Defecation Protocol

Feb. 21, 2008 by Erik Andersen

Preparation of assay plates

1. Take plates out of 4ºC for at least a day to dry them on the bench at room temperature.
2. Determine the concentration of OP50, grown overnight, and dilute to an OD of 1 or 10. When using an OD of 1, spot 50 µl of bacteria on the plate, spread with a little spreader to cover most of the plate and wait 24 hours. When using an OD of 10, spot 50 µl of bacteria on the plate, spread with a little spreader and wait until it is dry (usually 1 hr).
3. The plates are kept at the assay temperature for at least one hour prior to the assay.

Preparation of worms

1. Pick L4 hermaphrodites 24 hrs prior to assay time to a standard 6 cm plates. I usually pick around 30 hermaphrodites of each genotype.
2. The worms are incubated at the assay temperature for 24 hrs prior to the assay.

Defecation assay

1. Pick adult hermaphrodites 10 minutes prior to each assay to assay plates.
2. To increase the efficiency, offset the next animal to score from the first by about 5 minutes. Doing this offset allows for nearly constant scoring of defecation from worm to worm.
3. Alternate genotypes to score during the assay.
4. Score five animals of each genotype for five cycles each.
5. Each cycle period is timed from pBoc to pBoc. Expulsion step is monitored and recorded as well.