CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

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 CaCl2
3. 1ml 1M MgSO4
4. 10ml 0.1M IPTG
5. 25 ml 1 M KH2PO4, pH 6.0 (to make: 136 g KH2PO4, 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)