CBG
Max Planck Institute
of Molecular Cell Biology
and Genetics

Steps

Day -2

1. Make sure you have the worm plates (diameter 14cm) with NGM-agar
2. Start a 2l culture with E.coli (plain DH5α or similar)

Day -1

1. Sterilize the (2) 1l centrifuge bottles with 30%EtOH and then UV. Use only polycarbonate (milky) bottles, not polypropylene (clear), which would craze under UV.
2. Collect the bacteria, pellet them down 5' at 4000xg in the above-mentioned bottles, resuspend in as little MQ water (always sterile) as needed. [form now on, do everything in the hood, to avoid all possible fungal infection].
3. Crack 2-3 chicken eggs in a beaker and run them through a syringe fitted with a 1.2mm diameter needle, to homogenize; mix with the bacteria. Plate =8ml per plate, spread it well; leave the plates for 1/2...1h in the hood (running flow) to dry them out and then put them O/N in a 37C incubator, to let the bugs grow. You will need at least 12 plates. [we found that you don't necessarily need eggs, and they tend to smell bad and make you popular]

Day 1

1. Put 4 plates in a 25C incubator and the rest in the cold room.
2. Collect adult, egg-laying worms from two small (6cm diameter) plates at 25C in a little bit of MQ water and spread them onto the four plates. Let the worms grow at 25C until they go to dauer larvae (about 8-10 days, check the state every two days or so, by scooping up a small sample for observation under dissection microscope).

D Day

1. Get the rest of the egg-plates from the cold room into the 25C incubator.
2. Add about 15-20ml of sterile MQ water onto each worm plate and leave them for 10-15minutes. This will "extract" the dauers (if they burrowed in the agar).
3. Collect the "slime" in 50ml conical tubes. Rinse thoroughly the lids with cold water as well, there's lots of dauers there. (Cold water/buffer minimizes thrashing (of the worms) resulting in a tighter pellet). Pellet them down at 120-160xg (800-1000rpm in the Heraeus) for 2-3 minutes; **disable the brake** from 100rpm downwards [Hint: on the Heraeus depress shortly the Set button; the display starts blinking; press and hold Brake and use the +/- buttons to have the display show 100. Press Set again.]
4. Remove supernatants by aspiration and repeat the operation 1-2 times.
5. **Killing non-dauers by SDS-lysis:** resuspend the pellet from the previous step in 1% SDS (about twice the pellet volume). Gently rock at RT for 15 minutes. Pellet everything down at 120-160xg (800-1000rpm in the Heraeus) for 2-3 minutes. Wash 2-3 times with cold water.
6. **Sucrose-flotation:** over the pellet from the previous step add 60% ice-cold sucrose (equal volumes). Spin in the centrifuge for 5min at 50xg (500rpm) and (without stopping) another 5min at 1000xg (2400rpm). Collect the upper layer of the supernatant (about 5ml) with a wide-bore pipette. The upper layer contains living dauers; discard the rest, which contains mostly (>80%) fragments and killed worms; don't be shocked if you apparently lose a lot. Wash 2-3 times with ice-cold M9.
7. Re-plate the dauers (about 300,000 dauers/plate) on pre-warmed plates at 25C and let them grow at 25C for 28-30 hours in order to get egg-laying worms.

Solutions

1. MQ water sterile, cold, about 700ml
2. SDS 1%, about 100ml
3. 60% sucrose, cold, about 200ml
4. M9 sterile, cold, about 200ml

Bookings

1. NGM-Agar plates, 145mm diameter, about 15 (a stack) at least. Have them made with 1.5x agar (1.5% instead of 1%)
   Haereus for about 4 hours