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Microbiome Assay with 96WP Updated April 2020

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Behavioural Genomics

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Protocol status: Working
We use this protocol and it's working

Created: Apr 29, 2020

Last Modified: Apr 29, 2020

PROTOCOL integer ID:
36368

-10 days

- 1 Pick 10 L4s onto 10x90mm OP50 seeded plates on Monday (10 L4 per plate so total 100 L4s)

-6 days

- 2 At 2pm on day of bleaching (eg 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 5days as the worm behaviour is not consistent post this time frame)

-3 days

- 4 If tracking is intended to be performed on the following Thursday, then refeed the arrested L1s onto 4 OP50 seeded 150mm plates on the Monday (+72hrs post bleaching, at 2pm following the protocol *Bleach synchronization of C elegans*)
- 5 Store the refeed L1 plates at 20C incubator

-2days

- 6 At least 2days prior to tracking day (e.g. Tuesday if tracking is planned on Thursday) make about 250ml no peptone NGM
- 7 Dispense 200ul of agar into each well of the 96WP using the integra viafill following the protocol *Dispensing agar into multi well 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)-Note:

- 9 Measure the weight of the plates with lids on and record average plate weight on day of pouring
- 10 Grow an overnight culture of the bacterial strain from the (shuffled) 96WP library stock plates, 2days prior to tracking (eg on a Tuesday afternoon if tracking is to be intended on Thursday) following the protocol *Growing overnight bacterial culture in 96WP*.
- 11 Cover the plates with breathable seals and shake at 180-200rpm in a shaking incubator
- 12 Separately inoculate OP50 and incubate at 37C overnight in a shaking incubator at 200 rpm

-1day

- 13 Take the bacterial cultures out of the incubators in the morning, and store at 4C until used for seeding later that day
- 14 Take agar-filled 96WPs out of the cold room and dry under in the drying cabinet for about 2-3hrs (Measure the weight of 3 plates before drying without the lid)
- 15 After drying measure the weight of the 3 plates again. Ensure that the plates lose between 3-5% of their original weight following drying.
- 16 Seed the dried plates with the bacterial culture grown the night before using the ViaFlo

- 17** Turn the ViaFlo ON (Make sure to book it beforehand)
- 18** Edit the protocol SAUL to have the mixing stage consisting of 15 cycles to initially mix the bacterial cultures and to resuspend each strain
- 19** Load the tips (long 12ul tips), Adjust the Z height to the one that worked best in the test runs (See lab notes)
- 20** Mix the bacterial plate using the ViaFlo
- 21** Change the tips
- 22** Edit protocol SAUL again to have mixing stage be of 1 cycle when actually seeding plates
- 23** Run the protocol- To dispense 10ul volume in each well
- 24** Change tips every time a new plate is seeded

- 25 After all the plates are seeded dry them under the hood for another 1hr
- 26 After drying put the plates in a closed box upside down in the cold room (4C)

Day 0: Tracking day

- 27 On the day of tracking prepare the worms following the protocol *Preparing worms for the COPAS (wormsorter)*
- 28 Dispense the worms using the COPAS following the protocol *Using the worm sorter (COPAS)*
- 29 Dry the plates for 30minutes under the hood
- 30 After drying, place the plates under the Hydra rig, leave for a further 30mins to acclimatize, and then record for 15mins, following the protocol *Tracking on the Hydra rigs*
- 31 Record with the lids on and lid side facing up inside the rig. Make sure to wipe the lids with lint-free tissues before placing them under the rig. Also use the script that involves using blue light in the recording
- 32 Record each plate at four time points: 1hr, 3hrs, 5hrs, and 23.5hrs (next day). Between 1hr, 3hr & 5hr the plates are kept in the cave outside the rigs

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- 33** Store the plates in 20C incubator overnight between tracking 5hrs and 23.5hrs timepoint
 - 34** Discard the plates in the biological waste bins post tracking