

## Procedure

### Day -2

1. Pour worm plates with NGM-agar, 50mg/l Amp, 1mM IPTG
2. Start an overnight inoculus in LB-Amp (50mg/l) in a shaker

### Day -1

1. Get the plates in a hood or a warm room (the 30C room is just perfect), to dry out well
2. Dilute 100x the o/n inoculus, and grow 8-12h at 37C, in LB-Amp
3. Pellet the bacterial culture. Resuspend in 1/10th of the initial volume
4. Spot appropriate amounts on plates (about 60-100µl per 4cm plate)
5. Leave the plates o/n at room temperature to dry and induce the dsRNA production

### Day 1

1. Put worms (young L4s or bleached eggs) on plates and have fun
- Based on Kamath et al., Genome Biology 2000, 2 (1): research0002.1-0002.10

### Do NOT

1. use Tet for growing the bacteria or on plates
2. induce at high temperatures (37C)

## Solutions

### NGM-Agar

1. 3 g NaCl
2. 2.5 g Bacto-Peptone
3. 17 g agar - 21 g agar

autoclave in 1l water

Cool to 55C, and add (using sterile technique and swirling):

1. 1ml cholesterol (5mg/ml in ethanol)
2. 1ml 1M CaCl<sub>2</sub>
3. 1ml 1M MgSO<sub>4</sub>
4. 10ml 0.1M IPTG
5. 25 ml 1 M KH<sub>2</sub>PO<sub>4</sub>, pH 6.0 (to make: 136 g KH<sub>2</sub>PO<sub>4</sub>, add water to 900 mL, adjust pH to 6.0 with concentrated KOH, add water to 1 L. autoclave).

Pour plates about half-full, and flame the agar surface to remove air bubbles (or else worms will burrow).

### Ampicillin

50-100mg/ml in water/EtOH (50:50), sterile filtered

### IPTG

100mM (2.38g/100ml in water, sterile filtered)

## Steps

1. Transform L4440-XX plasmid into HT115 (DE3) bacteria – plate on LB/amp/tet
  2. Inoculate 50 ml LB/amp/tet w. 0.5 ml O/N culture or directly from plate
  3. Grow at 37°C until OD<sub>600</sub> reaches 0.4
  4. Induce with IPTG to a final concentration of 2 mM
  5. Continue growth for 4 hours
  6. Prepare small NGM plates w. 40 µl IPTG (168 mM) + 2.5 µl amp
  7. Spin down bacteria – resuspend in 10 ml LB/amp/2 mM IPTG
  8. Inoculate NGM plates w. 100 µl bacteria suspension
  9. Use plates immediately or store at RT.
- Inoculate plates with worms as you prefer – I have seen phenotypes after 17 hours at 25°C