

Video Article

Generation of Transgenic *C. elegans* by Biolistic Transformation

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Abstract

The number of laboratories using the free living nematode *C. elegans* is rapidly growing. The popularity of this biological model is attributed to a rapid generation time and short life span, easy and inexpensive maintenance, fully sequenced genome, and array of RNAi resources and mutant animals. Additionally, analysis of the *C. elegans* genome revealed a great similarity between worms and higher vertebrates, which suggests that research in worms could be an important adjunct to studies performed in whole mice or cultured cells. A powerful and important part of worm research is the ability to use transgenic animals to study gene localization and function. Transgenic animals can be created either via microinjection of the worm germline or through the use of biolistic bombardment. Bombardment is a newer technique and is less familiar to a number of labs. Here we describe a simple protocol to generate transgenic worms by biolistic bombardment with gold particles using the Bio-Rad PDS-1000 system. Compared with DNA microinjection into hermaphrodite germline, this protocol has the advantage of not requiring special skills from the operator with regards to identifying worm anatomy or performing microinjection. Further multiple transgenic lines are usually obtained from a single bombardment. Also in contrast to microinjection, biolistic bombardment produces transgenic animals with both extrachromosomal arrays and integrated transgenes. The ability to obtain integrated transgenic lines can avoid the use of mutagenic protocols to integrate foreign DNA. In conclusion, biolistic bombardment can be an attractive method for the generation of transgenic animals, especially for investigators not interested in investing the time and effort needed to become skilled at microinjection.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2090/>

Protocol

Overview

Traditionally transgenic worms were generated by microinjecting transgene DNA into the *C. elegans* germline^{1,2,3,4}. While successful, this approach required specialized equipment and the development of experience with worm anatomy and the microinjection technique. More recently biolistic bombardment has been developed as an alternate approach which uses DNA-coated gold particles to introduce foreign DNA into the germline^{5,6}. This approach requires less time investment in terms of practice to become successful.

The biolistic bombardment of *C. elegans* to generate transgenic worms has low efficiency at the level of the individual worm so success requires a large quantity of animals. These can be grown in several ways, but we use egg plates to reduce the work and number of plates involved. Our protocol starts with the preparation of egg plates and worms and then focuses on the bombardment protocol using the Bio-Rad PDS-1000/He Hepta System. We adapted our protocol in part from work of Berezikov et al⁷.

With bombardment, the *unc-119* gene is typically used as a marker for transgenic animals. Worm strains carrying this mutation are severely uncoordinated and barely move, are dumpy in appearance, and are unable to form dauer larvae⁸. The dauer is an alternate larval stage which is used to delay development to a reproductive adult under adverse conditions, and the entry into dauer is regulated by multiple genes⁹. Several strains carrying the *unc-119* gene are available from the *C. elegans* Genetics Center (CGC, Minneapolis, MN). The original DP38 strain (*unc-119(ed3)*) also carries an unrelated dauer-formation constitutive (*daf-c*) mutation which could affect downstream experiments. Several labs have outcrossed this mutation, and the HT1593 strain is available from the CGC. We find that transgenic animals are somewhat easier to identify with the DP38 strain and tend to use this strain for creating transgenic animals. When necessary, we use HT1593 to outcross the transgenic worms to remove the *daf-c* mutation. Getting the DP38 worms to grow is one of the slowest steps in the protocol so we maintain a stock of growing plates while preparing the transgenes.

Expression of the *unc-119* marker along with the gene of interest allows easy identification of the animals expressing the transgene based on rescue of normal motility and body size⁵. The *unc-119* gene can be obtained from several sources, and vectors using the *unc-119* genomic DNA, *unc-119* promoter and cDNA, and the genomic DNA for the smaller *C. briggsiae* gene are used^{8,10}. We recently described a simple protocol to add an *unc-119* cassette to any plasmid containing an ampicillin resistance gene by homologous recombination¹¹ and a method to modify worm

fosmids to carry the *unc-119* gene by Cre-lox recombination to generate transgenic animals¹². The plasmids are available from Addgene Inc. (Cambridge, MA).

While the effort involved in performing a single bombardment is significant but manageable, the additional work involved in performing multiple bombardments on a single day is minimal. Consequently, we routinely cluster the production of transgenic worms to decrease the work involved in generating each.

1. Egg Plate Preparation

The *unc-119* mutant worms are difficult to grow on standard NGM plates because with their impaired mobility they tend to starve on parts of a plate while other parts of the plate still contain food. Egg plates solve this problem as the thick food layer on egg plates allows *unc-119* worms to crawl more easily and to take over all the plate⁷. The egg plates also have the benefit of supporting the growth of a large number of worms so fewer plates are needed¹³. Usually 5 egg plates are sufficient to grow worms for one bombardment. The recipe below makes roughly 50 plates but can be halved to make fewer if desired.

The egg plates can become easily contaminated so we use both antibiotics and antifungal drugs in the plates and only use eggs generated by hypochlorite treatment for seeding the plates.

Day 1:

1. Prepare LB (400 mL) in a 500mL bottle. Autoclave for 20min.
2. Pour ~50 10 cm NGA plates using 1 liter NGA with 2% agar. We add nystatin 10 mL per liter and streptomycin to 200 µg/mL¹³.
3. Grow a 40 mL overnight culture of OP50-1 in LB with 200 µg/mL streptomycin. This strain is streptomycin resistant and available from CGC.
4. Autoclave a 500mL bottle for next day.

Day 2:

1. Separate yolks of 10 chicken eggs into the sterile bottle. We use a yolk separator (available at household stores, including Target). Shake.
2. Bring volume to 400mL with LB. Shake
3. Incubate at 60°C for 1 hour.
4. Cool to room temperature.
5. Add 40mL of OP50-1. Shake
6. Distribute 5-8 mL of mixture per plate.
7. Allow plates to settle at room temperature overnight.

Day 3:

1. Gently pour off remaining liquid from plates. Allow to dry with lids on at room temperature overnight.

Day 4:

1. Put plates into a box or plastic bag and store at 4°C until needed. These plates appear to be OK to use for 1-2 months.

2. Seeding Egg Plates

1. Expand the DP38 worms on 6 cm NGA plates by adding concentrated OP50 to recently starved plates. For a single bombardment, we use ~6 small plates to seed 5 egg plates. For multiple bombardments, we use the small plates to instead seed 2 enriched peptone plates before seeding the egg plates.
2. Isolate eggs from the DP38 worms via the use of hypochlorite^{13,14}. Resuspend the eggs in sterile S-basal or M9 buffers^{13,14}.
3. Add the eggs (>10,000 per plate) to the appropriate number of egg plates. Grow the worms on egg plates at 20°C for 7-10 days to use for bombardment. The plates will be ready to use once the worms have begun to clear the food from the plates. These plates are really hard to starve and growing for longer durations will improve worm yield.

Bombardment

We prepare the gold stock ahead of time and keep it stored at 4°C for use. Also needed are unspotted and spotted 10 cm NGA plates. The unspotted plates need to be poured well in advance and allowed to thoroughly dry to facilitate absorption of the liquid added with the worms.

On the day of the bombardment, we wash off the worms first then start preparing the DNA-coated gold particles. We then add the worms to the unspotted NGA plates and finish washing the gold particles and transferring them to the macrocarriers.

Gold particle preparation:

1. Weigh 60mg of gold particles in a 1.7mL tube and soak in 70% ethanol for 15min. Spin briefly to precipitate the gold particles.
2. Wash 3 times in sterile water and spin briefly each time to collect the gold.
3. Resuspend the gold in 1mL of 50% sterile glycerol. This is the stock gold particle solution and can be stored for months at 4°C.

Worms:

1. Float the DP38 worms off the egg plates with S-basal or M9 buffer and transfer them to a 50mL conical tube.

- Keep the tubes on the bench until the worms are in the bottom. Then aspirate the buffer and add fresh S-basal or M9. Wash 2 or 3 times, until buffer is relatively clear of debris. Keep the worms at this step until the DNA coating is completed.
- Aspirate and leave approx 2-3 mL of worms in buffer per bombardment. Transfer to a 10 cm unspotted NGA plate on ice. It is important minimize the transferred volume as the NGA plate will need to dry completely before bombardment. Be sure to cover the entire surface of the plate with the worms.
- Allow plate to dry on ice. The ice will prevent the worms from clumping while drying.

This step is important: the plate should be dry and the worms evenly dispersed on the NGA plate. Keeping the worms on ice prevents them from moving on the plate and aggregating into piles.

DNA preparation (for one bombardment):

- Mix in a 1.7mL tube:
 - 50 μ l gold particles. Resuspend thoroughly before adding.
 - 50 μ l DNA (10-15 μ g). We see little difference between circular or linear DNA. Usually we use Qiagen midiprep cartridges for DNA purification (Qiagen Inc., Valencia, CA).
 - 50 μ l 2.5M CaCl_2 . This solution is stable for several weeks at room temperature.
 - 20 μ l 0.1M spermidine.
The spermidine is unstable in aqueous solution. We make 16 μ l spermidine aliquots and store at -20°C . We then add 1mL of water right before use to obtain a 0.1M solution. This solution must be prepared fresh for each bombardment.
We usually vortex the gold particles in an 1.7mL tube at a low speed and then add the CaCl_2 , DNA and spermidine/protamine dropwise while vortexing. For the people that have transfected with calcium phosphate this technique is familiar.
We have also used protamine instead of spermidine with very good results¹⁵. We prepare a fresh solution (1mg/mL) in water and use 50 μ l instead of the 20 μ l of spermidine. If we use protamine, we usually incubate the mixture at room temperature for 10 minutes instead of placing on ice. Protamine does not need to be kept frozen and is a powder instead of a liquid.
- Incubate the mixture on ice for 30min, and periodically mix to keep the gold particles in suspension. Then spin briefly and aspirate the supernatant.
- Wash with 300 μ l 70% ethanol. Spin briefly.
- Wash with 1mL 100% ethanol. Spin briefly.
- Resuspend in 170 μ l 100% ethanol.

Preparation of macrocarriers:

- Rinse 7 macrocarriers (Bio-Rad Laboratories, Hercules, CA) in 2-propanol. Put them on tissue paper and let them dry at room temperature.
- Add 20 μ l of DNA/gold mixture at the center of each macrocarrier. Be sure to vortex right before pipetting to keep the gold in suspension.
- Let the ethanol evaporate.

3. Bombardment

Be sure to perform a blank bombardment before the experiment to flush helium through the system.

- Turn on vacuum pump and close vacuum pump trap. We use an oil-free vacuum pump.
- Turn on helium tank. Check that pressure is ≥ 2200 psi.
- Wet rupture disk (1350 psi) in 2-propanol, placing in retaining cap for hepta adapter.
- Screw adapter into PDS-1000 system and tighten with the supplied torque wrench.
- Place the 7 macrocarriers into the holder and seat them with the supplied tool. Then put a hepta stop screen and the bottom on to the holder. Flip the holder over and place on the second shelf from the top. The gold spotted side of the macrocarriers must be facing down at this point.
- Align the holes in the top of the macrocarrier holder with the outlets of the hepta adapter.
- Place the NGA plate coated with worms (lid off) on the lowest shelf in bombardment chamber.
- Completely open the vacuum flow-rate knob on the PDS-1000. Press vac button until chamber reaches 26 In Hg. Then switch to hold position to maintain the vacuum.
- Press and hold the fire button until disk ruptures. This will happen when pressure reaches 1350psi. Sometimes this takes 5-20 seconds to occur. Release the button.
- Release vacuum from chamber (vent position), and remove plate.
- Turn vacuum and helium off.
- Fire several times until the line does not contain helium (helium gauge should mark 0 psi).
- Turn off the PDS-1000 system.

Worm recovery after bombardment:

- Leave the bombarded plate at 20°C for 20 minutes.
- Float the worms from the plate with 10 mL S-basal or M9 buffer.
- Put 0.5mL worms on 20 - 10 cm spotted NGA plates.
- Leave at 20°C or room temperature ($22-24^\circ\text{C}$) for about 2 weeks before checking for rescued worms.
- Screen for worms with the *unc-119(+)* phenotype with a dissecting microscope. The optimal time for screening is after the plates have starved since the rescued worms have survival advantage over non-rescued worms.
- Generate one individual line from each 10 cm plate that contained rescued worms.

4. Representative Results

The success of the protocol with regards to obtaining transgenic animals depends on the particular transgene. For promoter:GFP reporter transgenes we have obtained up to 20 lines. A more typical result is 3-10 lines. Up to 30% of the transgenic lines are integrated lines, but this is random and we will perform multiple bombardments on a single day if an integrated line is particularly desired.

Discussion

Biolistic bombardment is a simple method to introduce foreign DNA into many organisms, including *C. elegans*^{1,5,6,7,16}. It relies upon gold particles forming a complex with DNA in the presence of CaCl₂. Cationic polyamines, such as spermidine, protect DNA from nuclease degradation *in vivo*. Since spermidine is a labile molecule, it is important to store it in small aliquots at -20°C, and make the solution right before performing the bombardment. As we described in the bombardment protocol, protamine can be used instead of spermidine to deliver foreign DNA¹⁵. Protamine has the advantage of being more stable at room temperature and being a powder instead of a viscous liquid.

The *unc-119* rescue gene can be placed either in cis on the same plasmid as the transgene or on a separate plasmid that is mixed with the transgene prior to coating the gold particles. While the mixing of one or more plasmids is usually successful, we and others have found that bombardment of worms with multiple plasmids can create transgenic animals carrying some, but not all the plasmids^{6,11}. To facilitate placing the *unc-119* gene on the transgene plasmid, we recently described a protocol using homologous recombination to insert the *unc-119* gene into the ampicillin resistance gene of almost all plasmids¹¹.

Further, to facilitate moving transgenes from the DP38 strain into worm strains lacking the *unc-119* mutation, we also generated a plasmid with *unc-119* fused to mCherry¹¹. The pan-neuronal mCherry fluorescence can then be used to track the presence of the transgene in a variety of genetic backgrounds¹¹.

Some transgenes might be difficult to detect after bombardment due to weak expression or a stage specific expression. The presence of the transgene can be verified often via the use of PCR using the transgenic worms. For transgenes with weak expression, performing additional bombardments can lead to the identification of lines with stronger expression. Alternately, the use of a compound or confocal microscope can often help with visualizing the expression of fluorescent proteins.

Disclosures

No conflicts of interest declared.

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