Bombardment

Aim: obtain transgenic strains with low-copy integration.

Materials: One synchronized *unc-119(ed3)* liquid culture with a large number of L4s or young adults (the most effective for bombarding).
unc-119 rescuing vector, containing the sequence of the gene of interest.
2.5 M $CaCl_2$
0.1 M spermidine

Protocol:

DNA preparation:
Linearize 10-15 μg of DNA. DNA cleaning after restriction digest is not necessary. Final volume of DNA should be 50 μl.

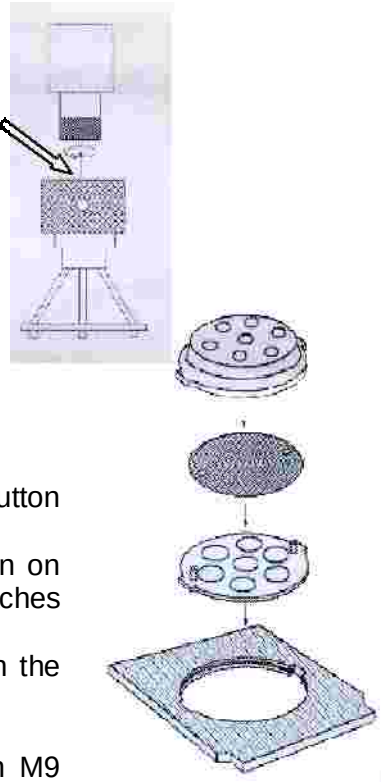
Preparation of gold particles:
Weigh 60 mg of gold particles (1 μm, Bio-Rad) into a siliconized ED (Eppendorf tube).
Add 70% EtOH (ethanol), vortex at least 5 min in Vortex Genie 2.
Soak at least 15 min.
Spin briefly (3-4 sec) and discard s/n (supernatant).
Wash 3 times: Add 1 ml of sterile water, vortex 1 min. Allow particles to settle 1 min. Spin briefly, remove s/n.
Resuspend in 1 ml of 50% sterile glycerol.
The final concentration of gold is 60 mg/ml. Prepared ld slurry can be stored 1-2 months at 4°C or longer at –20°C. There is enough gold for 9-10 bombardments.

Preparation of worms:
Prepare one 10 cm NGM plate. The plate should be dry enough to quickly absorb all liquid from a pellet of worms. So dry the plate for some time at 37°C.
Place the dried plate on ice to cool down.
Wash worms with M9 in a 50 ml tube.
Allow worms to settle for 10 min, remove s/n containin younger animals and most of bacteria.
Repeat the procedure several times. The goal is to obtain 2 ml sediment of worms clean from large pieces of debris and L1s.
Remove s/n and transfer 2 ml of worms to the prechilled dry NGM plate.
Allow the worm liquid to distribute uniformly on the p Keep the plate on ice. If the plate is not kept on ice, worms will start to crawl and form clumps.
Once the liquid is absorbed by agar, a uniform layer of worms will form.
While plate with worms is drying, proceed to DNA coating.

Coating gold particles with DNA:
Vortex gold beads (60 mg/ml) for at least 5 min. Take 100 μl into siliconized ED by pipetting up and down.
Spin briefly and remove s/n.
Add the following components with resuspension by vortexing after each step:

50 μl of DNA (10-15 μg)
50 μl 2.5 M $CaCl_2$
20 μl 0.1 M spermidine.

Incubate 30 min on ice with resuspension by vortexing 7-8 min. During incubation, rinse 7 macrocarriers and a 1350 psi rupture disk in isopropanol and allow hem to dry on a tissue paper.
Spin briefly the DNA-gold mix and remove s/n.
Add 300 µl of 70% EtOH, vortex, spin, remove s/n.
Add 500 µl of 100% EtOH, vortex, spin, remove s/n.
Add 170 µl 100% EtOH, (vortex).
Place macrocarriers into hepta-adapter holder. Distribute 20 µl of beads on each macrocarrier. Be careful to well resuspend by pipettin Allow them to dry.
At this point, the plate with the worms should be ready for bombardment.



Bombardment:
Put the 1350 psi rupture disk it in the hole between the ube and the rest of the machine. Screw the tube gently and tighten with the dynamometric key (the large piece of metal must touch he black plastic).
Once the beads on the macrocarriers have dried, assemble the macrocarrier holder. It must be placed at the second s the top. The holes in the tube must be aligned with the 7 holes in the macrocarrier holder.
Place the plate with the worms in the fourth shelf from the top.
Close the door and switch on the machine and the vacuum pump.
Open the Helium source to obtain a pressure of about 2000 kPa.
Close the vent flow rate tap and open the vacuum flow tap. The button must be on Vac.
Vacuum until pressure reaches 25 in. Hg vac. Switch the middle button on Hold. Push on the button Fire without releasing it until the pressure reaches 1350 psi and breaks the rupture disk.
Close the vacuum flow rate tap and open the vent flow tap. Switch the middle button on Vent.
Switch the machine off and wash the material with EtOH.
After bombardment, allow worms to recover for 30 min, ash off with M9 buffer and distribute to 20-25 5 cm NGM plates seeded with OP50.

Selection of transformants:
Allow worms to grow and starve for 10-14 days. Usually, transformants could be identified already within 5 days after bombardment, but most of them would be transient, therefore it is necessary to wait until *unc-119* worms die. Stable transformants with wild-type phenotype are easily identified on starved plates. Only one worm per plate should be selected to avoid redundancy and ensure independence of transformed lines.