Microinjection into Rice Protoplasts and Mouse ES Cells with Single-cell Manipulation Supporting Robot

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Single-cell manipulation supporting robot (SMSR) has been developed for the high throughput injection of genes, proteins, and drugs into various single-cells. The concept of SMSR is to let the experimenter concentrate his/her attention only on the microinjection by facilitating other associated works.

An experimenter can drive a pair of XYZ-micromanipulators and an XY-automatic stage with a pair of joystick controllers. A switch box for the driving mode selection is set near the joystick. Each joystick has a head switch. Clicking the head switch alters the control mode from the right XYZ-micromanipulator to the XY-automatic stage and vice versa. An injection micropipette is attached to the right micromanipulator and the axial movement of the injection micropipette is controlled with the Z-direction drive controller.

Just on the injection, the moving speed, the minimum step of moving distance, the braking time, and the push-pull moving mode of the tip of the pipette are important parameters. According to the experiences of trained experimenters, 3 speed levels have been provided for SMSR. Consequently SMSR has enabled a smooth and delicate manipulation of the injection micropipette.

The simultaneous microinjection of 2 plasmids (p35S-DsRed, pRCC1-EGFP) into 50-100 cells of rice protoplast was preformed nonstop. The work speed reached as high as 100 cells per 1 h in the case of adhesive cells (Fig.1). In contrast, it was only 6 cells per 1h in the robot-less work. The fluorescent intensity was quantitatively expressed by means of an image analysis program. When DsRed was observed at 24 h, then elicitor was added to induce RCC1 gene. In the successful case, EGFP was observed at 48 h .

The success rates were 5-10% (5/71, 8/70, 4/70) in the case of non-adhesive cells and 7-8% (8/100, 7/100) in the case of adhesive cells. According to the experience of our laboratory in the last decade, this level was same as the success rate achieved by the most skillful expert without any robot. It should be noted that the same level of success rate could be achieved at much higher speed with SMSR.

Another challenge was the microinjection into mouse ES cells. At first, in order to get used to the properties of ES cells, several experimenters tried the microinjection without SMSR. After sufficient trials, some of them succeeded in the microinjection of pCMV-EGFP and obtained fluorescent ES cells for the first time. The success rate varied depending upon the skill of respective experimenters. The number of cells that emitted EGFP fluorescence at 24h in successful cases was 3/274, 7/252, 1/34, and 1/40. In total, the success rate was 2% (12/600). Seven cells out of the successful 12 cells were fluorescent at 48h and 2 cells maintained fluorescence even at 72h.

Then the microinjection was done with use of SMSR. The microinjection could be done very rapidly at 0.43-0.50 min/cell. The number of fluorescent cells at 24h in successful cases was 1/46, 1/432, 1/573, and 2/751. In total, the success rate was 0.3% (5/1802). Four cells out of the successful 5 cells are shown in Fig.2.

SMSR has enabled a much higher throughput of microinjection than ever and has made a long lasting work of microinjection a much less hard work..

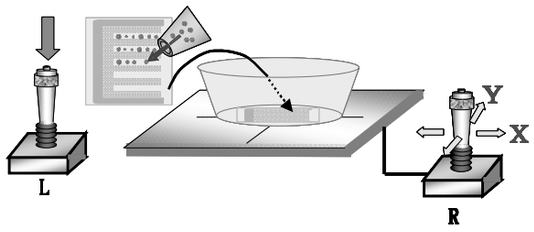

Fig.1 Numbering of adhesive cells. At first, 20-30 cells were sucked in a pipette with the tip diameter of 100mm that was attached on the right manipulator. Then the cells were pushed out one by one and placed in line along the gap of a comb shape frame. After the incubation for 24 h, the XY-addresses of respective cells were registered. XY-automatic stage was driven so that an arbitrary cell came to the center of the microscope view. Then the head switch of the left joystick was clicked. Thus the XY-address of the cell was registered and displayed on a TV monitor. The same protocol was repeated for 50-100 cells.

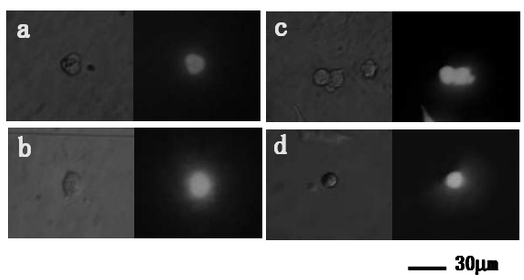

Fig.2 Expression of EGFP gene microinjected in mouse single-ES cells using SMSR. EGFP gene expression was observed at 24 h. The concentration of plasmid in the micropipette: 5 (a), 25 (b), 150 (c, d) ng/ml.