BTX Applications

Tech-Trends

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BTX Microinjection of Zebrafish Embryos

A Fully Automated Robotic System for Microinjection of Zebrafish Embryos

Introduction: Despite their relatively large size (~600 µm or ,1.2 mm including chorion), zebrafish embryos have a delicate structure and can be easily damaged, making automated, high-throughput injection difficult. Specific challenges include: (i) to quickly (i.e., in seconds) immobilize a large number of zebrafish embryos; (ii) to automatically, robustly identify cell structures for vision-based position control and account for size differences across embryos; and (iii) to coordinately control two microrobots to maximize operation speed. Addressing these challenges, the objective of this research was thus to develop an effective massive sample preparation method and create a system that is capable of injecting a large number of embryos in the short time window.

Discussion: The operation speed of the automated system (15 embryos with unremoved chorion per minute) compares favorably with the speed of manual injection (10–20 embryos/minute). The embryo holding device permits the completion of immobilizing zebrafish embryos into regular patterns within seconds while manually pushing embryos into agarose trenches, as in the state-of-the-art zebrafish embryo injection, costs minutes. The achieved survival rate of 98% is consistent with the best survival rate achieved by proficient injection technicians. The high survival rate results from efforts in minimizing embryo lysis, by fine tuning parameters such as the micropipette tip size (~10 µm), suction pressure (2–7 InHg), injection speed (2.1 mm/s), and retraction speed (4.1 mm/s), which were determined from trials on another 300 zebrafish embryos during system development. The achieved success rate of 99% demonstrates that the automated system is capable of repeatedly depositing materials at a desired destination inside zebrafish embryos.

Conclusion: The automated microrobotic system is a reliable tool for determining new gene functions and more generally, for facilitating large-scale molecule screening.

A Fully Automated Robotic System for Microinjection of Zebrafish Embryos

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Microinjection procedure: The outbred zebrafish embryos, which were not de-chorionized, were cultured in embryo media that contained 10l reverse osmosis water, 3 g instant ocean salt mix, and 10 ml methylene blue solution (stock = 1 gm/l). For the ease of visually inspecting the injection effectiveness, fluorescent dyes (Rhodamine B, 100 mM) were injected into 350 embryos. Glass capillaries (1.2 mm in outer diameter, TW120F-4, WPI) were heated and pulled using a pipette puller (P-97, Sutter).

Microjection parameters:

Tip diameter:	10 µm
Suction pressure:	2-7 InHg
Injection speed:	2.1 mm/s
Retraction speed:	4.1 mm/s

45-0751 MicroJect 1000 Plus System (PLI-100): MicroJect 1000 pico-injector with Injection, balance, clear/fill and hold pressure. Includes one footswitch, input/output hoses, holding hose, one pipette holder and input adaptor for hoses, power cord and manual.

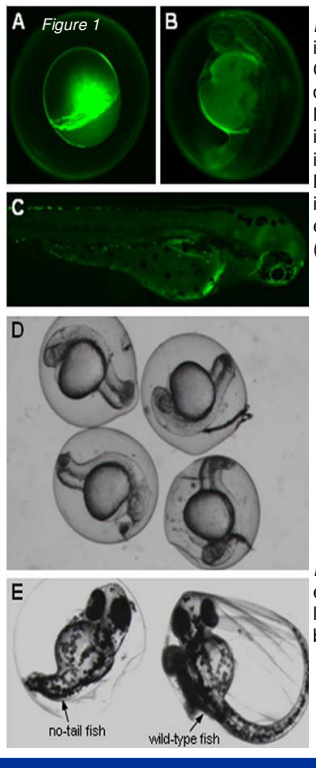

Figure 1: Development of zebrafish embryos injected with fluorescent dyes and ntl-MO. A-C show embryos injected with fluorescent dyes, D-E show embryos injected with ntl-MO. Dye injected embryos are shown immediately following injection (A), 24 hr after injection (B), and 48 hr after injection (C). (D) Ntl-MO injected embryos 24 hr following injection. (E) Comparison of ntl-MO injected embryo (left) with uninjected control embryo (right) 48 hr following injection.

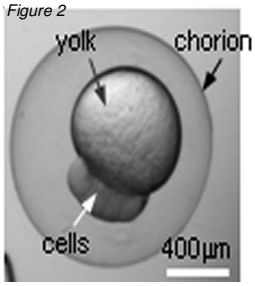

Figure 2: The structure of a zebrafish embryo. Although the embryo is relatively large, it is highly deformable and care must be taken in injection to avoid cell damage.

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