Materials and Solutions

Reagents, Materials

- macro carriers (Bio-Rad)
- rupture disks (1350 psi, Bio-Rad)
- 1.5ml low retention tubes
- gold powder (Chempur 0.3-3 micron)
- 1xM9 buffer
- 2.5M $CaCl_2$
- 0.1M Spermidine (Sigma-Aldrich)
- 50% glycerol
- 100% ethanol
- 70% ethanol
- 70% isopropanol
- LB medium
- OP50 bacteria
- C600 bacteria
- NGM plates (5cm and 8cm)
- peptone plates (8cm)

Gold stock solution (60mg/ml)

1. weigh 30mg (0.03g) into a low retention 1.5ml tube
2. add 1ml 70% ethanol
3. vortex 5 minutes
4. soak 15 minutes
5. spin down
6. discard supernatant
7. wash 3 times in 1ml sterile water
8. resuspend in 500μl of 50% sterile glycerol

10xM9 buffer

1. 300ml H2O
2. add:
    - Na_2HPO_4 70g

- KH_2PO_4 30g
- NaCl 5g
- NH_4Cl 10g

3. fill up to 1l with H_2O and autoclave

Bleach Solution

- 99ml water
- 6ml 5M KOH
- 15ml bleach (Natriumhypochloritl□sung / Sodium hypochlorite)

Bacteria preparation

Grow OP50 E.coli bacteria in LB medium overnight at room temperature. Grow C600 E.coli bacteria in LB medium overnight at room temperature. Take one single colony per 200ml bottle.

Plate seeding

Seed small 5cm NGM plates with 100μl OP50 bacteria medium, large 8cm NGM plates, with 500μl OP50 bacteria medium. Let them dry overnight. We dry them in a 30°C room. Seed large peptone plates with 1000μl C600 bacteria let them dry for 1 to 2 days at 30°C.

Worm growing and bleaching

Maintain DP38 [unc119 (ed3)] on small NGM plates seeded with 100μl OP50 at 20°C.

Pick about 10 adult worms per small NGM plate. After 5-6 days DP38 worms stick together in "clumps" (L1-L3) that's when they are best for seeding the peptone plates. The bacteria shouldn't be eaten up completely and there should be no dents in the agar.

For bombardment pick 1-2 clumps and spread the worms on 3 different spots on peptone plates seeded with C600 E.coli. DP38 don□t move to well so this way you make sure that the worms colonize the entire bacteria lawn. Grow worms for 7 days at 25°C. They are best for bleaching when the bacteria are almost eaten up and when there are mostly adult worms with many eggs on the plate.

Use 34 plates per bleaching.

Bleaching

1. add 4ml of 1xM9 to each plate, swirl to loosen worms, then remove suspension to 2 50ml conical tubes
2. centrifuge until speed reaches 1000rpm, then turn off
3. aspirate supernatant
4. resuspend each worm pellet in about 50ml 1xM9 (wash worm pellet)
5. centrifuge until speed reaches 1000rpm, then turn off
6. aspirate supernatant
7. resuspend each worm pellet in about 15ml bleach solution
8. centrifuge until speed reaches 1000rpm, then turn off
9. aspirate supernatant
10. resuspend each worm pellet in about 30ml bleach solution

11. vortex tubes vigorously for at least 10min
    - solution should become slightly viscous and yellowish
    - put 2μL onto a slide and check under microscope
    - if there are lots of eggs still trapped in adult carcasses, vortex for another 2min, then check again
12. when most eggs are loose, centrifuge until speed reaches 1500rpm
13. aspirate supernatant
14. wash each egg pellet in 50ml 1xM9, centrifuge until speed reaches 1500rpm, aspirate supernatant and repeat this step
15. combine the egg pellets and add 1xM9 until total volume is 20ml
16. mix, then put 2 samples of 2μL onto a slide and count the number of eggs (drawing a grid on the slide makes this easier)
17. use these counts to estimate the total number of eggs and use this estimate to calculate volume needed
18. plate 25000 eggs in 400μL per plate and 15 plates per bombardment with the hepta adapter
19. let the worms grow at 25°C for 2 days, at 20°C for 3 days, at 18°C for 4 days or at 16°C for 5 days

Dry large unseeded NGM plates over night at 30°C, store at 4°C.

Day of Bombardment

Wash 7 macrocarriers per bombardment with 100% ethanol, let dry.

Coating gold particles with DNA

1. vortex gold stock solution for 5min
2. use low retention tubes for coating
3. 10μl DNA (Maxi-prep at least 1mg/ml)
4. add 100μl of gold stock solution (60mg/ml)
5. vortex 1min on lowest setting
6. add 100μl of 2.5M CaCl2
7. add 40μl of 0.1M spermidine
8. vortex 30min on lowest setting
9. let the particles settle
10. spin down 1min
11. discard supernatant
12. wash with 300μl 70% ethanol
13. spin down 1min
14. discard supernatant

15. wash with 500µl 100% ethanol
16. spin down 1min
17. discard supernatant
18. add 170µl of 100% ethanol
19. mix with a pipette and distribute 20µl to each macro carrier (distribute what's left evenly on the carriers)

Washing worms

1. during the 30min DNA binding wash the worms off from the peptone plates with 1xM9 buffer into a 50ml Falcon tube (1 tube per bombardment)
2. let them settle and remove supernatant
3. wash once more with 1xM9
4. let them settle and remove supernatant leaving no more than 2ml
5. put very dry and unseeded NGM plate on ice
6. pipette worms on the cooled NGM plate let them completely dry leaving the worm plate on ice

Bombardment procedure PDS-1000/He (Bio-Rad)

a. turn on the PDS-1000/He, helium and vacuum

b. before sample bombardment do one dry run to make sure that all the lines are drained from air and filled with helium

c. dry run

    1. dip rupture disk in 70% isopropanol
    2. place rupture disk into the hepta adapter
    3. place the adapter into the bombardment chamber and tighten with torque wrench
    4. close chamber
    5. press the vacuum button to the upper position
    6. wait until vacuum reaches about 28 inches (26 or 27 inches work as well)
    7. press vacuum button to the lower position to hold the vacuum
    8. press the fire button
    9. release the fire button after you hear a popping noise
    10. press the vacuum button to the middle position to vent the chamber
    11. unscrew the hepta-adapter and take out the burst rupture disk

d. Sample bombardment

1. place DNA-loaded and dried macrocarrier into the hepta macrocarrier holder secure them with the red plastic seating tool, put on stopping screen and macrocarrier coverlid
2. dip rupture disk in 70% isopropanol
3. place rupture disk into the hepta adapter
4. place the adapter in to the bombardment chamber and tighten with torque wrench
5. position the macrocarrier holder
6. place the completely dried and cold worm plate sitting on the target shelf into the bombardment chamber
7. bombard the same way as described above
8. let the bombarded plate sit at room temperature for 1h
9. wash worms with approximately 5ml 1xM9 off the plate
10. spread the washed off worms evenly onto 20 large NGM plates
11. let the worms grow at 25°C for 2 weeks
12. start screening