

# Lawn-entry assay

## Microscope settings

32.0x magnification  
mRFP filter  
Fluorescent bulb turned on and brightness set to 100%  
30fps

## pylon Viewer settings

Binning: 1  
Gain: 5.0  
30,000 exposure

### Saving recording:

As sequence of images, 1 Frame at a time, stop after 10 minutes

## Protocol

### Syncing AML-283 adults

- AML-283 takes ~66-67 hours until laying of first eggs - ideally looking for **65hr** adults.
  - Set up one set of syncs Monday evening and one set of syncs Tuesday evening for data collection on Thursday and Friday morning-afternoon respectively.
1. Take two seeded plates - one is used as a lawn to remove unwanted worms while picking and the second is used for egg-laying.
  2. From a healthy plate of AML-283, pick between 15-20 gravid hermaphrodites and allow to crawl on the lawn before transferring onto the egg-laying plate (ELP). Preferably pick adults of the same age i.e. day 1-2 adults.
  3. Allow the hermaphrodites to lay eggs for 2 hours.
  4. Remove all hermaphrodites from the ELP.
  5. Incubate at 20C.
  6. Check the progress of the ELP on day 2.
  7. Pre-egg laying adults should be grown after 65hrs.

### Seeding testing plates

- 6cm NGM plates should be made fresh at the beginning of each week and put into 4C storage (if necessary) after drying overnight.
- Use 1.75ul OP50-GFP to seed a lawn exactly in the center of the 6cm plates.
- OP50-GFP should be cultured fresh the night before seeding.

### Setting up recording

1. Put a 5ml drop of M9 0.5cm away from the lawn and opposite each other. Measurement should start from the center of the lawn outwards.
2. Pick 10 pre-egg laying hermaphrodites and immerse them in one drop of M9. Replenish the M9 if the drop is beginning to dry. Put another 5 worms in the other drop.
3. Replenish both M9 drops before setting up the microscope camera. Place the plate under the microscope and find the focus of the pharynx as best as possible.
4. Center on the bacterial lawn, making sure the outside is completely visible.
5. Before recording, take a picture of the bacterial lawn.
6. Begin recording once the first pharynx comes into view.

## Camera settings

(No way to upload .pfs file yet)

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