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Microinjecting Cells Using a Constant-Flow Microinjection System

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INTRODUCTION

The efficient delivery of exogenous DNA to cells for expression and function studies is an essential technique of modern cell biology, and direct delivery of genetic material by microinjection remains a reliable means of transfection. In the system described here a constant flow of sample is delivered from the tip of the pipette, and the amount of sample injected into the cell is determined by how long the pipette remains in the cell. The system can be assembled at relatively low cost using commercially available components:

Typically, a pressure regulator that can be adjusted for two pressures (back pressure and injection pressure), a capillary holder, and coarse and fine micromanipulators. A constant-flow system, by design, injects less reproducible cell volumes than does a pulsed-flow system. However, with practice, this difference can be made negligible.

RELATED INFORMATION

In addition to techniques for **Plating Cells for Microinjection** (Dean and Gasiorowski 2011a), two methods are described to prepare injection pipettes (**Preparing Injection Pipettes on a Flaming/Brown Pipette Puller** [Dean and Gasiorowski 2011b] and **Preparing Injection Pipettes on a PUL-1 Micropipette Puller** [Dean and Gasiorowski 2011c]). Once the DNA samples are loaded (see **DNA Sample Preparation and Loading Sample into Pipettes for Microinjection of Cells** [Dean and Gasiorowski 2011d]) they can be delivered either using the system described here or by **Microinjecting Cells Using a Pulsed-Flow Microinjection System** (Dean and Gasiorowski 2011e). The article on **Nonviral Gene Delivery** (Dean and Gasiorowski 2011f) presents additional information on this and other transfection procedures.

MATERIALS

RECIPES: Please see the end of this article for recipes for reagents marked with <R>.

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Cells to be injected

*See **Plating Cells for Microinjection** (Dean and Gasiorowski 2011a) for details on preparation.*

Growth medium appropriate for microinjected cells

Equipment

Footswitch for controlling injection pressure (World Precision Instruments [WPI] 3260)

Microinjection needles, filled with solution to be injected

*Prepare as described in **DNA Sample Preparation and Loading Sample into Pipettes for Microinjection of Cells** (Dean and Gasiorowski 2011d).*

Micromanipulator, manual (e.g., WPI M3301R)

Micropipette holder, 1.0 mm (WPI MPH6S)

Microscope

Nitrogen gas

PicoNozzle kit (WPI 5430-10)

PicoPump (WPI PV820 or PV830)

Tissue culture incubator

METHOD

1. Attach a tank of nitrogen gas to the pressure inlet on the PicoPump.
2. Ensure that the injection pressure is off. Attach a footswitch to the PicoPump via the Remote plug.

The footswitch will be used to control injection pressure, and thus solution flow. Depressing the switch activates the injection pressure.
3. As a starting point, set the holding pressure to 4 pounds per square inch (psi) and the injection pressure to 40 psi.
4. Insert a needle into the 1.0-mm micropipette holder so that the end of the needle just goes through the gasket. Screw the head to the base, locking the needle into the holder.

When the system is pressurized, the needle can become a hazardous projectile. Make sure that the needle is secured into the holder and the injection pressure is not activated before attaching the needle in Step 5.

5. Attach the needle/micropipette holder to a PicoNozzle. Place the PicoNozzle onto the micromanipulator.

6. Advance and lower the needle so that it is just above the medium and over its objective.

7. Lower the needle into the medium.

Make sure that the hold pressure is high enough to prevent the medium from entering the needle, but low enough so that the sample is not being wasted.

8. Focus the microscope on the cell monolayer.

Start with a 10× objective for microinjections. If there is not enough resolution to distinguish nuclei from cytoplasm, change to a 20× objective for the injections themselves.

9. Lower the needle to just above the cells to be injected. Depress the footswitch to ensure that the needle is not clogged and that the sample will flow easily.

The injection pressure might need to be increased or decreased, depending on the sample flow that is seen in the medium.

10. When injections are to begin, press the footswitch to start the sample flowing.

11. Lower the needle rapidly into the cell to be injected and raise it as soon as fluid has been delivered.

Because the time of injection is not set or uniform, leave the needle in the cell until a very slight change in refractive index of the cell or fluid delivery can be detected. It is important not to leave the needle in the cell too long or the cell will die. (Ideally, it should be in the cell for <0.5 sec; with practice, this is easy to master.) The easiest way to do this is to turn the micromanipulator joystick one-quarter turn so that the needle goes down into the cell and almost immediately turn it one-quarter turn in the opposite direction to raise the needle. All micromanipulators can be adjusted to vary the distance traveled by one turn.

12. Continue until all injections are completed or the needle clogs and has to be cleared or replaced.

See Troubleshooting.

13. Incubate microinjected cells under appropriate growth conditions.

See Troubleshooting.

TROUBLESHOOTING

Problem: The needle becomes blocked so that the solution will not flow into the cell.

[Step 12]

Solution: Even with painstaking attention to filtering samples and centrifuging them to remove particulates, clogging will occur. Consider the following:

1. Make sure that the needle-pulling process produces quality needles from which solution can flow. If not, discard the needle and replace it with another one.
2. Increase the injection pressure to get the needle flowing again (i.e., blow out the blockage). Tap the tip to dislodge any particulates (e.g., cell debris or extracellular matrix that becomes attached to needles during the injection process). Try increasing the injection pressure while gently tapping the needle against a cell-free area of the coverslip.
3. Drag the needle across one of the etched spots on the coverslip. Although dragging the needle can cause it to break, a broken needle and a clogged one are equally useless; it is worth the risk if the blockage can be dislodged.
4. If these methods do not work, replace the blocked needle with a new one.

Problem: Microinjected cells become damaged or die.

[Step 13]

Solution: Although great care is taken during the microinjection process to treat the cells gently, a fraction of the injected cells will die or will fail to express the gene product(s).

Three parameters can be adjusted to try to decrease cellular damage and death:

1. Reduce the amount of time a microinjection needle is left in a cell. This will reduce the injected sample volume. Excess sample volume within a cell can result in damage.
2. Reduce the injection pressure, which will also reduce the injected sample volume. Excessive injection pressures can damage cells; fluid exiting a 0.5- μ m diameter tip is expelled at a pressure great enough to damage cellular architecture and function.
3. Adjust the depth of needle penetration in injected cells. Because coverslips are uneven, cells grow at slightly different heights across the coverslip. An injection depth that might be fine for one group of cells could push too far into another set of cells, damaging them. To circumvent this, pay close attention to the injection z-limit.

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