Chapter 2

Transgenesis in *Caenorhabditis elegans*

Matthias Rieckher*, Nikos Kourtis*, Angela Pasparaki, and Nektarios Tavernarakis

Summary

Two efficient strategies have been developed and are widely used for the genetic transformation of the nematode *Caenorhabditis elegans*, DNA microinjection, and DNA-coated microparticle bombardment. Both methodologies facilitate the delivery of exogenous DNA into the developing oocytes of adult hermaphrodite animals, which then generate transgenic worms among their progeny. Although both approaches share the common underlying principle of introducing foreign DNA into the germline of *C. elegans*, they offer distinct transformation outcomes. In this chapter, we present DNA microinjection and bombardment methods for transgenesis in *C. elegans* and provide time-tested procedures for their implementation. We also discuss their relative advantages as well as their limitations and evaluate their potential for a range of applications.

Key words: Biolistic transformation, Chromosomal integration, DNA-coated microparticle bombardment, Extrachromosomal arrays, Genetic transformation, Germline, GFP, Microinjection Nematode, transgenic animals

1. Introduction

Caenorhabditis elegans is a small (approximately 1 mm) soil-dwelling, free-living nematode worm. In the laboratory, animals feed on an *E. coli* diet and complete a reproductive life cycle in 2.5 days at 25°C, progressing from fertilized embryos through four larval stages to become hermaphroditic adults, which then live for about 2 weeks and lay ~300 eggs. While the dominant sexual form is the hermaphrodite (genotype: XX), males (genotype X0) can also be propagated and used to construct strains carrying multiple mutations *(1)*. Importantly, *C. elegans* strains can be

* Matthias Rieckher and Nikos Kourtis contributed equally.

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stored indefinitely in liquid nitrogen, making it feasible for any laboratory to possess an unlimited collection of mutants. Because *C. elegans* can reproduce by self-fertilization it is possible to raise genetically identical populations that do not undergo inbreeding depression. The simple body plan, the transparent egg and cuticle, and the nearly invariant developmental plan of this nematode have facilitated exceptionally detailed developmental and anatomical characterization of the animal (see http://www.wormatlas.org/).

C. elegans has proven instrumental in providing insights into the molecular mechanisms underlying numerous biological processes, such as cell death, cell cycle, sex determination and neuronal function, as well as in the genetic dissection of signalling pathways that were subsequently found to be conserved in mammals. Similarly, *C. elegans* has contributed decisively to our understanding of human neurodegenerative disorders ***(2)***, cancer ***(3)***, and ageing ***(4)***. The availability of a fully sequenced worm genome, in which 60–80% of the genes have a human counterpart, the wealth of information available at Wormbase (http://www.wormbase.org/ ***(5)***) relevant to gene structures, mutant and RNAi phenotypes, microarray data and protein-protein interactions, and the availability of numerous mutant strains, have led to the rapid adoption of the worm as a model organism.

DNA manipulated *in vitro* can be introduced into animals for functional assays. Vectors are available for identification of transformants **(Table 1)**, cell-specific expression, and generation of fusions to numerous marker genes, such as *E. coli* β-galactosidase, the firefly luciferase, and the jellyfish Green Fluorescent Protein (GFP) so that individual cells can be visualized in stained or living animals ***(6–11)***. Transformation is also routinely used to rescue mutant genes, to analyse regulatory elements, to ectopically express altered genes and reporters, and ultimately to investigate gene function *in vivo*. In the following sections we describe the two widely used methods for obtaining transgenic *C. elegans* animals, DNA microinjection, and DNA-coated microparticle bombardment, aiming to provide the essential information required for their successful implementation.

1.1. Microinjection

Microinjection is the most widely used transformation method in *C. elegans*. Transgenic animals are generated by injecting appropriate DNA fragments or plasmids. DNA is commonly injected into the distal gonad syncytium **(Fig. 1)** or directly into the developing oocytes ***(12, 13)***. Injected DNA forms large extrachromosomal arrays incorporating between 50 and 300 copies, which are inherited by the progeny of injected animals ***(10, 11)***. Identification of transgenic individuals is facilitated by the use of several available transformation markers, which are co-injected along with the DNA of interest **(Table 1)**. A varying number of

Table 1
***C. elegans* transformation markers**

Plasmid	Phenotype	Comments	Disadvantages	References
pDPMM0016b	Rescues *unc-119(ed3)*	*unc-119(ed3)* mutants are uncoordinated and dauer-formation defective. Only transformants carrying stable integrated transgenes survive starvation. Useful for enrichment of integrants after bombardment	*unc-119(ed3)* is difficult to grow. Selection process takes a long time	*(17, 26)*
pC1 or pBX	Rescues *pha-1(e2123ts)*	Temperature-sensitive strain. Allows selection of transformed worms at the restrictive temperature. Convenient for obtaining large numbers of transgenics	Requires *pha-1(ts)* mutant background. Selection requires growth at 25°C which can be problematic for some temperature-sensitive transgenes	*(27)*
pGK10	Expresses GFP in muscles of the worm using the SERCA gene promoter	Select for worms with bright stained muscles	Not suitable for the characterization of GFP expression patterns	*(28)*
pRF4	Dominant roller	The *rol-6(su1006)* mutant phenotype is easy to detect	Not suitable for studies of locomotion and other behaviours. Larval lineage analysis is problematic. Mating efficiency reduced	*(11)*
pPD10.46	Dominant twitcher	The *unc-22 antisense* mutant is easy to detect	Similar to *rol-6(su1006.)* More difficult to detect	*(9)*
pPD122.11	GFP expression in pharyngeal muscles	The *myo-2::GFP* mutant is a dominant visible fluorescent marker	Requires fluorescent stereo microscope. Interferes with other GFP-fusion-based expression studies	*(11)*
pTG96_2	GFP expression in somatic cells	*sur5::GFP* is a dominant visible fluorescent marker	Similar to pPD122.11	*(29)*
plin-15EK	Rescues *lin-15(765ts)*	*lin-15(765ts)* develop multiple vulvae (muv) at 22.5°C growth temperature, selection of *lin-15(wt)* transformants, less labour for maintenance	Requires *lin-15(765ts)* mutant background. Overexpression of *lin-15(wt)* might lead to misdevelopment of the nervous system	*(30)*

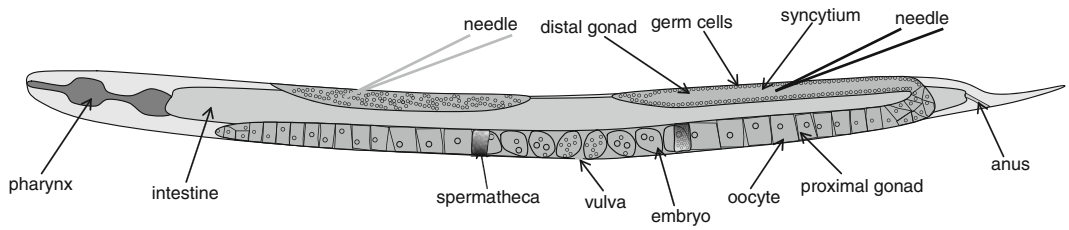

Fig. 1. Schematic drawing of *C. elegans*, indicating the sites of microinjection. The needle injects DNA at the syncytium of the two distal gonad arms, containing mitotic and undifferentiated germ cells. An angle of about 40° between needle and worm facilitates entry through the cuticle. Injected DNA is taken up by developing oocytes which will ultimately get fertilized in the spermatheca to give rise to transgenic embryos.

F1 progeny that carry the desired extrachromosomal array can thereby be identified by characteristic phenotypes associated with each transformation marker. For example, the dominant *rol-6(su1006)* allele induces a distinctive rolling phenotype in transgenic F1 progeny. Extrachromosomal arrays are inherited in a non-Mendelian manner to subsequent generations. Typically, a varying percentage (between 5 and 80%) of next-generation progeny will carry the extrachromosomal array ***(11)***. To overcome low-transmission problems and the consequent, gradual loss of extrachromosomal arrays, they can be incorporated into a chromosome by inducing non-homologous, double-strand DNA break repair. We describe a method for integrating extrachromosomal arrays carried by transgenic *C. elegans* lines in the last section of this chapter.

1.2. Bombardment

The relatively recently developed, DNA-coated microparticle bombardment method (biolistic transformation) is also used to transform *C. elegans**(14–16)***. Gold microparticles are first coated with the DNA to be introduced to animals and then 'shot' into the worms at high speeds, using a specialized biolistic bombardment instrument or 'gene gun'. The commercially available microparticle bombardment devices most commonly used are the Bio-Rad Biolistic® PDS-1000/He Particle Delivery system, with or without the Hepta adapter and the Bio-Rad Helios Gene system ***(14–18)***. A helium gene gun can also be constructed in the laboratory, using off-the-shelf custom parts at a low cost. Instructions for obtaining parts or required accessories and for building the device can be found at Ralf Schnabel's laboratory home page (http://www.ifg.tu-bs.de/Schnabel/ce-home.html).

DNA bombardment offers distinct features and possibilities compared to microinjection **(Table 2)**. Contrary to transgenics obtained by microinjection, which, in their majority, carry large repetitive extrachromosomal arrays, a significant number of transformed individuals generated by bombardment are integrants

Table 2
Features and comparison of transformation methods in *C. elegans*

Microinjection	Bombardment
Transformation of individual hermaphrodites	Transformation of large worm populations
Generally less time consuming	More time consuming
Extrachromosomal arrays carrying ~80–300 copies of the transgene	Low-copy extrachromosomal or integrated lines and homologous recombinants
Frequently extrachromosomal array expression shows extensive mosaicism	Integrated transgenes are expressed in reproducibly consistent manner
Germline expressed genes are silenced	Germline expression of many transgenes remain stable
Transgenic animals are easily identified in a short period of time after injection	Suitable marker for strong selection is necessary
Widely used	Less common
Less expensive: A light DIC (Nomarski) microscope is standard in a *C. elegans* laboratory	Expensive equipment
Inexpensive consumables	Expensive consumables
Requires considerable practice for consistent results	Easier to perform, with less delicate steps

containing a low number of transgene copies ***(15)***. Biolistic bombardment has also been used successfully for homologous gene targeting in the worm ***(18)*** and for expressing transgenes in the *C. elegans* germline, a tissue refractory to expression of genes on conventional extrachromosomal arrays due to germline silencing of highly repetitive DNA ***(15)***. In addition, microparticle bombardment has been utilized to facilitate the routine use of dsRNA hairpin constructs (hpRNAi) ***(19)*** and to target tissues refractory to other RNAi delivery methods ***(20)***.

The major advantage of the microparticle bombardment technique over the germline injection method is that stable integrated transgenic strains can be obtained directly. Moreover, germline expression of many transgenes remains stable, increasing in that way the ability of researchers to investigate the regulation of germline development and function ***(15)***. In addition, low-copy integrants are likely to circumvent the problems associated with mosaic loss of the extrachromosomal arrays. Integrated transgenic lines created by microparticle bombardment express GFP reporter constructs in reproducible and consistent patterns ***(15)***. Finally, the method can be easily scaled up, allowing for isolation of strains with homologous gene replacements ***(18)***. This capacity enables the implementation of powerful

knock-in and knock-out methodologies. Transformation of *C. elegans* by microparticle bombardment also has limitations that should be taken into consideration before choosing which method to implement **(Table 2)**. The preparation of nematodes for gene bombardment is significantly more laborious compared to microinjection, and transgenic animal generation takes significantly longer. In addition, gene bombardment requires access to specialized equipment and reagents. A suitable transformation marker for strong selection is necessary, since large populations of worms must be bombarded to generate the relatively rare desired integration events **(Table 1)**. Attention should be paid to avoid markers that interfere with the function of the gene of interest. In addition, it is still difficult to express dose-sensitive genes because even in integrated lines, expression of the gene of interest may not reflect that of the endogenous gene. Finally, during the integration events, the transgene may disrupt or interfere with the expression of genes located at the site of integration. Here, we describe the most commonly used microparticle bombardment approach for the transformation of *C. elegans*, using the Bio-Rad Biolistic PDS-1000/He Particle Delivery system with and without the Hepta adapter *(15–18)*.

1.3. Integrating Extrachromosomal Arrays

Transgenic animals obtained by microinjection (and occasionally by bombardment) carry and inherit the exogenous DNA as an extrachromosomal array, which results in mosaic expression and gradual loss between successive generations unless actively selecting for transgenic animals *(11)*. To decrease mosaicism and alleviate loss of extrachromosomal arrays they can be integrated; this also facilitates genetic manipulations and screening experiments with transgenics *(10, 21)*; *see* **Note 5**). Integration is triggered by causing chromosomal breaks through the use of mutagenic chemicals (e.g. Ethylmethane Sulphonate) or irradiating animals with an X-ray, gamma-ray, or UV source. The protocol described here refers to the use of an irradiation source *(22)*. Selection is based on the selection markers used for the initial transformation **(Table 1)**. It is advisable to carry out the integration procedure with 2 or 3 independent transgenic lines carrying the extrachromosomal array.

2. Materials

2.1. Microinjection

1. Wormpick: Cut 3 cm of platinum wire (90% platinum, 10% iridium wire, 0.010 in. diameter; e.g. Tritech Research, Los Angeles, CA) and flatten one end using pincers or a light hammer. Break off the thin part of a glass Pasteur pipette. Melt

the glass at the site of breakage on a Bunsen burner and attach the sharp end of the platinum wire. When using the wormpick always sterilize the tip over a flame.

2. 10× TE (Tris/EDTA) buffer: 100 mM Tris–HCl of pH 7.4–8.0 and 10 mM EDTA of pH 8.0 in distilled H_2O. Autoclave and store at room temperature.
3. 10× microinjection buffer: 2% polyethylene glycol (PEG 6000), 200 mM potassium phosphate, 30 mM potassium citrate (adjust to pH 7.5) in distilled H_2O. Autoclave and store at room temperature.
4. NGM agar plates with bacterial lawn: For 1 L NGM agar, combine 3 g NaCl, 2.5 g bactopeptone, 0.2 g streptomycin, and 17 g agar. Autoclave including stir bar and cool to 55°C while stirring. Add 1 mL 1 M $CaCl_2$ (stock: 14.7 g $CaCl_2$ in 100 mL distilled H_2O, autoclaved), 1 mL 1 M $MgSO_4$ (stock: 12.03 g $MgSO_4$ in 100 mL distilled H_2O, autoclaved), 1 mL cholesterol (5 mg/mL in 100% ethanol; *Caution*: flammable), 1 mL nystatin (Sigma, Cat. No. N1638; 10 mg/mL in 70% ethanol; *Caution*: flammable), and 1 mL KPO_4 (stock: 102.2 g KH_2PO_4 and 57.06 g K_2HPO_4 in 1 L distilled H_2O, autoclaved). Use 60 mm Petri dishes and pour 11.5 mL of NGM agar per plate (adjust volume to size of Petri dishes). Let cool until agar hardens. Streak a drop (~50 µL) of an *Escherichia coli* OP50 liquid culture in the centre of each plate and grow at 37°C for 8 h or at room temperature overnight.
5. M9 buffer: 22 mM KH_2PO_4, 22 mM Na_2HPO_4, 85 mM NaCl, and 1 mM $MgSO_4$. Sterilize by autoclaving for 15 min.
6. Recovery buffer: 0.1% salmon sperm DNA, 4% glucose, 2.4 mM KCl, 66 mM $CaCl_2$, 3 mM Hepes, pH 7.2.

2.2. Bombardment

1. Enriched peptone plates: For 1 L, combine 1.2 g sodium chloride, 20 g peptone, and 25 g agar. Autoclave and cool to 55°C. Add sterile 1 mL cholesterol (5 mg/mL in ethanol; *Caution*: flammable), 1 mL 1 M $MgSO_4$, and 25 mL 1 M potassium phosphate, pH 6.
2. M9 buffer: 22 mM KH_2PO_4, 22 mM Na_2HPO_4, 85 mM NaCl, and 1 mM $MgSO_4$.
3. NGM plates: 3 g NaCl, 2.5 g Bactopeptone, 0.2 g Streptomycin, and 17 g agar. Autoclave. Let cool to 55–60°C and add 1 mL cholesterol (5 mg/mL in ethanol; *Caution*: flammable), 1 mL 1 M $CaCl_2$, 1 mL 1 M $MgSO_4$, 1 mL Nystatin, and 25 mL phosphate buffer, pH 6.
4. Materials, reagents, and solutions required for preparing gold beads include: 1 µm-diameter gold beads (Bio-Rad, Hercules, CA; store in a dry, non-oxidizing environment to minimize agglomeration), 0.1 M spermidine (Sigma; free base, tissue

culture grade; filter sterilized; store at –20°C), sterile 2.5 M $CaCl_2$, dehydrated ethanol, and 50% sterile glycerol.

5. High purity and concentrated (~1 mg/mL) DNA is required for transformation. Purification using a commercial kit is recommended.
6. We describe the procedure of microparticle bombardment using the Bio-Rad Biolistic PDS-1000/He particle delivery system (Bio-Rad). The basic transformation protocol can be scaled up by using the Hepta adapter (Bio-Rad).
7. Information about setting up and operating the bombardment apparatus can be found in the manufacturer's manual. Supplies required for the biolistic transformation include: 1,350 psi rupture discs for the conventional system or 2,000 psi rupture discs (Bio-Rad) if using the Hepta adapter, biolistic microcarriers (Bio-Rad) and Hepta stopping screens (Bio-Rad).

2.3. Integrating Extrachromosomal Arrays

For animal irradiation, an X-ray, a gamma-ray (^{137}Cs; 2,000–4,000 rad), or a UV source can be obtained from commercial sources. A UV crosslinker with the appropriate UV bulbs (254 nm) and energy measurement sensor offers the most convenient and widely used alternative (Bio-Link Crosslinker, Vilber Lourmat, Marne-la-Vallée, FR or Stratalinker, Stratagene, La Jolla, USA).

3. Methods

3.1. Microinjection

3.1.1. Preparing Agarose Pads

Animals are fixed stably on the surface of an agarose pad for injection.

1. Prepare 10–50 mL of 2% agarose solution in distilled H_2O. Boil in a microwave oven until agarose is dissolved and cool down the solution in a water bath (60°C) or at the bench for a short while.
2. Align a number of microscope cover glasses (24 × 50 mm; Paul Marienfeld GmBH, Lauda-Königshofen, Germany) next to each other on the bench and fix at both ends with two layers of adhesive tape.
3. Put a drop of melted agarose (~100 µL) by pipetting in the centre of the cover slide and flatten by dropping a microscope slide on top, immediately (75 × 25 × 1 mm; Paul Marienfeld GmBH, Lauda-Königshofen, Germany). The double-layered tape on both sides of the microscope cover glass serves as a spacer to ensure adequate thickness of the agarose area.

4. Let the agarose harden for at least 5 min and then remove the cover slide from the top of the agarose by carefully slipping it off. Dry the agarose pad before use by leaving on the bench at room temperature for several hours or in a vacuum oven at 100°C for about 1 h. The pads can be stored indefinitely until used.

3.1.2. Preparing DNA Samples

Any DNA including plasmid, phage, or linear DNA as obtained from a PCR reaction can be used for transformation of *C. elegans*. Standard methods are used for purification of DNA for injection ***(23)***. We typically prepare plasmid DNA using a standard alkaline lysis protocol combined with a final purification step using a commercial purification column, followed by isopropanol or ethanol precipitation. DNA is dissolved in either distilled H_2O or TE buffer. Note that DNA prepared by boiling lysis or by phenol-chloroform extraction might be ineffective due to remaining impurities ***(21)***. A critical factor for successful transformation is the final concentration of the DNA. Samples are routinely injected at a concentration of between 50 and 100 µg/mL ***(11)***. Occasionally, lower DNA concentrations may be used to alleviate toxicity problems or lethality due to gene overexpression ***(21)***. We inject DNA dissolved in distilled H_2O or 1× TE buffer. A special microinjection buffer can also be used and is particularly advantageous in cases of direct injection into oocytes ***(13)***. Care should be taken to remove dust and other microparticles which could clog the injection needle.

1. Set up DNA samples for injection to a final volume of 20–50 µL DNA in distilled H_2O, or 1× TE buffer, or 1× microinjection buffer, at a final concentration of 100 µg/mL.
2. Centrifuge the solution in a microcentrifuge at maximum speed for 10–15 min, preferably at 4°C to remove dust and other microparticles.
3. Carefully pipette 3–5 µL from top of the DNA solution to a clean reaction tube and use it to directly load injection needles. The remaining DNA sample can be frozen at –20°C for later use.

3.1.3. Preparing and Loading Microinjection Needles

Pulling a Microinjection Needle

To pull injection needles use borosilicate glass capillaries (Borosil 1.0 mm OD × 0.5–0.75 mm ID; Capillary tubing FHC Inc., Bowdoin, ME; FLG10, or equivalent) and a needle puller (Microelectrode Puller PN-30, Narishige Group, Tokyo, Japan, or equivalent). Pulling needles with proper tips is essential for successful and efficient microinjection: The aim is to pull needles which are sealed at the tip and are opened later by breaking off the closed end of the tip. The tip of the needle should taper constantly and quickly (5–7 mm in length). Stubby tips may result in openings too wide after milling and tips tapering too long and steady might not be stiff enough. Depending on the type of glass

capillaries and needle puller individual settings for making an optimal needle need to be acquired by trial and error. The adjustable parameters of the needle puller are usually heating time, coil temperature, and pulling force which determines pulling speed. Needles can be stored for infinite time in a clean Petri plate modified with wax or double-sided tape as holder. Pre-pulled microinjection needles can also be obtained from several companies.

1. Fix one glass capillary (100 mm) on the needle puller using the two fixing screws. About 4 cm of the capillary should protrude over the sliding unit and about 1 cm at the fixed part of the holder. Close the acrylic hood covering the heating filament.
2. Turn on the machine and choose appropriate settings. For the PN-30 Puller, use magnet (main) 70, magnet (sub) 60 (together these values affect the pulling force and speed of the sliding unit), and heater 80 (determines the temperature of the platinum heater), as the starting values.
3. Start the pulling process of the needle.
4. Open the acrylic hood and the screws. Remove the needle carefully and check the tip under a stereo microscope at high magnification.

Loading the Needle

1. Prepare capillary mouth pipettes: Hold a glass capillary micropipette (Einmal-Mikropipetten intraMARK (100 μL), Brand GMBH, Wertheim, Germany) at the tips and heat its centre over the flame of a Bunsen Brenner until the glass begins to melt. Take the micropipette off the flame and carefully pull it apart, creating a very thin middle part. Break the micropipette at the thin middle part to generate two mouth pipettes.
2. Dip the tip of a mouth pipette into the DNA sample and allow the liquid (estimated amount is about 0.2–0.5 μL) to enter by capillary force.
3. Stick the thin tip of the mouth pipette into the backside opening of a microinjection needle and push it down towards the tip. When close to the tip, blow the liquid out of the mouth pipette and into the injection capillary. Tilt the injection needle to allow the liquid to settle at the tip. More than 50 worms can be injected with one loaded needle.

Breaking the Tip of the Needle

1. Take an agarose pad (*see* **Subheading 3.1.1**) and slightly scratch the edge of the agarose area with a scalpel. Fix the loaded needle to the needle holder of the injection system attached to the inverted DIC (Nomarski) microscope (for microscope details *see* **Note 1**).
2. Focus on the cragged region of the agarose area using the 10× lens and align the needle at a 90° angle to the rough edge of agarose.

3. Change to the 40× lens and bring the tip of the needle and the agarose edge to the same focal plane. Carefully move the edge of the pad towards the needle until the tip bends.
4. Gently tap the sliding sample stage of the microscope towards the needle and break it, creating an opening <1 μm. Apply nitrogen gas pressure to the needle and allow some of the liquid to flow out (*see* **Note 2**). Check for excessive or limited flow which indicates a too-wide or too-narrow opening respectively.
5. Lift the needle along the z-axis into a safe resting position. For an alternative method to break open the needle *see* **Note 3**.

3.1.4. Preparing and Fixing Animals for Microinjection

The number of worms fixed and injected each time on a single agarose pad depends on the experience of the experimenter. It is recommended to start with one animal at a time and progressively raise the number of worms manipulated simultaneously. The limiting factor is the gradual desiccation of animals, which occurs after mounting to the dry agarose pads and depends on the thickness of the agarose layer and the size of the worm *(13)*. Worms shrink and die about 10–15 min after fixation.

1. Prior to the day of microinjection, move worms to be injected, at L4 larval stage, from a freshly grown culture to seeded NGM plates. Let animals grow overnight at 20°C.
2. One hour prior to microinjection, move worms to 15°C, which results in lower locomotion activity making animals easier to handle.
3. Working under a stereo microscope, place a drop of halocarbon oil (Halocarbon oil 700, Sigma-Aldrich Co., St. Louis, MO, Sigma; or Series 700 Halocarbon oil, Halocarbon Products, River Edge, NJ) on a glass slide next to the agarose pad area. Alternatively, Heavy Paraffin Oil can be used (BDH Chemicals, Poole, England; Gallarol-Schlesinger, Carle Place, NY). Transfer the desired amount of worms into the oil (dipping the wormpick into the oil simplifies collecting and transferring the worms). Let animals move in the oil for some minutes to get rid of bacteria attached to their cuticle.
4. Transfer the worms in another drop of oil placed on top of the agarose and gently press them to the agar surface. Alternatively, worms can directly be transferred along with some oil to the clean surface of the agarose. Note that it is impossible to inject moving animals, so be sure that they are attached to the agarose properly.
5. Animals are carefully arranged into position, with their distal gonad arms facing the side of the needle tip (*see* **Fig. 1** and **Note 1**). When injecting several worms at a time align them along their anterior–posterior axis on the pad. These prearrangements simplify the process of injection. Quickly proceed with the final steps of the procedure.

3.1.5. Microinjecting Animals

This step is the most delicate of the protocol: Worm and microinjection needle need to be positioned correctly by using the gliding stage and micromanipulator attached to the inverted DIC (Nomarski) microscope (*see* **Note 1**). For successful microinjection aim at the syncytium of the distal part of one of the gonad arms (*see* **Fig. 1**). Harshly moving the needle into the worm will damage the cuticle or other organs of the animal. Working fast is important to avoid desiccation.

1. After mounting animals on the agarose pad place it on the gliding base of the light microscope and localize the first worm to be injected through the 10× lens. Bring the roughly focused needle tip next to the worm and move the gliding base in order to get a sharp angle of about 20° to 40° between the needle and worm.
2. Focus the worm under the 40× lens and locate the distal gonad arms. Focus on the gonadal syncytium and bring the tip of the needle to the same focal plane next to it (*see* **Fig. 1**).
3. Using the gliding stage gently move the worm towards the needle. Tap the stage gently to move the needle tip into the gonad.
4. Apply nitrogen gas pressure (*see* **Note 2**) to the needle to push the DNA solution into the gonad. Observe the flow of the liquid and the characteristic ballooning of the gonad, which has a maximum capacity estimated to 1–10 pL. Gently remove the needle by moving the gliding stage while constantly applying pressure, which prevents backpressure from the gonad clogging of the needle. For recovering a clogged needle *see* **Note 4**.
5. Repeat for other worms on the agarose pad. Injecting between 10 and 20 animals is usually sufficient to obtain several independent transgenic lines; however, this number can vary considerably for different DNA samples.
6. After injecting all animals on the pad lift the needle along the z-axis into a resting position, remove the agarose pad and quickly proceed with the recovery of the injected worms.

3.1.6. Recovery of Injected Animals

1. To recover the worms, put a drop (about 50 µL) of M9 buffer or recovery buffer (*see* **Note 5**) on top of the halocarbon oil above the agarose area they are attached to. Animals will detach and float away from the agarose and into the recovery buffer.
2. After about 5 min of recovery in the buffer, transfer them with a wormpick into another drop of recovery buffer placed on a NGM agar plate next to the bacterial lawn. Transfer no more than five injected worms to a single plate to allow for easy selection of transgenic individuals among their progeny.

3.1.7. Selecting Transformed Animals and Maintaining Transgenic Nematode Lines

Transformed animals are selected among the F1 progeny of injected worms based on specific phenotypes associated with the appropriate co-injection markers used during injection. The most commonly used co-injection markers for both microinjection and bombardment are presented in **Table 1**.

1. After microinjection let the animals grow at 20°C for about 3–4 days until F1 progeny develop into young adults.
2. Pick transgenic F1 animals, each to one fresh, seeded NGM agar plate. Allow F1 animals to grow and examine their progeny for transformed individuals (*see* also **Note 6**). Keep plates with transgenic F2 animals. These represent independent transgenic lines carrying different extrachromosomal arrays.
3. To maintain transgenic lines, periodically transfer ~10 animals from each plate containing transgenic worms to fresh seeded NGM plates.

3.2. Bombardment

3.2.1. Preparing Animals for Bombardment

Microparticle bombardment transformation requires large numbers of animals *(15–18)*. Growth conditions vary depending on the strain selected to transform **(Table 1)**.

1. Create a master plate by evenly dispersing ~100 worms of the appropriate strain to be transformed on an enriched peptone plate, and completely spread with NA22 bacteria. Let worms grow until they have starved for 2–3 days (~7 days total for *unc-119(ed3)*). At this stage, the plate contains a large population, which consists mainly of L1 larvae. Always maintain master plates for *unc-119* mutant strains, which are slow growing.
   (a) When using the Bio-Rad Biolistic PDS-1000 device without the Hepta adapter, wash off the starved L1 larvae with M9 buffer and transfer approximately one-sixth of the worms onto a 100 mm enriched peptone plate. Grow worms at the appropriate temperature until they become young adults (*see* **Note 7**). Each plate will be used for approximately 1–2 bombardment(s). It is recommended to prepare five or more plates for each transformation.
   (b) For the Hepta adapter protocol, wash off the starved L1 larvae with M9 buffer from the master plate and transfer approximately one-sixth of the worms onto each of six 100 mm enriched peptone plates. Grow worms to starved L1 larvae at the appropriate temperature. Wash off worms from the six plates with M9 buffer. Resuspend worms in 60 mL M9 buffer. Disperse worms evenly on 60 enriched peptone plates. Grow until they become young adults (*see* **Note 7**).

2. Wash off young adults with M9 buffer and transfer to a centrifuge tube. Allow worms to settle by leaving the tube on the bench for 5 min. Remove supernatant and transfer the worm pellet to a dry (1 week post-bacterial spreading) bombardment plate by using a 200 µL micropipette with the end of the tip cut off.
   (a) If using the Bio-Rad Biolistic PDS-1000 device, transfer worm pellet onto a round-shaped bacterial lawn, seeded on dry 60-mm NGM plates. A 0.25-mL worm pellet, harvested from five 100-mm enriched peptone plates, is sufficient for five to six bombardments.
   (b) If using the Bio-Rad Hepta adapter, transfer the worm pellet onto dry 100 mm rich media plates completely seeded with bacteria. A 2–4 mL worm pellet, harvested from sixty 100 mm enriched peptone plates, is sufficient for six to seven bombardments.
3. Leave the covers off the bombardment plates to allow them to dry. To prevent worms from moving off the bacterial lawn, pre-chill plates to 4°C and keep them on ice.

3.2.2. Preparation of Gold Particles

1. Weigh 18 mg of 1 µm gold beads into a siliconized 1.5 mL microcentrifuge tube (*see* **Note 8**).
2. Add 1 mL 70% ethanol. Vortex for 5 min, allow beads to settle for 15 min, short spin in a microcentrifuge, and remove supernatant (*see* **Note 9**).
3. Add 1 mL sterile water. Vortex for 1 min, allow beads to settle for 1 min, short spin in microcentrifuge, and remove the supernatant.
4. Repeat **step 3** three times.
5. Resuspend the gold bead pellet in 300 µL 50% sterile glycerol (*see* **Note 10**).
6. Vortex beads for 5 min and add 10 µL prepared gold beads in a siliconized microcentrifuge tube (*see* **Note 11**).
7. While vortexing on medium speed, add the following reagents per bombardment:
   a. 1 µL DNA (concentration ~1 mg/mL; *see* **Note 12**).
   b. 10 µL of 2.5 M $CaCl_2$.
   c. 4 µL of 0.1 M spermidine.
   Vortex for at least 3 min, allow beads to settle for 1 min, spin briefly in a microcentrifuge, and remove supernatant.
8. Add 30 µL 70% ethanol, flick tube to mix, spin briefly, and remove supernatant (*see* **Note 13**).
9. Add 30 µL 100% ethanol, flick tube to mix, spin briefly, and remove supernatant.

10. Resuspend in 10 µL 100% ethanol. Vortex for at least 3 min to ensure that all large clumps of beads are broken up (*see* **Note 14**).
    a. If not using the Hepta adapter, spread approximately 10 µL of the gold bead/DNA suspension over the central region of a macrocarrier and let dry. Prepare one macrocarrier per bombardment.
    b. If using the Hepta adapter, scale up preparation by preparing three tubes of gold beads/DNA each of which contains a final volume of 100 µL DNA/bead suspension (for each tube increase by tenfold the reagents listed in **steps 6–10**). Transfer 6–7 µL of the beads onto a macrocarrier, spread it around with your pipette tip, and let dry. Only spread on area around the hole in the holder. Repeat for all macrocarriers.

3.2.3. Bombardment Using the Biolistic PDS-100/He Particle Delivery System with the Hepta Adapter

1. Set up the Bio-Rad Biolistic PDS-1000/He particle delivery system with the Hepta adapter following the manufacturer's instructions.
2. Wipe down all the gene gun components with 70% ethanol, then autoclave the Hepta adapter and the macrocarrier holder components. Wipe down the bombardment chamber with 70% ethanol.
3. Place the seven macrocarriers onto the Hepta adapter macrocarrier holder using the special tool.
4. Place a 1,500–2,000 psi rupture disc soaked in isopropanol in the retaining cap of the Hepta adapter, and tighten the adapter onto the chamber.
5. Place Hepta stopping screen and macrocarrier holder in chamber as described in the instrument manual.
6. Secure the uncovered worm plate into lowest shelf of the chamber using a rolled piece of adhesive tape.
7. Evacuate chamber to 26 in. Hg, press fire button, and hold until disc ruptures. Release vacuum and remove plate. Repeat for all six plates.

3.2.4. Bombardment Using the Biolistic PDS-100/He Particle Delivery System Without the Hepta Adapter

1. Set up the Bio-Rad Biolistic PDS-1000/He particle delivery system without the Hepta adapter. The manual accompanying the instrument provides detailed information on the procedure and its requirements.
2. Wipe down all the gene gun components and the bombardment chamber with 70% ethanol.
3. Set up a 0.25 in. gap distance between the rupture disc holder and the macrocarrier launch assembly.
4. Place a 1,350 psi rupture disc into the retaining cap and tighten with the provided torque wrench.

5. Using the macrocarrier insertion tool, place DNA-coated macrocarrier into the macrocarrier holder and screw into the macrocarrier launch assembly according to the manufacturer's instructions.
6. Remove lid and place worm plate on the second target shelf from the bottom.
7. Evacuate chamber to 27 in. Hg, press fire button, and hold until disc ruptures. Release vacuum and remove plate.

3.2.5. Recovery of Animals After Bombardment

1. After bombardment, allow worms to recover for ~1 h.
2. Wash worms off of the bombardment plate with M9 buffer and transfer them to two or more 100 mm enriched peptone plates per bombardment (*see* **Notes 15** and **16**).
3. If using the Hepta adapter, wash off worms from six to seven bombardment plates with M9 buffer, pellet and transfer to 80 (diameter: 100 mm) enriched media plates.
4. Incubate plates at appropriate temperature. Screening for transformed lines depends on the specific transformation marker and selection strategy used.

3.3. Integrating Extrachromosomal Arrays

1. Move 20–30 freshly grown, transgenic worms (at the L4 larva stage) to a clean NGM agar plate and irradiate. Irradiation conditions need to be adjusted empirically for optimal results (~30–70 mJ total energy, ~50% lethality).
2. Transfer irradiated worms to fresh, seeded NGM agar plates (3–5 on each plate). Let them grow at 20°C for 3–4 days.
3. Pick about 500 transgenic F1 progeny from all plates and transfer them each to an individual seeded NGM plate. Let them grow at 20°C for 3–4 days.
4. Pick 2–4 adult worms from individual F2 lines on a single plate. After another 4–5 days observe the F3 generation for integration: Transmission of the transgenic animal phenotype should be close to 100% (for an alternative growing and selection method *see* **Note 17**).
5. To minimize undesirable mutations due to irradiation treatment, integrated lines should be backcrossed several times *(22, 24)*.

4. Notes

1. Working with and microinjecting *C. elegans* requires a stereoscopic microscope and an inverted DIC (Nomarski) microscope with two lenses (10× and 40×), and a gliding stage,

a micromanipulator with fine controls, and a needle holder attached to a nitrogen tank through a pressure adjusting system and an electromechanical valve for controlling pressure applied to the needle. Microscopes and complete systems can be obtained from companies such as the Carl Zeiss AG, Jena, Germany, or Nikon Corporation, Tokyo, Japan, and others. Several sources for obtaining additional information about microscopy for *C. elegans* work are available both online and in the literature *(25)*.

2. The standard nitrogen gas pressure depends on the system used and the type of needle that has been pulled. For long and thin tips it is necessary to raise the pressure; for short and stubby tips apply a lower pressure.
3. The tip of the needle can also be unsealed by dipping into 15 μL of hydrofluoric acid (HF) for about 1 s. Stop the reaction by immediately rinsing it in a drop of distilled H_2O. The size of the opening can be controlled by the time in the HF *(11)*. Depending on the type of needle puller, needles with an open tip can be produced (some trial and error may be required to obtain the proper settings on the machine).
4. If a needle gets clogged during injection the tip can be carefully broken again. Sometimes it is enough to just apply some higher pressure to recover the needle. It is advisable to prepare more than one needle at a time as a replacement backup.
5. After microinjection highly desiccated animals can be recovered more effectively by using a special recovery buffer for a longer time period (up to some hours).
6. Transgenic animals frequently display mosaic expression of transgenes, resulting in a variable phenotype. Mosaicism and low inheritance of extrachromosomal arrays may improve by maintaining the transgenic lines over several generations. It is recommended to obtain and study at least 3 independent transgenic lines.
7. Young adult hermaphrodites with 5–10 eggs inside transform best.
8. Siliconized microcentrifuge tubes prevent beads from sticking to the tube walls.
9. A vortex mixer with a tube holder is particularly useful when handling multiple samples (available from Fisher Scientific).
10. The bead stock can be stored at 4°C for at least 2 weeks. Vortex prepared gold beads for at least 5 min prior to use to ensure complete suspension.
11. Keep gold beads in suspension during transfers.
12. To optimize transformation efficiency try a range of DNA amounts (0.5–2×). When co-transforming plasmids, use equal amounts of DNA.

13. If a ring of beads forms on the tube wall, scrape it off with pipette tip.
14. Resuspending the beads may be difficult of crosslinking by DNA. Vortex manually instead of using the turbomixer. To test resuspension, put 1–2 μL on a slide and view under a compound microscope. You should see single beads and small clusters.
15. Save original bombardment plates, as these will occasionally also yield transformants.
16. You may examine worms under the microscope; beads are visible at 100×.
17. Instead of transferring a high number (~500) of worms to a single plate, worms from **step 2** can be grown to starvation on the same plates (7–14 days, depending on the size of the plate and bacterial lawn). F2 progeny are generated on the same plates. The agar substrate containing starved animals is then cut into pieces of similar size (about 1 cm^2) and transferred to 9-cm NGM plates each. After 2–3 days, 15–20 adult transgenic worms are selected and moved to individual plates. The progeny of these animals are screened for genetic transmission frequencies approaching 100%, which indicates stable integration events. Once integrants are identified, proceed with **step 5**.

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