

Biolistic transformation in *C. elegans* using *unc-119* rescue

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Adapted from Shai Shaham, pers. communication, and Praitis et al., 2001

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Day 1: Master Plate

Seed 50-100 *unc-119* (*ed3* or *4*) worms on an enriched peptone plate with NA22 bacteria spread evenly over the entire plate (use enriched peptone plates with NA22 bacteria throughout this protocol). Either pick worms on several different spots or evenly spread out worms, suspended in M9 buffer. Let the worms grow until they have starved for 2-3 days (~7 days total). The plate now contains numerous worm colonies, which consist mainly of L1s. Always have master plates ready when planning bombardments since *unc-119* worms are slow growing.



Day 7: Amplify worms to 6-7 plates

Wash worms off the master plate with M9 buffer. Repeat the wash step until most of the bacteria are gone. Resuspend worms in 6-7ml M9 buffer and spread 1.0ml onto each of 6-7 plates. Allow worms to grow and starve to L1s (2 days). These plates now contain on the order of $2-4 \times 10^6$ L1s.



Day 9: Amplify worms to 60 plates

Wash off worms from 6-7 plates with M9 buffer until most of the bacteria is gone. Resuspend worms in 60 ml buffer. Spread worms evenly by holding 5 plates a time and gently rotating the stack.

Let the worms grow for 2.5-3 days at 20°C or 2 days at 25°C (until they become young adults).

Day 12: DNA preparation.

Weigh 35-50 mg of 1 μm gold beads (Biorad) into a **siliconized** 1.5 ml eppendorf tube.

Add 1ml 70%EtOH. Vortex 5 minutes. Soak for 15 minutes. Pellet and remove sup.

Add 1ml sterile water. Vortex 1 minute. Soak for 1 minute. Pellet and remove sup.

Add 1ml sterile water. Vortex 1 minute. Soak for 1 minute. Pellet and remove sup.

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Resuspend in 500 μl sterile 50% glycerol. This bead stock can be used for 2 weeks (or more) and should be stored at 4 °C.

Vortex mix for 5 minutes. Remove 100 μl of bead suspension into 3 eppendorf tubes. Make sure to keep the gold beads in suspension. You may choose to vortex the suspension at medium speed, stop, and then immediately transfer 100 μl s to each tube.

For each of the three tubes add in order while vortexing on medium speed:

10 μl DNA (1 mg/ml)

100 μl 2.5 M CaCl_2

40 μl 0.1 M spermidine (free base, tissue culture grade)

Vortex 2 minutes. Soak for 1 minute. Pellet and remove sup.

Add 280 μl 70% EtOH. Flick tube to mix. Pellet and remove sup.

Add 280 μl 100% EtOH. Flick tube to mix. Pellet and remove sup.

Add 96 μl 100% EtOH and resuspend by gently flicking tube. This is your prepped DNA. Do this before prepping worms (below).

Day 12: Worm preparation

Wash the 60 plates with M9 buffer into 50ml tubes (~400mls/60plates). Spin at low speed for 1 minute; wash worms until the M9 solution becomes clear and finally transfer worms to a 15ml tube. Spin again at low speed for 1 minute. This should give you a pellet of 2-4mls packed worms. Remove liquid and resuspend worms in M9 to a total volume of 10mls.

Day 12: Bombardment

Using a short pasteur pipette suck about 1.5 mls of worms and add them onto the surface of a dry (1 week post bacterial spreading) enriched peptone plate. Add them drop wise starting at the center and then spiraling around until you reach the edge of the plate. Repeat until all worms are plated (6 plates).

Leave the covers off the plates to evaporate the liquid. This should take no more than 15 minutes. If it takes more, then your enriched peptone plates are too wet.

While the plates dry prepare the gene gun. We use the BioRad Biolistic PDS-1000/He particle delivery system with the Hepta adaptor. This adaptor saves an enormous amount of time and effort. You can find the illustrations and definitions of the various jargon terms (like macrocarriers, and macrocarrier holder) in the BioRad manual. Read this manual to be familiar with the procedures described below.

Hook the vacuum tubing to the lyophilizer (our vacuum source) port (with plastic adaptor). Open vacuum port. Turn on gene gun. Open helium tank valve (check to make sure that He tank pressure is ≥ 2200 psi). Perform a test bombardment by wetting a rupture disk (**1500-2000 psi**) in isopropanol, placing in retaining cap for hepta adaptor, and tightening onto bombardment chamber.

Close door. Pull vacuum to 27 In. of Hg. Once it reaches 27 in. of /Hg press hold. Press Fire button and hold until disk ruptures. Release vacuum (Vent position), open door, unscrew retaining cap and discard the ruptured disk.

Place 7 macrocarriers onto the hepta adaptor macrocarrier holder using the special tool. Vortex your DNA preparation at medium speed with cap open. Stop and immediately (quickly) transfer 6 μ l of beads onto a macrocarrier and spread it around with your pipet tip. Only spread on area around the hole in the holder. Repeat for all macrocarriers. **Let EtOH evaporate** (this only takes a few minutes).

Place a rupture disk soaked in isopropanol in the retaining cap and tighten. Place hepta stopping screen and macrocarrier holder in chamber as described in manual. Place uncovered worm plate (taped to the sample holder using a rolled piece of adhesive tape to create double sided tape) into lowest rung in bombardment chamber.

Evacuate chamber to 26 In Hg. Press Fire button until disk ruptures. Release vacuum (Vent position), and remove plate.

Repeat above for all 6 plates.

Turn off the vacuum. Close the helium tank valve – make sure no pressure is left in the line. Turn the gene gun power OFF.

**Wipe down all of the gene gun components with 70% ethanol, then autoclave the hepta adaptor and the macro carrier holder components. Wipe down the bombardment chamber with 70% ethanol.

Day 12: Plating worms

Wash bombarded worms off plates with M9. Place worms in 50ml tubes. Spin 1 minute at low speed. Resuspend worms evenly in 2 tubes with 80 mls of M9 media.

Plate worms, 1ml per plate, enriched peptone plates **with nystatin** and seeded with NA22 bacteria. Should end up with ~80 plates.

Once plates have dried, place them in a 25° C incubator (we found this to be important for GFP expression of several germline genes, you can grow worms at 20 for non-GFP transgenes) and let sit for 10-14 days (preferably in a plastic containers to avoid plates drying out).

Day 22-26: Screening

Scan plates for WT animals. You should find about 10-20 plates with WT animals with anywhere from 1 to many WTs per plate.

Clone out (to individual plates) 3-5 WT worms per plate. You can use regular NNGM worm plates seeded with OP50 at this step. Place the plates a 25° C for 4 days, and then examine the progeny for GFP expression.

Reagents

Biolistic PDS-1000/He particle delivery system	Bio-rad	1652257
Hepta Adapter, biolistic	Bio-rad	1652225
Biolistic Macrocarriers	Bio-rad	1652335 (500 ct)
1 μ M gold beads	Bio-rad	1652263 (0.25gm)
Rupture Discs	Bio-rad	1652333 (2000psi)
Hepta Stop Screen	Bio-rad	1652226 (50ct)
Spermidine(tissue culture grade)	Sigma	S-4139 (5.0 gm)
Nystatin	Sigma	N1638 (100mls)

Enriched Peptone Plates (1 Liter)

1.2g sodium chloride

20g peptone

25g agar

water to 1 liter

autoclave- cool to 55° C- then add sterile:

1ml cholesterol (5mg/ml in EtOH)

1 ml 1M MgSO₄

25 ml 1M potassium phosphate (pH 6.0)

Enriched Peptone Plates with nystatin (1 Liter)

Same recipe as above and add 10mls Nystatin suspension 10,000 units/ml