Procedures

(PDF file containing more pictures is available on request)

A. Preparation of micropipettes for DNA injection

1. Pull 75 mm glass capillary microhematocrits (Drummond Scientific, Broomall, PA) using a micropipette puller P-97/ IVF (Sutter Instrument, Novato, CA) under the following conditions: pressure, 500; heat, 800; pull, 30; velocity, 40; time, 1.

2. Cut off pulled pipettes with forceps at ~ 1.2 cm from the shoulder of the pipettes.

3. Mark tips of cut pipettes with a water-resistant magic marker in order to clarify their ends.

4. Mark bodies of the pipettes every 5 mm length using the same marker as above.

(1 span with 5 mm corresponds to 5 µl.)

5. Sterilize the pipettes under the UV lamp in a clean bench for ~15 min.

B. DNA preparation

1. Purify plasmids using the EndoFree Plasmid Kit (QIAGEN, Hilden, Germany) according to the manufacture's protocol with the following minor modifications.

∗Wash the QIAGEN-tip capturing DNA with Buffer QC three times, instead of twice.

∗After 70% ethanol rinse, suspend the DNA pellet with a small amount (300 µl for the Maxi Kit) of TE, and precipitate again by adding NaOAc and ethanol. Then, suspend DNA pellet with 1 mM Tris-HCl (pH7.5), 0.1 mM EDTA.

2. For microinjection, dilute the DNA solution with PBS to a final concentration of 0.1 to 1 mg/ml.

∗Brighter fluorescence was obtained by injection of higher concentrations of DNA, and the intensity of fluorescence seemed to reach plateau at 0.5 mg/ml.

C. Electroporation

1. Anesthetize a timed-pregnant mouse with an intraperioneal injection of 10% Nembutal solution (Nembutal diluted with saline to 10%).

2. Put the mouse on a working plate with the abdomen upside.

3. Cover the abdomen with a piece of folded gauze (7 cm × 15 cm) which has a ~ 3 cm- long slit in its center.

4. Clean the gauze with 70% ethanol.

5. Cut the abdominal skin and wall by scissors.

*While the skin can be cut for ~ 3 cm at the midline, the midline of the abdominal wall should not be cut for well healing.

6. Take out the uterus carefully by pinching gaps between embryos (but not either the placenta or embryos) with ring-forceps.

∗During the surgery, the uterus must be kept wet by dropping warm saline*(saline should be prewarmed at 37°C).
∗It is important to take care not to damage either the placenta or the blood vessels connecting with the uterus.

In utero electroporation

7. Inject 1-3 µl of the DNA solution into the ventricle using a mouth-controlled pipette system with the micropipette.

∗We can inject the solution approximately into an aimed site, because the surface of the telencephalon is visible through the uterin wall, by illumination of a fiber-optics light source.

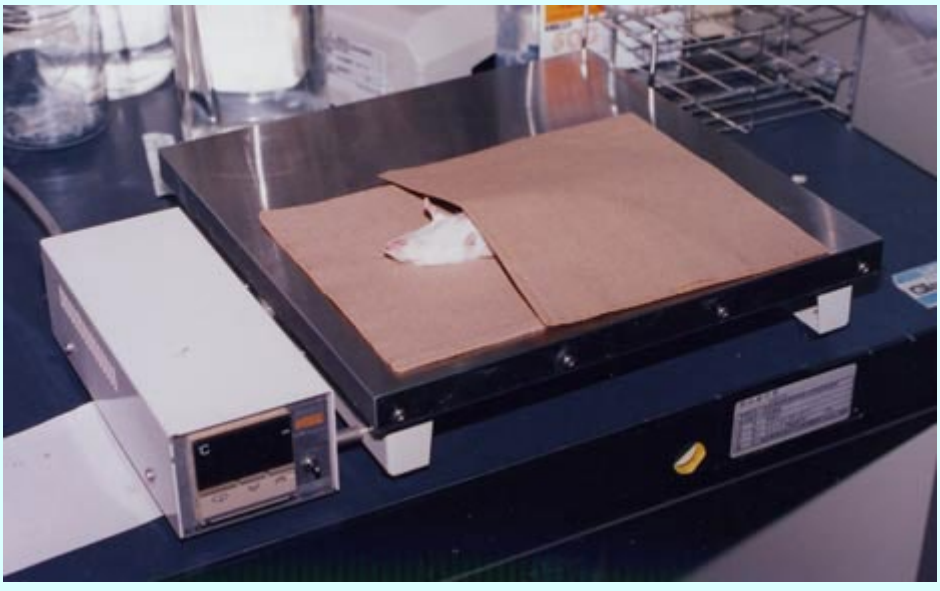
8. Hold a DNA-injected embryo in parallel along its antero-posterior axis through the uterus with forceps-type electrodes, and deliver electric pulses to the embryo.

∗The electric pulses are generated using ElectroSqurePorator T820 (BTX, San Diego CA) and each pulse is for duration of 50 ms.
∗Fill a gap between the electrodes with warm saline*, before electric pulses.
9. Put back the uterus carefully into the abdominal cavity.

10. Fill the cavity with warm saline*.

11. Sew up the abdominal wall and skin with the surgical needle and thread.

12. Warm the mouse on a hot plate at 38°C, until the mouse recovers from the anesthetic.



Exo utero electroporation

7. Cut the uterine wall along the antiplacental side carefully not to break the yolk sac.

*Embryos should be clearly visible through the yolk sac after cutting the uterine wall.

8. Inject 1 to 2 μl of the DNA solution into the ventricle.

9. Hold a DNA-injected embryo through the yolk sac and deliver electric pulses as above.

10. Without sewing the uterine wall, put the embryos back into the abdominal cavity.

11. Fill the cavity with warm saline*.

12. Sew up the abdominal wall and skin as above.

13. Warm the mouse as above.