Microbiome Assay 96WP

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1 Works for me
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Behavioural Genomics

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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 (makesure not to exceed 5days as the wormbehaviouris 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 3pmfollowing the protocolBleach synchronization of C elegans

5 Store the refed L1 plates at 20C incubator

Dispensing low peptone NGM on 96WP for imaging

6 At least 2days 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 integraviafillfollowing the protocolDispensing agar intomultiwellplates

8 Let the agar dry and store the plates at 4C (lid side down) until used (plates can be stored foruptoa week prior to use)

Making liquid bacterial culture

9 Streak the bacterial strain of interest on appropriate LB plateatleasta week prior to tracking using the protocolStreaking bacteria from frozen glycerol stock(a freshly streaked plate can be stored at 4C and used forupto1 month)

10 Grow an overnight culture of thebacterial strain 3days prior to tracking (egon a Tuesday afternoon if tracking is to be intended on Thursday) following the protocolGrowing 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 theOpenTrons,definingthe necessary labware (1 flat-bottom 96wp [source plate], 2 Whatman96wp [destination plates],2 pipette tip racks 10ul),as well aspipette parameters (aspirating/dispensing volume), andtheseries of commands to be executedby the robot.Before using the robot,firstmake sure that the scriptwill run successfully by callingitusing 'opentrons_simulate'.

15 ConnectOpenTronsto laptop/desktop computerviaUSB, andopen theOpenTronsapp(Wi-Fihas 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 forrobot ID'OT2P20180526A07' and click the slider button to connect.

17 Proceed to 'PROTOCOL'sidebartab,click 'Open' and select theOpenTronsscript you wish to execute.

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18 Proceed to ‘CALIBRATE’ sidebar tab, and follow the on-screen instructionstocalibrate the robot, first placingempty(dummy)labware (1 flat-bottomed 96WPs, 2 Whatman 96WPs, 2OpenTrons10ul tip-racks) in the correct slots in the robot deck, and then adjustingthe 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 plateas 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

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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

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