Applications

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Microinjection of DNA, RNA and tracer dyes into early fish embryos

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Abstract

Microinjection techniques are widely applied in developmental biology for the analysis of early developmental processes such as gastrulation, neural induction and patterning or organogenesis. Microinjection experiments into vertebrate embryos (e.g. mouse, frog, fish) allow to generate transgenic animals by injection of DNA [1, 2]; to interfere with specific developmental processes by DNA [3], RNA [4], morpholino oligo [5] or small Hairpin DNA [6] injection, or to follow the fate of individual cells by the injection of fluorescent lineage tracer dyes.

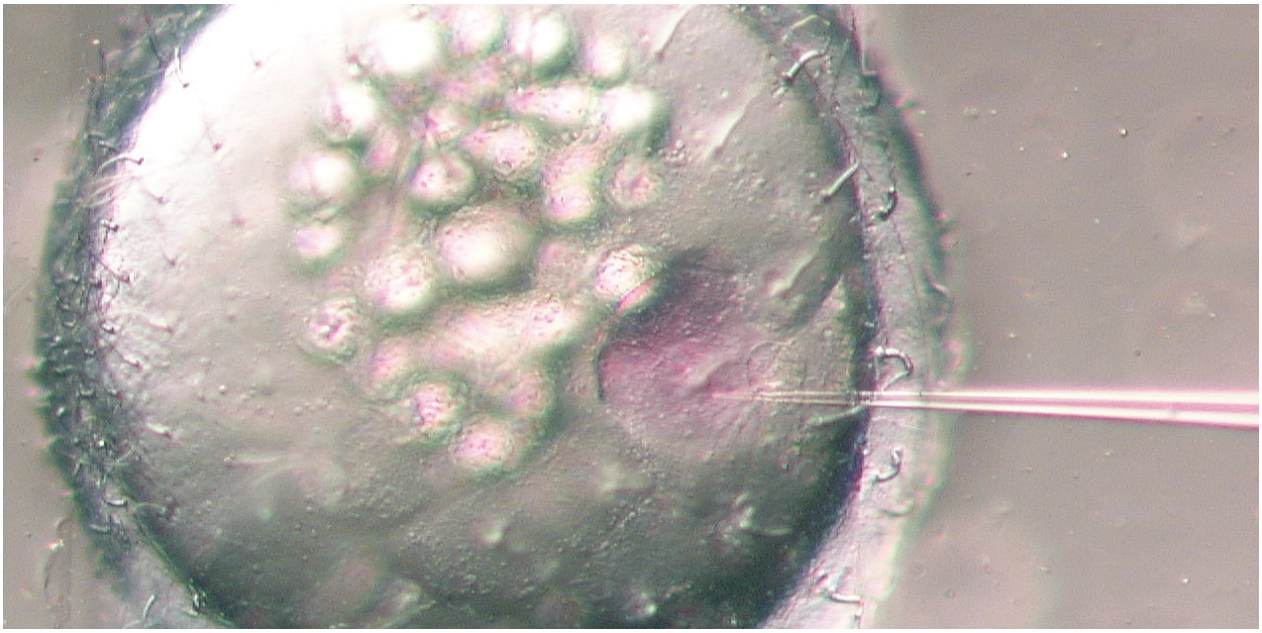

Fig. 1: Microinjection into early fish embryo

Introduction

Gene transfer technology in fish has made a great gain in the last decades. Zebrafish (*Danio rerio*) and Medaka (*Oryzias latipes*) are well-established model organisms used in developmental biology research for the analysis of early developmental processes. Microinjection is one of the leading methods for the production of transgenic fish. In this Application Note, we describe the sample preparation and the injection procedure which is performed with the aid of an Eppendorf Micromanipulator InjectMan NI 2 under a standard dissecting microscope.

Experiments

Medaka and Zebrafish matings are set up as described [7, 8]. Embryos are collected latest 20 minutes after successful mating or as soon as eggs are laid and fertilized. Single embryos are transferred and aligned into the trenches of an agarose mold type injection plate [1] (Fig. 2) using a Pasteur pipette (approximately 20 embryos/trench). Aiming for transient assays or for the generation of stable transgenic lines, the embryos must be at the one-cell stage for consistent results. Medaka embryos may be injected in Yamamoto's embryo rearing medium, chilled to 4 °C to slow down development. It is not necessary to remove the chorion prior to microinjection, as it can be easily penetrated with the injection needle. However, embryos tend to move inside the chorion, so each embryo has to be oriented properly just prior to the injection.

Probe preparation and loading

- All probes (DNA, RNA, dyes) are centrifuged twice at full speed (min. 10,000 x *g*) for 5 min, 90 % of the supernatant is transferred to new, dust-free tubes.
- Probes are loaded from the back to injection needles with Eppendorf Microloaders (2 - 5 µL).

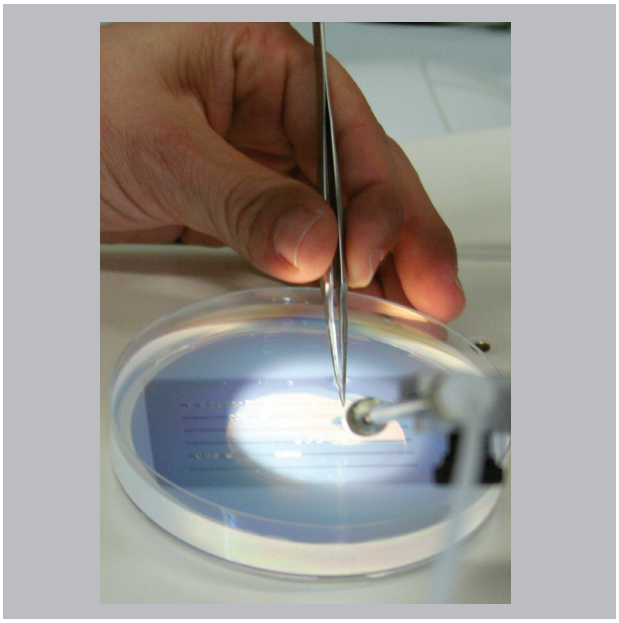

Fig. 2: Agarose mold type injection plate

1) DNA

For transgenesis, the meganuclease system can be used [1, 2]. Plasmid DNA is prepared and purified using a high-purity plasmid preparation kit. DNA concentration and purity can be checked by spectrophotometry. The ratio of $A_{260/A280}$ should be between 1.8-2.0. For transgenesis experiments, DNA is co-injected with the meganuclease (I-SceI). Due to the low stability of the meganuclease, aliquots of enzyme solution should be prepared (e.g. 2 µL)

Fig. 3: Workstation for microinjection into fish embryos; InjectMan NI 2, Zeiss SteREO Discovery V12 and FemtoJet express

upon arrival and stored at -80 °C. The microinjection solution should be prepared shortly before injection and kept on ice. Medaka and Zebrafish may be injected using an identical solution: DNA 10-20 ng/µL, I-SceI buffer 0.5x (optional for Medaka: Yamamoto buffer 0.5x), I-SceI enzyme 0.3 U/µL, ddH_2O ad 30 µL. Results in Medaka are improved by adding Yamamoto buffer. For consistent results it is crucial to inject into the cytoplasm of the embryos and not into the yolk.

- 250 – 500 pL (approx. 1/6 of the cell volume) of plasmid DNA at a concentration of 10 µg/mL (transgenesis experiments) to 50 µg/mL (mosaic transient expression) are injected into the cytoplasm of early embryos.

2) RNA

- Capped mRNA is synthesized *in vitro* using an Ambion "mMessage mMachine" kit.
- RNA is purified through standard purification columns, precipitated and resuspended in RNase-free water.
- The RNA is injected in 1x Ringer´s solution at concentrations of 50 µg/mL up to 1 mg/mL (i.e. from 25 pg to 500 pg RNA per cell).

3) Tracer dyes

- Tracer dyes such as FITC-dextran or rhodamine-dextran are injected at a concentration of 1.5 % in 1x Ringer´s solution.

Eppendorf workstation setup

Devices:
InjectMan NI 2
FemtoJet express
Universal Stand
Consumables:
Eppendorf Femtotips (Medaka) and Femtotips II (Zebrafish)
Eppendorf Microloader
Eppendorf Safe-Lock micro test tubes

Injection procedure for Zebrafish embryos

- For the Eppendorf FemtoJet express microinjector, the starting settings are 80-100 hPa for compensation pressure and 500-700 hPa for injection pressure. The operating mode is set to “manual“.
- The optimal injection time (to inject 15 - 20 % of the cell volume) is established empirically.
- FemtoJet express and InjectMan NI 2 are connected via the interface cable. The InjectMan NI 2 menu function “synchron pressure“ is active. The mode of movement is set to "axial". The microcapillary (e.g. Femtotips II) is brought down and the injection pressure is triggered by pressing the joystick button. Dye or DNA/RNA is injected as long as the button is pressed.
- Once the tip of the microcapillary enters the cytoplasm, up to 500 pL of injection solution containing 10^5 to 10^7 molecules of DNA or RNA are injected.
- Injected embryos are transferred to hatching solution and kept at 28 °C until hatching.

Injection procedure for Medaka embryos

- For the Eppendorf FemtoJet express microinjector, the starting settings and the operating mode are similar to the ones mentioned above.
- The Menu function of the InjectMan NI 2 is set to "stepinject on", and the movement is "axial".
- FemtoJet express and InjectMan NI 2 are connected via the interface cable. The Menu function “synchron immediate“ is active. The mode of movement is set to "axial". The capillary (e.g. Femtotips) is brought down close to the embryo and the injection movement and synchronous pressure is triggered by pressing the joystick button. Dye or DNA/RNA is injected as long as the button is pressed, then the capillary is retracted.
- Injected embryos are transferred to hatching solution and kept at 26 °C until hatching.

Solutions

10x (Yamamoto's isotonic BSS; Ringer´s solution)

NaCl	7.5 g
KCl	0.2 g
$CaCl_2$	0.2 g
$NaHCO_3$	0.02 g

fill up to a final volume of 100 mL with distilled water; adjust pH to 7.3

Hatching solution

NaCl	0.1	%	(w/v)
KCl	0.003	%	(w/v)
$CaCl_2$ x 2 H_2O	0.004	%	(w/v)
$MgSO_4$ x 7 H_2O	0.016	%	(w/v)
methylene blue	0.0001	%	(w/v)

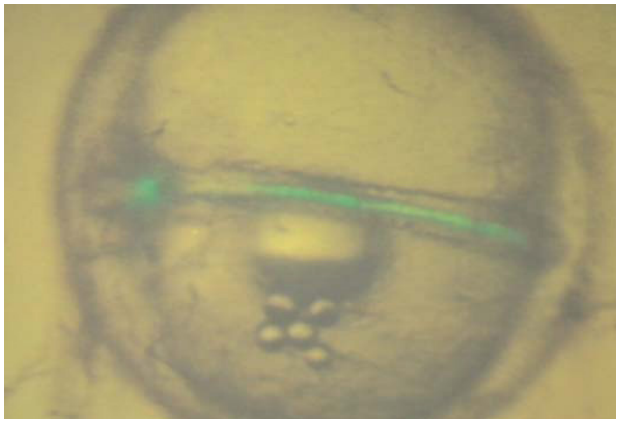

Fig. 4: Developing Zebrafish embryo expressing GFP

Microinjector FemtoJet express

If your research demands injecting volumes greater than 100 picoliters and/or longer series at higher pressures - increasingly seen in functional genomics and developmental biology applications - the FemtoJet express with its external pressure supply delivers the precise and continuous pressure required. Applying the same quality design and compatibility features as our original FemtoJet, the express model is your marathon device for those more demanding applications.

Fig. 5: FemtoJet express, programmable injector with external pressure supply

Literature

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Ordering information

Article	Description	Order no. international	Order no. North America
InjectMan® NI 2	Dynamic micromanipulator for microinjection	5181 000.017	920000029
Foot control	for triggering inject function	5247 623.002	920005098
FemtoJet® express	Programmable microinjector with external pressure supply,	5248 000.050	920010521
Femtotips®	20 sterile glass capillaries for microinjection, 0.5 μm inner diameter	5242 952.008	930000025
Femtotips II	20 sterile glass capillaries for microinjection, 0.5 μm inner diameter	5242 957.000	930000043
Microloader	Pipette tip for filling the microinjection capillaries, set of 2 x 96 pcs.	5242 956.003	930001007
Universal capillary holder*	Including Femtotip adapter and grip head 0 for Femtotips, Femtotips II or microcapillaries	5176 190.002	920007392
Grip head 0*	2 x for microcapillaries, outer diameter 1.0 – 1.1 mm	5176 210.003	920007414
Grip head 1	2 x for microcapillaries, outer diameter 1.2 – 1.3 mm	5176 212.006	920007708
Grip head 2	2 x for microcapillaries, outer diameter 1.4 – 1.5 mm	5176 214.009	920007716
Grip head 3	2 x for microcapillaries, outer diameter 0.7 – 0.9 mm	5176 207.002	920007406
Universal Stand	suitable for the assembly of manipulators independent of the microscope tripod used	5181 250.005	920007406
Research® Plus	0.5 – 10 μL	3120 000.020	3120000020
Centrifuge 5430	incl. standard rotor, aerosol tight, 30 bores for 1.5/2.0 ml microcentrifuge tubes	5427 000.410	022620511

* Universal capillary holder and Grip Head 0 are contained in delivery package of FemtoJet® express

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