IN UTERO ELECTROPORATION

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) 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 0.5mg/ml.

C. Electroporation

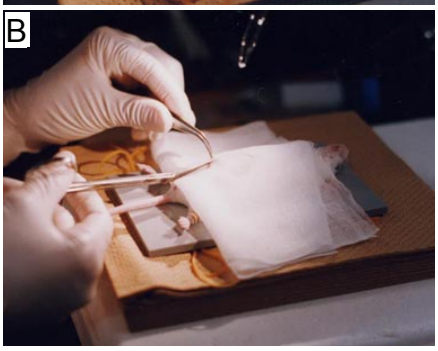
1. Anesthetize a timed-pregnant mouse with an intraperioneal injection of Avertin (125-250 mg per kg of body weight). Five to 10 minutes after injection, toe pinch and corneal reflex methods are used to access the surgery plan.

2. Put the mouse on a working plate with the abdomen upside. Wash with 70% ethanol. Shave the fur over the abdomen using a razor blade.

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

4. Clean the gauze with 70% ethanol.

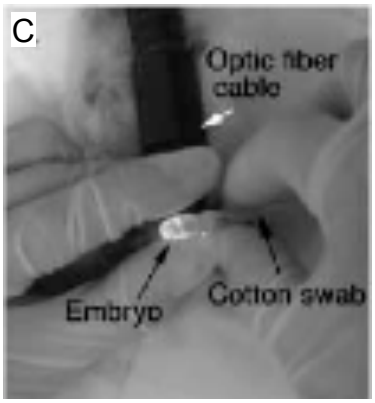
5. Make an incision (2.5 cm or less) in the abdominal cavity using fine scissors (Fig. B). (While the skin can be cut for ~ 3 cm at the midline, the midline of the abdominal wall should not be cut for well healing.)



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

7. During the surgery, the uterus must be kept wet by dropping prewarm (37°C) saline .

8. It is important to take care not to damage either the placenta or the blood vessels connecting with the uterus.



9. Hold the fiberoptic light with the index and middle finger, and place the uterus between the fiberoptic light cable and thumb. Squeeze the uterus gently to push the embryos closer to the uterine wall (Fig. C).

10. Inject 1-3 μl of the DNA solution into the ventricle using a mouth-controlled pipette system with the micropipette (Fig. D and E).

11. 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 (Fig. E).

12. The electric pulses (5x at 50V each) are generated using ElectroSqurePorator T820 (BTX, San Diego CA), and each pulse is for duration of 50 ms with 1s interval.

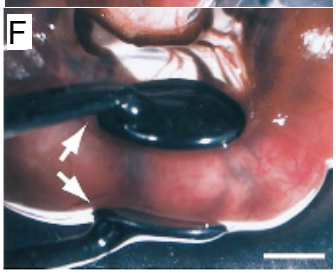
13. Fill a gap between the electrodes with warm saline, before electric pulses.

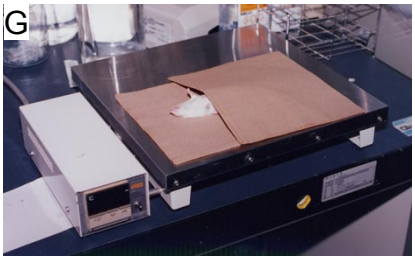
14. Put back the uterus carefully into the abdominal cavity.

15. Fill the cavity with warm saline.

16. Close the surgical incision in the uterine wall with suture and close the skin with a 9-mm Autoclip.

17. Keep the animal warm until recovery from anesthesia (~2 hours) (Fig. G).





E. Troubleshooting

The injection site is difficult to visualize
Visualization is achieved by placing the fiberoptic light at a specific angle relative to the embryo. Rotate the embryos into position by rubbing the surface of uterus with a cotton swab soaked in PBS.

The micropipette damages the uterine wall
The size of the electrodes must be precisely controlled. In order to prevent damage to the uterus, 1 mm from the tip should be no more than 50 μm.

Problem: Electroporation is <50%
Electroporation should be optimized for the age of the embryo and the area of the embryo undergoing electroporation. To maximize transfection efficiency, alter the duration of the pulse, the number of electric pulses, or the pulse voltage as necessary.

Viability is low
Shorter surgical times give better viability. A maximum surgery time of 30 minutes per pregnant dam is critical.

F. Materials

Reagents

Avertin
DNA solution (1 μg/μl)
Ethanol, 70%
Fast Green FCF protein staining reagent (Sigma-Aldrich)
Phosphate buffered saline (PBS)
Pregnant mice (embryonic day 10.5 or older)

Equipment

Warm plate
Fiberoptic light (Leica)
Micro-manipulator (KD scientific)
Pulse generator (BTX 830)
Micropipette puller
Glass capillary tubes (Stoelting Co.)

Instruments

Autoclip, 9 mm (FST)
Cotton gauze
Cotton swabs
Forceps (FST)
Ring forceps (FST)
Razor blade
Suture, 3-0 DEXON II
Scissors

G. Reference

Saito, T. and Nakatsuji, N. (2001). Efficient gene transfer into the embryonic mouse brain using in vivo electroporation. *Dev Biol* **240**, 237-46.
Shimogori, T. and Ogawa, M. (2008). Gene application with in utero electroporation in mouse embryonic brain. *Dev Growth Differ* **50**, 499-506.