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Microbiome Assay

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1 Works for me dx.doi.org/10.17504/protocols.io.5zmg746

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1 Preparing worms

1. Chunk N2s on 3-4 plates on Wednesday, also book the hood for next Wednesday morning to dry the seeded plates
2. Bleach the N2s on Friday and keep the tube in the rotator inside the 20C incubator
3. On Monday refeed the arrested L1s to be young adults on Thursday (Tracking Day1), do the same on Tuesday for the worms to be young adults on Friday (Tracking Day2)

2 Preparing Bacterial culture

1. On Tuesday, grow an overnight culture of the bacteria to be tested by inoculating a single colony in LB broth (1 single colony+100ml LB Broth), in the shaking incubator at 200-220rpm
2. On Wednesday morning, take the culture off the incubator and seed 2 maintenance plates per strain with 100ul of the liquid culture, leave the plates under the hood to dry
3. Store the rest of the bacterial culture in a falcon tube at 4C to be used the following day for tracking i.e. Thursday
4. Repeat steps 1-3 on Wednesday for tracking on Friday.

3 Pre-conditioning L4s

1. The day before tracking Wednesday/Thursday, wash 2 plates of worms refed either Monday/Tuesday respectively with M9 and spin at 2500 for 2 mins
2. Remove the supernatant with a Pasteur pipette leaving about 250ul in the tube
3. Pipette about 20ul of the L4s on to the seeded plates with the bacteria to be tested and leave at 20C overnight

4 Tracking

1. On the day of tracking measure the OD of the bacterial culture stored at 4C
2. Match the starting ODs for the cultures being tested on the same day and note them down
3. Measure the weight of three imaging plates and record the average
4. Label the imaging plates according to the tracking plan
5. Seed 12 plates with 20ul of each bacterial culture (6 plates for the preconditioned L4s and 6 plates for young adults) and leave to dry
6. For the young adults:
   -Wash 2 plates with M9 and spin at 2500rpm for 2mins
   - Remove as much supernatant as possible and pipette the remaining worms on to an unseeded maintenance plate
   -Once the M9 has dried, pick 5 adult worms onto each imaging plates and image right away
7. For the preconditioned L4s, just pick from their individual plates and image right away