

Microinjection into the gonads of *C. elegans* using Eppendorf InjectMan® 4

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Abstract

Caenorhabditis elegans is a well-established model organism for a wide variety of research fields. The simple and highly conserved structure, together with its short generation time and transparent body, renders *C. elegans* a perfect experimental system for genetic alterations.

Microinjection is an established method for generating transgenic *C. elegans* strains. In this Application Note we describe the microinjection procedure using the Eppendorf InjectMan 4 micromanipulator.

Introduction

The nematode *C. elegans* is an established model organism for a wide variety of research fields. The simple and highly conserved structure, together with its short generation time and transparent body, renders *C. elegans* a perfect experimental system for genetic alterations. A further and major increase in popularity came in 1998, when it became the first multicellular organism (and the second eukaryote after *Saccharomyces cerevisiae*) for which a complete genome sequence was obtained [1]. The generation of transgenic *C. elegans* strains by microinjection has been an established method for more than twenty years [2]. This technique is considered an easy approach to introduce nucleic acids into worms. By microinjecting reporter-gene constructs (e.g. green fluorescent protein, GFP) into *C. elegans*, localization and expression studies can be performed. Through microinjection, protein domains and RNA or DNA regulatory elements can be studied.

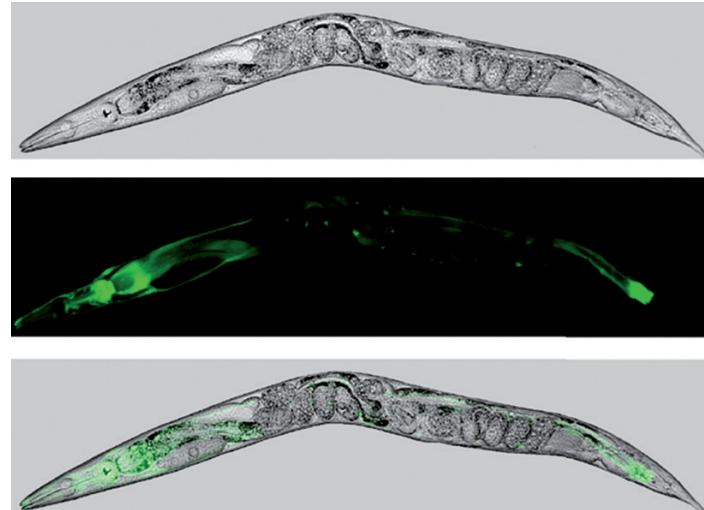


Fig. 1: Successful microinjection of the construct »*promGST44::GFP::pPD95.77*« using InjectMan 4.

Furthermore, a novel *in vivo* approach enables the analysis of protein-protein interaction (DCLA, differential cytolocalization assay). Microinjection of overexpression construct allows gain-of-function analysis or the rescue of deletion mutants. *C. elegans* is a rather large object for microinjection and one can employ relatively simple workstations to achieve sufficient results. Nevertheless, by using the Eppendorf InjectMan 4 micromanipulator (see Figure 2) during a test period in our lab, we achieved higher transgenic rates than with our standard system, the 1st generation TransferMan® micromanipulator.

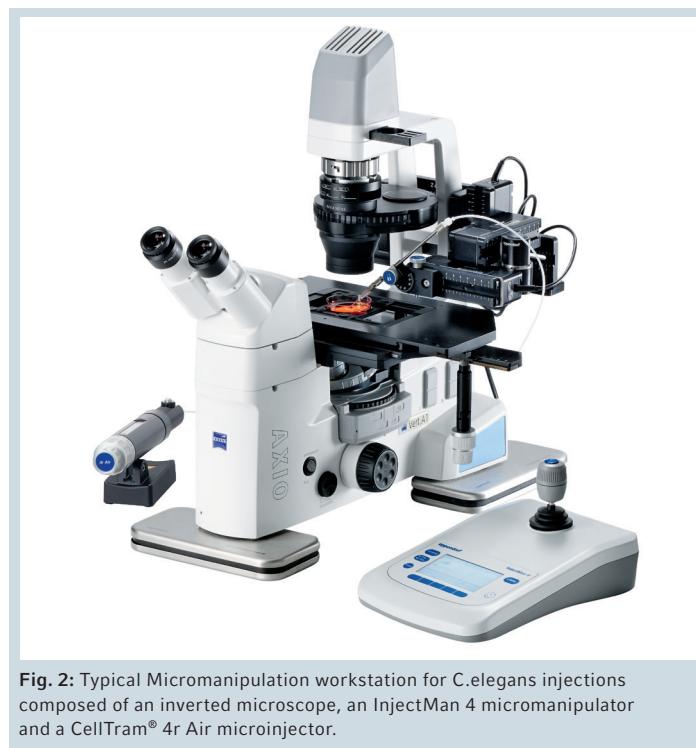


Fig. 2: Typical Micromanipulation workstation for *C.elegans* injections composed of an inverted microscope, an InjectMan 4 micromanipulator and a CellTram® 4r Air microinjector.

Materials and Methods

Equipment

- > Fluorescence Microscope IX50 (Olympus®, Japan)
- > Manual injector made in-house from a 50 mL syringe which is connected to a pressure tube and an Eppendorf Universal capillary holder. The syringe is fixed by a support stand and a Lab Jack (Scissor Jack®). Pressure is applied by lifting the Lab Jack.
- > Biofuge® Fresco (Heraeus®, Germany)
- > InjectMan 4 (Eppendorf®, Germany)

Materials

- > Microloader (Eppendorf, Germany)
- > Femtotips® (Eppendorf, Germany)
- > Cover slips 24 x 32 mm, # 1 (Knittel Glaeser, Germany)
- > Agarose, low melt (Biozym®, Germany)
- > Nematode growth medium (NGM) agarose (Protocol, see [3])
- > Halocarbon oil 700 (Sigma-Aldrich®, Germany)
- > Plasmids pRF4 [2] and pBX [4] are available from Addgene® (<http://www.addgene.org/>)
- > Plasmid »*promGST44::GFP::pPD95.77*«

Worm strains

- > N2 var. Bristol
- > pha-1 (e2123)III [2]
- > Worm strains are available from the *Caenorhabditis Genetics Centre* (CGC, <https://www.cbs.umn.edu/cgc>). Feeding bacteria *E. coli* OP50 can also be obtained from CGC.

Methods

Preparation of injection matrix

Microinjection of worms was performed in halocarbon oil on agarose pads. For the preparation of agarose pads, cover slips were laid out on a clean surface (aluminum foil), without overlapping edges. Agarose (0.16 % in water) was boiled and 100 µL were applied to each cover slip. Cover slips were dried overnight at room temperature.

Preparation of injection mixture

The generation of transgenic worms was either performed by microinjection of a single plasmid (pBX) or by co-injecting a marker plasmid (pRF4) for ease of selection. Offspring were selected via the expression of a co-injected pRF4 vector (a *rol-6* containing plasmid) leading to the roller phenotype. This phenotype develops a severe movement deficiency which is easily observed (see example in Figure 3). A second approach to establish almost stable transgenic lines uses *pha-1* mutant worms. The transcription factor *pha-1* is required for the morphogenesis of the pharynx. In worms carrying the *pha-1* mutation the pharynx fails to undergo differentiation. This interferes with embryonic development at 25 °C, thus facilitating the selection of transgenic worms which are co-transfected with the rescue plasmid pBX. Offspring were then further selected using the GFP signal encoded by the construct vector. For single injection, 120 µg/µL of the plasmid was used. The injection mix for co-injection contained 80 µg of the desired plasmid DNA (construct »*promGST44::GFP::pPD95.77*«) and 50 µg of the pRF4 or pBX-plasmid, respectively. The injection mixture was centrifuged for at least 30 min at 10,000 x g at 4 °C prior to use. The Femtotips were loaded with 2 µL of the mixture. Bubbles in the tip were carefully avoided.

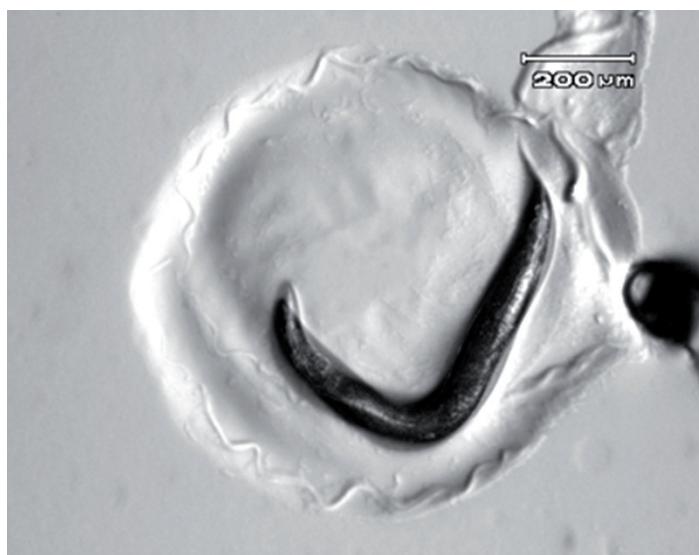


Fig. 3: Phenotype of *rol-6* worms.

Settings of InjectMan 4

The Eppendorf micromanipulation system has a set of special features. The application-specific masks of the InjectMan 4 micromanipulator facilitate the individual workflow process (see Figure 4a). For the microinjection into *C. elegans* we used a personalized program (My application, Figure 4b). Within this application mask, 5 softkeys are freely programmable according to individual needs. The first key was assigned to axial movement (used at fine speed of approx. 20 µm/s). The second key was assigned to the Clean function. This function can be activated to clean the needle from particles adhering to its outer surface by moving the needle out of the medium and back to the working position. The joystick of the InjectMan 4 can be assigned to activate different injection modes either coupled with the programmable Eppendorf injectors like the FemtoJet® (automated injection) or as independent injection movements. For our set-up the joystick key was set/programmed for the activation of »Step injection« (20 µm increments, 500 µm/s speed, axial movement). Injection movement is triggered by pressing the key, injection can be performed as long as the key is pressed, and the needle is retracted by releasing the key. The axial movement, a combination of the X- and Z-motor movements, was set to an injection angle of 15°.

A

Choose Your Application

For permanent storage, press the softkey for 3 seconds (changeable in menu)

| | | | | |
|-------------------|------|---------------|-------|------------|
| | | | | |
| Adher. inject. | ICSI | Dev. biol. | Phys. | My app. |

B

| fine | Joystick key = Inject |
|-------------------------------|--|
| | X = 1061 µm Y = 8520 µm Z = -1482 µm |
| Step inject My application | |
| | |
| Axial | Clean |

Fig. 4a+b: a) Display of InjectMan 4 with application-specific operating masks to be chosen. b) Personalized settings in the application mask »My application«; Softkey 1: Axial – axial movement of the motors can be activated; Softkey 2: Clean – triggering of Clean function – the retraction of the needle to remove particles from the outer surface of the needle.

Injection of worms

The agarose pad was fixed on a metal disk and coated with a small layer of halocarbon oil. Young adult worms were picked and transferred into the oil. Worms were attached to the pad by pressing the head and tail firmly onto the pad using a worm pick (wire-tipped or eyelash). Up to eight worms were attached in a straight line. After fixation, the disk was placed on the microscope stage and the capillary holder was mounted onto the micromanipulator. In general, an injection angle of 15° was applied. The Femtotip microcapillary was inserted into the universal capillary holder of the manual injector. After immersion in the oil, the microcapillary was held close to the worms and the microscopic amplification was set to 200-fold. The permeability of the microcapillary was checked first. If the microcapillary spilled sufficient injection mixture directly after applying the pressure, the actual injection was initiated. Here the microcapillary was positioned shortly above the germ line distal core region (see Figure 5). The InjectMan 4 was set to axial movement and the microcapillary was carefully driven onto the worm's cuticula. On touch, the function »Step injection« was applied by keeping the joystick key pressed.

If the gonads were successfully penetrated, flow pressure was applied. Following injection, the joystick key was released retracting the needle quickly out of the worm. The microcapillary was removed out of the workspace under the microscope using the Home function. The injected worms were carefully transferred onto NGM plates seeded with OP50 bacteria. Here they were washed with M9 and the oil was pushed to the sides. The plates were then incubated for three days and the offspring were checked for either *rol*-phenotype (if co-injected with pRF4, see Figure 1), for survival at 25 °C (if co-injected with pBX) or for GFP signal (if GFP fusion was used as reporter).

Troubleshooting

In case the capillary was blocked, we drove it back and forth through the oil on the agar pad by using the Clean function. If this was not successful, we applied small vibrations by touching the microscope with fingertips to move the needle. If the needle failed to unblock, we drove it gently into the agar pad to remove its upper tip. When the function »Step injection« failed to transfer the needle inside the worm, small taps were applied by fingertips onto the apparatus to penetrate the cuticula.

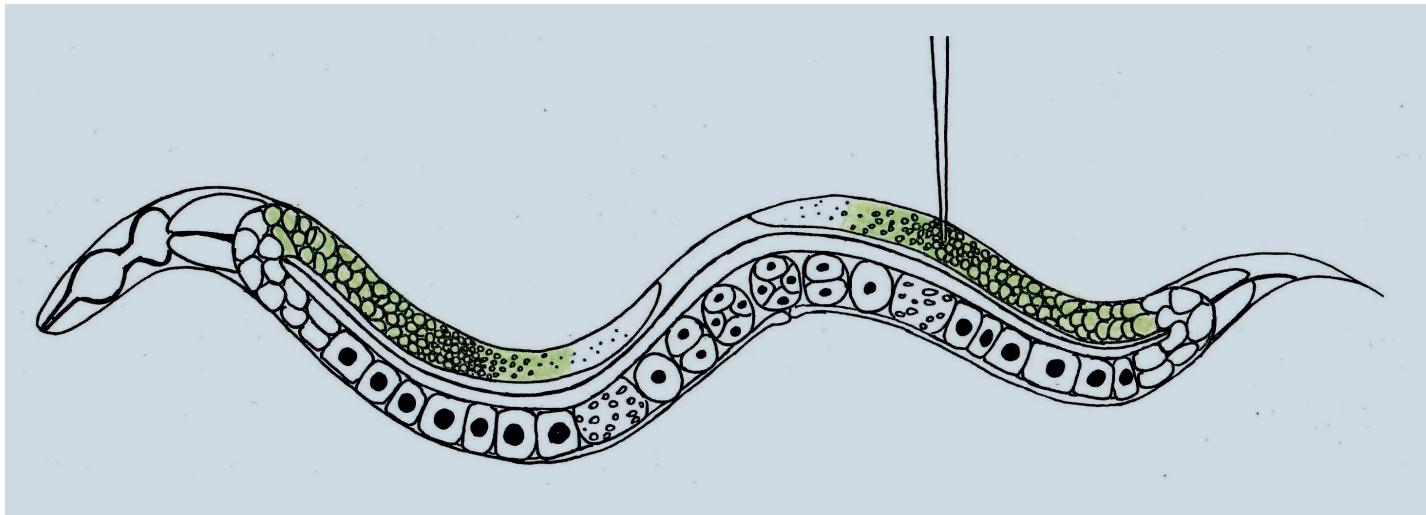


Fig. 5: Microinjection of young adult hermaphrodite into the distal gonad region (colored green).

Results and Discussion

During the testing period of the InjectMan 4 in our laboratory, our success rate for obtaining a positive transgenic worm was greatly improved from 1:50 to about 1:30 in comparison with the regularly used predecessor Eppendorf manipulator model TransferMan. Typically 20 % of F1 transgenic offspring will transmit the array to the F2 generation (see Figure 1). We believe that the increased rate of achieved transgenes can be accredited to the overall improved performance of the micromanipulator InjectMan 4. In detail, by using the

»Step injection« function with individually programmed injection distance and speed, we were able to further optimize our injection process. Being able to inject in an axial or horizontal mode, simply by activating/deactivating a softkey, one can adjust easily to the different sizes or anatomies of worms within one injection experiment. In addition, the device is nearly neutral to external vibrations, a feature which definitely ensures a higher survival rate of injected worms.

Literature

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Ordering Information

| Description | Order no. international | Order no. North America |
|--|-------------------------|-------------------------|
| InjectMan® 4 , Micromanipulator for semiautomatic injection into adherent cells or serial microinjection into small organisms and early stage embryos | 5192 000.019 | 5192 000.027 |
| Femtotips® , Sterile injection capillary, 0.5 µm inner diameter, set of 20 | 5242 952.008 | 930000035 |
| Microloader , Pipette tip for filling Femtotips or other microcapillaries, sterile, 0.5 - 20 µL, 2 racks x 96 pcs. | 5242 956.003 | 930001007 |
| CellTram® 4r Air , Manual pneumatic microinjector, with gears 1:1 and 1:10, for holding and injection | 5196 000.013 | 5196000013 |
| CellTram® 4r Oil , Manual hydraulic microinjector, with gears 1:1 and 1:10, for holding and injection | 5196 000.021 | 5196000021 |
| Capillary holder 4 , For mounting Femtotips®, Femtotip II or capillaries with an outer diameter of 1 mm | 5196 081.005 | 5196081005 |
| Microscope adapter , For mounting the InjectMan® 4 onto an inverted microscope | Available on request | Available on request |

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