracked the next day



Tracking using Hydra rigs

- 34 On the morning of the tracking day, take out the prepared 96WPs from the 20C incubator and keep under a hood for 1hr to dry out any remaining M9 from the dispensed worms, also to get rid of any condensation
- 35 After drying, place the plates under the Hydra rig and record for 15mins following the protocol *Tracking on the Hydra rigs*
- 36 Wait 1hr and then record the plates for another 15mins
- 37 Discard the plates in the biological waste bins post tracking