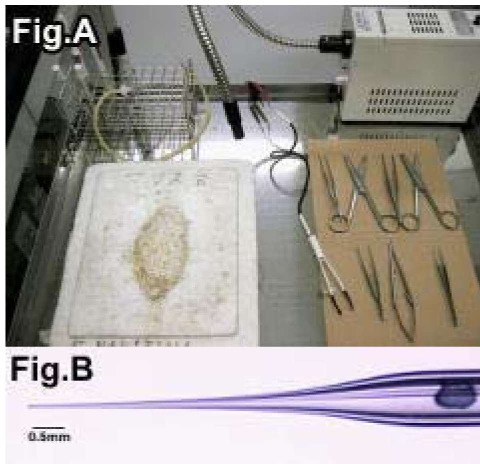
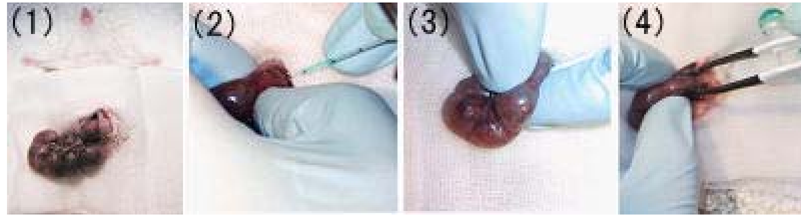
[In Utero] Gene transfer into embryonic brains using in utero electroporation technique



Instruments: Fig. A

- In Vivo Electroporator: NEPA21, CUY21SC or CUY21EDIT (Nepa Gene Co., Ltd.)
- In Vivo Electrode (Nepa Gene Co., Ltd.)
  CUY650P3 (Tweezers w/3mm diameter platinum disk electrode)
  CUY650P5 (Tweezers w/5mm diameter platinum disk electrode)
- Micropipette for DNA injection: Fig. B
  Make the micropipette by a micropipette puller
- Aspirator tube assembly (Drummond)
- Optical fiber light (Technolight, Kenko, #KTS-100RSV)
- Sterile gauze (K-Pine, 7.5cm x 7.5cm)
- Surgical instruments: Fine forceps x 2, Surgery scissors x 2, Ring forceps, Needle holder, Surgical tape
- Nylon suture (Nesco, #HT1605NA75)
- Silk suture (D&G, #112451)



(5) Electroporation Setting

Age	Electrode (diameter)	Voltage	Pulse On	Pulse Off	Number of Pulses
E12.5	3mm	33V	30msec	970ms	4
E13.5	5mm	30V	50msec	950ms	4
E14.5	5mm	33V	50msec	950ms	4
E15-	5mm	35V	50msec	950ms	4

◆ Procedure (In Utero Electroporation)

(1) A 2 cm midline incision is then made in the abdominal wall along the linea alba using a set of forceps and scissors. A piece of sterile gauze with a hole cut in the center is placed over the incision, and one uterine horn is drawn out through the hole in the gauze.

(2) After observing the orientation of the embryos through the wall of the uterine horn, a micropipette is inserted into the lateral ventricle, and 2-5μl of plasmid solution is injected by expiratory pressure using the aspirator tube assembly. When CAG-EGFP is used, a concentration of 1μg/μl is sufficient to visualize the migrating neurons.

(3) After the injection, DNA solution containing 0.01% FastGreen can be seen through the uterine wall (➞).

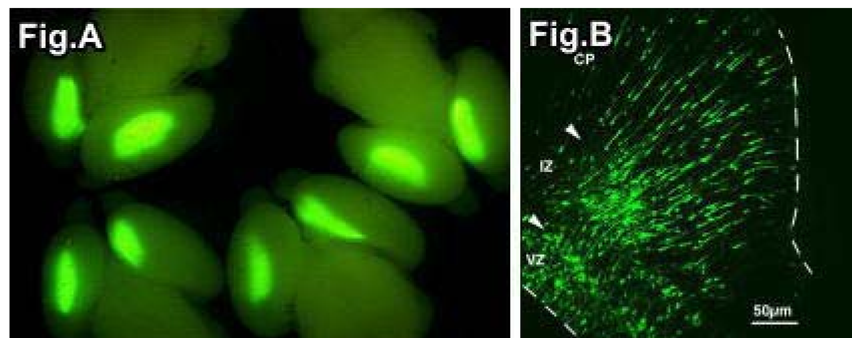
(4) After soaking the uterine horn with PBS, the head of embryo is pinched with a tweezers-type electrode, and electronic pulses are applied with the electroporator.

(5) Electroporation parameters for ICR mouse.
If viability was prioritized over transfection efficiency, the number of pulses can be changed to two.

The actual current is displayed on the screen of the electroporator*. Make sure that the current would become 30-60mA. The current varies according to how the electrode applied or wetness of the uterine horn. Examine the gap between electrodes and the electrode contact areas to fit the current in the appropriate range. If the current is still above the range after the examination, change the voltage setting.
*NEPA21, CUY21SC or CUY21E (Nepa Gene Co., Ltd.)

◆ GFP Expression

CAG-EGFP was injected into the both lateral ventricles of E14.5 mouse embryos and electronic pulses (33V, 50msec) were charged four times. 3 days later, the embryos (E17.5) were fixed and the brains were removed and examined under a fluorescence stereomicroscope (Fig. A).



Fluorescence was observed in the lateral region of the hemisphere onto which the anode had been placed and in the medial region of the opposite hemisphere. And brains were frozen and sliced and the fluorescent image was obtained with a confocal laser microscope (Fig. B). GFP positive cells into which DNA was transferred at the ventricular zone (VZ) migrated to the intermediate zone (IZ) and cortical plate (CP). The arrowheads show the border between VZ and IZ and the border between IZ and CP. Dashed line show the border of tissues.
VZ: Ventricular Zone, IZ: Intermediate Zone, CP: Cortical Plate

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