

In vivo electroporation in the embryonic mouse central nervous system

Tetsuichiro Saito

Department of Developmental Biology, Graduate School of Medicine Chiba University, Chiba 260-8670, Japan. Correspondence should be addressed to T.S. (tesaito@faculty.chiba-u.jp)

Published online 9 November 2006; corrected online 7 and 29 December 2006 (details online); doi:10.1038/nprot.2006.276

This protocol describes a basic method for *in vivo* electroporation in the nervous system of embryonic mice. Delivery of electric pulses following microinjection of DNA into the brain ventricle or the spinal cord central canal enables efficient transfection of genes into the nervous system. Transfection is facilitated by forceps-type electrodes, which hold the uterus and/or the yolk sac containing the embryo. More than ten embryos in a single pregnant mouse can be operated on within 30 min. More than 90% of operated embryos survive and more than 90% of these survivors express the transfected genes appropriately. Gene expression in neurons persists for a long time, even at postnatal stages, after electroporation. Thus, this method could be used to analyze roles of genes not only in embryonic development but also in higher order function of the nervous system, such as learning.

INTRODUCTION

Analyzing gene function in mice

The study of gene function and networks activity in the brain *in vivo* is a key issue, and many novel genes have been identified by genome projects. There are several ways to manipulate genes in mice¹. Transgenic and gene targeting techniques have generated numerous mouse lines in which altered genes are stably transmitted to next generation. Recombinant viruses, liposomes² and biolistic gene guns³ have been used to transfect genes into *in vivo* tissues. However, transfection by liposomes or biolistics is mostly limited to tissues adjacent to the vascular system and superficial tissues such as the skin. Construction of transgenic and gene-targeted mice and recombinant viruses is time-consuming and laborious. Moreover, it is not always easy to express a gene at the time and place required, as the number of transcriptional regulatory sequences, such as enhancers and silencers, that are available to restrict gene expression spatiotemporally is still limited. Thus, quick and easy methods of gene transfer in mice will greatly facilitate our understanding of gene function and networks *in vivo*.

In vivo electroporation: an overview

Electroporation has been widely used to introduce DNA into various types of cells and tissues: prokaryotic⁴ and eukaryotic cells^{5,6}, mouse muscle⁷, *in ovo* chick embryos^{8,9} and *in vitro* mouse embryos⁹. In most cases, electric pulses are delivered in a solution containing cells, or by inserting electrodes into tissues. Previously, we have shown that genes can be successfully transfected even into cells that are not in the proximity of electrodes by delivering electric pulses to the mouse embryo from outside the uterus, and that the transfected genes are expressed in a restricted area of the nervous system¹⁰. This *in vivo* electroporation has been successfully applied to analyze gene function^{10–16} and transcriptional regulation^{14,17}.

In this method, DNA is microinjected into the brain ventricle or the spinal cord central canal of the embryo, and square-wave electric pulses are delivered with forceps-type electrodes. Treated embryos survive in the uterus, are born and reared by their mother. Genes are transfected into cells that are located adjacent to the ventricle or the central canal^{10,11,18}. As many of the transfected cells are neuronal progenitors, they and their descendant neurons

express the transfected genes. To see the embryo more clearly during the procedure, the uterine wall can be cut before DNA injection¹⁰. Operated embryos that are connected to the placenta can survive in the yolk sac inside the pregnant mouse, and pups can be recovered by cesarean section and reared by a foster mother. Electroporation outside the uterine wall is described as *ex utero* electroporation, whereas *in utero* electroporation describes the procedure when the uterine wall is left uncut. *In vivo* electroporation describes both *in utero* and *ex utero* procedures. Genes have been successfully transfected to the telencephalon^{10,13,15–19}, diencephalon¹⁰, midbrain¹⁰, hindbrain²⁰ and spinal cord^{11,12,14}.

Advantages of *in vivo* electroporation

In vivo electroporation has the following advantageous features:

The procedure is simple and quick. *In vivo* electroporation for gene transfer into more than ten embryos can be carried out within 30 min.

Transfection efficiency is high (see **Table 1**). However, despite this high efficiency, cytotoxicity remains low. No significant increase in cell death has been detected after electroporation (ref. 18; TS, unpublished results).

Multiple genes on different plasmids can be simultaneously transfected into the same cells¹⁰, indicating that this method can be used to analyze combined function of genes. In contrast, transfer of multiple genes is not easy for recombinant viral systems, because the size of DNA that can be incorporated into viral particles is limited.

Plasmids that are larger than 10 kb can be successfully transfected^{10,18}.

Transfection is unidirectional. Only the side of the ventricle that is closest to the anode is transfected^{10,11}. This feature is useful for analyzing gene function, as the non-transfected side serves as a negative control on the same section.

Transfection is temporally restricted. Expression of a transfected gene is limited to particular types of neurons because generation of neuronal types is dependent on embryonic stage. In the cerebral cortex, progenitors that are transfected at a particular stage give rise to neurons of specific layers^{10,18}. Distinct genes can be expressed by neurons of different layers by double electroporation at two different stages¹⁸.



TABLE 1 | Optimal voltages at several embryonic stages of *in vivo* electroporation.

Embryonic stage	Voltage (V)	Surviving embryos (%)	EYFP ⁺ embryos (%)
E11.5	22	70.8 ± 6.4	82.6 ± 2.3
E12.5	30	86.9 ± 5.3	97.2 ± 2.8
E13.5	40	96.1 ± 1.4	93.8 ± 2.5
E15.5	45	92.2 ± 7.8	97.8 ± 2.2

Exo utero electroporation was performed for E11.5 and E12.5 embryos. Five electric pulses were delivered. To examine efficiency of *in vivo* electroporation, 2 μl of 0.5 μg μl⁻¹ of a reporter plasmid, pCAG-EYFP, was microinjected. Survival and EYFP⁺ rates (percentages) were calculated for every litter from the number of surviving embryos/operated and EYFP-positive/surviving embryos, respectively. The data are represented as mean ± s.e.m. EYFP, enhanced yellow fluorescent protein.

Operated *in vivo* embryos survive for months, in contrast with *in vitro* embryos, which develop normally in a culture only for 36 h¹.

Gene expression in postmitotic neurons usually persists for a long time, up to about 4 months after electroporation, possibly because transfected plasmids are not diluted in non-dividing neurons. The persistent gene expression in neurons implicates that this method can be used to analyze gene function not only in the embryo but also in the adult. One exception is cerebellar granule cells, which continue to divide actively even at postnatal stages, and expression levels of transfected genes decrease in accordance with their divisions (D Kawauchi and TS, unpublished results).

Purified plasmids are used for *in vivo* electroporation. Ubiquitous promoters, such as the CAG promoter, drive gene expression in all transfected cells¹⁰. Morphology and migration of neural progenitors and their descendant neurons are clearly visualized by using a fluorescent protein gene downstream of the promoter, and the protein has facilitated functional analysis of genes in those cells. Although transfection can be confined to areas close to the injection site of DNA¹⁰, gene expression can be further restricted to some types of cells by using other regulatory sequences. After electroporation, the *nestin* and *Sox2* enhancers activate gene expression specifically in neural progenitor cells¹⁷, as in the case of transgenic mice. The *Mbh1* enhancer functions in *Math1*-expressing cells¹⁴, consistent with *in vivo* expression and regulation of these genes.

In vivo analysis of transcriptional regulation, which mostly required construction of transgenic mice, is carried out more quickly by *in vivo* electroporation. Furthermore, quantitative assays of transcription can be carried out by using lysates of electroporated tissues¹⁴. These assays are very useful, especially when appropriate cell lines are not available.

Not only gain of function but also loss of function can be examined by *in vivo* electroporation. Gene function has been repressed in chicks

by introduction of double-stranded RNAs using *in ovo* electroporation²¹. Recently, mammalian genes have been knocked down by using *in vivo* electroporation to transfect cells with short interfering RNAs¹⁶ and plasmids that produce short hairpin RNAs^{22,23}. Expression of a transfected gene has been restricted to a specific embryonic stage by recombination-based elimination of the gene using the Cre recombinase-loxP system¹⁸. Similarly, genes could be spatio-temporally knocked out by electroporating *Cre* into mice carrying the genes that are flanked by loxP sites.

Forceps-type electrodes have been successfully used for other tissues. The testis and retina are transfected after DNA injection into the seminiferous tubule²⁴ and subretinal space²⁵, respectively. Cells adjacent to a closed space that does not allow extensive dilution of the injected DNA (such as the ventricle) will be good targets for *in vivo* electroporation, although all types of cells appear to be transfectable.

Limitations of *in vivo* electroporation

A drawback of *in vivo* electroporation is that strong electric pulses affect heart rhythm, which may lead to embryonic death. It appears to be important to minimize electric shocks to the heart for embryonic survival. Half-ring-type electrodes, which were designed not to cover the heart, have helped overcome this problem¹¹. Another drawback of the technique is that gene expression is limited to the operated mice and is not transferred to their offspring, except in the case of the testis.

Here, we provide a detailed protocol for carrying out *in vivo* electroporation to the central nervous system of embryonic mice. Any DNA construct (less than 14 kb in size so far tested) can be transfected using this method, and the protocol can be adapted to target various regions of the embryo. Thus, *in vivo* electroporation is a quick and relatively simple technique that can be readily modified to investigate many aspects of gene function.

MATERIALS

REAGENTS

- Mice (see REAGENT SETUP).
- EndoFree Plasmid Kit (Qiagen, cat. nos. 12362, 12381 and 12391)
- TE: 10 mM Tris-HCl (pH 7.5), 1 mM EDTA
- 70% ethanol/30% TE (vol/vol)
- 3 M sodium acetate (NaOAc)
- Ethanol
- 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA
- 10× PBS: 1.37 M NaCl, 27 mM KCl, 100 mM Na₂HPO₄, 20 mM KH₂PO₄
- 10% Nembutal (Nembutal (Abbott Laboratories)/saline (1:9, vol/vol)).

- **! CAUTION** Nembutal is a non-selective central nervous system depressant. It must be stored in a locked location and its use must be properly recorded.
- 70% ethanol (ethanol/H₂O (7:3, vol/vol))
- Saline, prewarmed at 37 °C
- Indigocarmine (Daiichi Pharmaceutical)
- **▲ CRITICAL** All reagents except mice must be sterile.

EQUIPMENT

- Glass capillary microhematocrit tube (Drummond Scientific, 75 mm)
- Micropipette puller (Sutter Instrument, cat. no. P-97/IVF)
- Watchmaker's #5 forceps
- Clean bench
- Operating board: an 85 mm × 135 mm plastic board with four small holes (distances between holes are shown in Fig. 1), through which rubber bands are passed
- Paper towel
- Gauze
- Scissors
- Curved forceps (Natsume Seisakusyo, cat. no. A14)
- Ring forceps (Natsume Seisakusyo, cat. no. A26)
- Peristaltic pump and tube: any type of pump and tube can be used, if they are fit with warm saline delivery (see below)

PROTOCOL

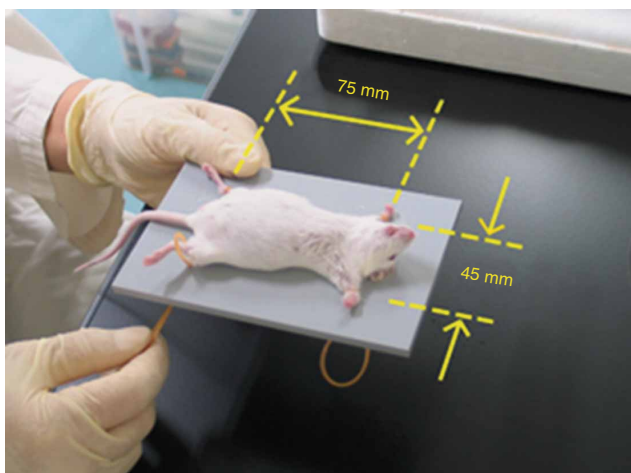


Figure 1 | A pregnant mouse on the operating board. The limb is fixed by pulling down the rubber band through the hole.

- Bath incubator: any incubator that is set at 37 °C can be used for warm saline delivery (see below)
- Mouth-controlled pipette (Drummond Scientific)
- Fiber optic light source
- Electroporator: CUY21Edit (Nepagene) or ElectroSquarePorator T820 (BTX)
- Switch box (Nepagene, cat. no. CU902)
- Forceps-type electrodes (Nepagene, cat. nos. CUY650P3, CUY650P5 and CUY650P10, diameters of which are 3, 5 and 10 mm, respectively)
- Petri dish
- Needled suture (Natsume Seisakusyo, cat. no. F17-50 braided silk)
- Slide warmer
- For further details, see <http://www.m.chiba-u.ac.jp/class/dev/protocol/apparatus.html>.

PROCEDURE

Preparation of animals for *in vivo* electroporation

- 1| Anesthetize a timed-pregnant mouse with an intraperitoneal injection of 10% Nembutal solution.
- 2| Place the mouse on its back on the operating board. Place the limbs through the rubber bands and fix in position by pulling rubber bands downwards (**Fig. 1**). Place a stack of paper towels under the operating board to absorb spilled saline.
- 3| Cover the abdomen with a piece of folded gauze (~70 mm × ~150 mm) that has an ~30-mm-long slit in its center.
- 4| Drench the gauze with 70% ethanol.
- 5| Pinch the skin through the slit with curved forceps and make an ~30-mm-long midline incision through the skin and then the abdominal wall with scissors.
- 6| Electroporation can be carried out *in utero* (option A) or *exo utero* (option B).

▲ **CRITICAL STEP** *Exo utero* electroporation is required for embryos that are E12.5 or younger to see them clearly. It can also be used for embryos older than E12.5, if necessary. It is advised to master *in utero* electroporation first, because *exo utero* electroporation requires more careful handling of the embryo.

(A) *In utero* electroporation

- (i) Attach ring forceps to the uterus at a gap between embryos and carefully pull the uterus out of the abdominal cavity (**Fig. 3**). Be careful not to attach the forceps to the placenta or embryos.

REAGENT SETUP

Mouse strains ICR mice (Clea, Japan) are used in most cases, because they bear many, usually more than ten, pups. Other mouse strains, such as C57BL/6, can also be used. The noon of a day when a vaginal plug is found is designated as embryonic day (E) 0.5. The day of birth is designated postnatal day (P) 0.

! **CAUTION** All experiments should be performed in accordance with the protocols approved by the institutional animal care and use committee.

Preparation of plasmid DNA Plasmids are purified using the EndoFree Plasmid Kit according to the manufacturer's protocol with the following minor modifications: (i) wash the QIAGEN-tip capturing DNA with Buffer QC (wash buffer in the kit) three times, instead of twice; (ii) after 70% ethanol rinse, suspend the DNA pellet with a small amount (300 µl for the Maxi Kit) of TE and precipitate DNA again with 1/30 vol of 3 M NaOAc and 2.5 vol of ethanol. Centrifuge, air-dry and suspend the DNA pellet with 1 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA. Before *in vivo* electroporation, dilute the DNA solution with 10× PBS and H₂O to a final concentration of 30–300 nM (0.1–1 µg µl⁻¹, if pCAG-EYFP is used) in PBS. pCAG-EYFP carries the *EYFP* (enhanced yellow fluorescent protein) gene downstream of the CAG promoter, and its size is 5.5 kb¹⁰.

▲ **CRITICAL** Plasmids, such as pCAG-EYFP, that carry a fluorescent protein gene will be useful to evaluate transfection efficiency, because their fluorescence is easily detected. Higher levels of gene expression are obtained by injection of higher concentrations of DNA, and expression levels appear to plateau at 150 nM (0.5 µg µl⁻¹, if pCAG-EYFP is used). Purified plasmids should be handled on a clean bench to avoid pathogenic contamination.

EQUIPMENT SETUP

Preparation of micropipettes for DNA injection Pull glass capillary microhematocrit tubes using the micropipette puller, P-97/IVF, under the following conditions: pressure, 500; heat, 800; pull, 30; velocity, 40; time, 1. Then, break pulled pipettes to an ~60 µm external diameter by pinching them with watchmaker's #5 forceps. Label tips of the pipettes with a water-resistant magic marker in order to find the tips easily. Draw lines every 5 mm on the pipettes using the marker to measure the volume of injected solution—5 mm corresponds to 5 µl. Sterilize the pipettes under UV light on a clean bench for ~15 min.

Warm saline delivery Warm saline is dropped from the end of a tube (**Fig. 2a**) by a peristaltic pump (**Fig. 2b**). The other end of the tube is inserted into a saline-containing bottle (**Fig. 2c**) in a 37 °C bath incubator (**Fig. 2d**). One saline drop should be ~0.1 ml. The pump should be controlled by an on/off foot switch to make your hands free.

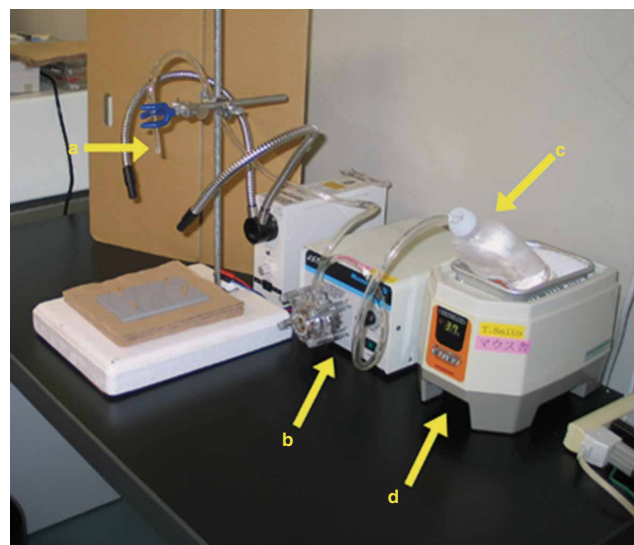


Figure 2 | Warm saline delivery.

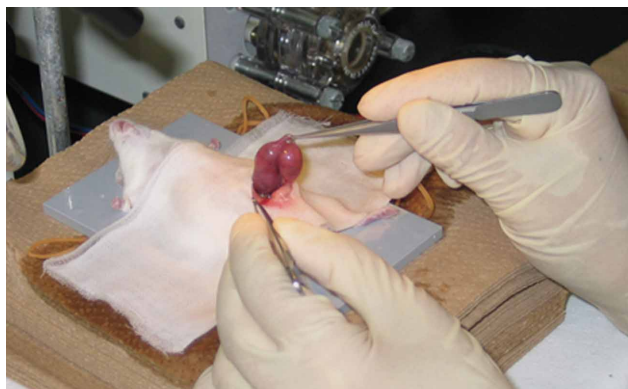


Figure 3 | The uterus should be carefully pulled out with ring forceps.

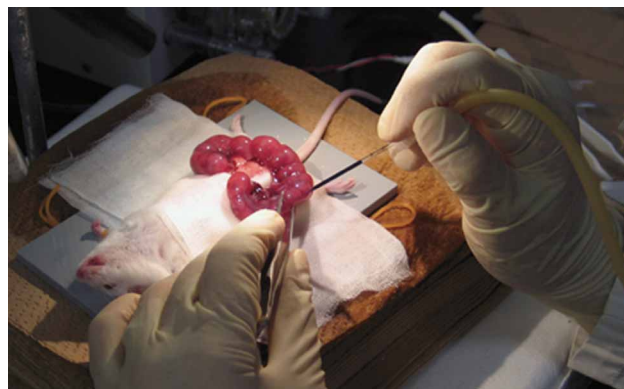


Figure 4 | DNA injection into the *in utero* embryo. Indigocarmine is used to show the micropipette.

▲ CRITICAL STEP During surgery, the uterus must be kept wet with warm saline from the delivery system. Damage to the placenta and blood vessels of the mesometrium should be minimized; be careful not to pinch them with forceps or scratch them on the gauze.

? TROUBLESHOOTING

- (ii) Inject 1–2 μ l DNA solution into the brain ventricle or the spinal cord central canal using the mouth-controlled micropipette under the illumination of a fiber optic light source (**Fig. 4**).

▲ CRITICAL STEP It is strongly advised to master injection first. A dye, such as Indigocarmine, is useful for practice, because it enables the site and shape of the ventricle to be clearly visualized if injection is successful. It is important to know how and where to insert the micropipette based on the locations of embryonic brain blood vessels, eye and nose. Try E14.5 embryos first, because they are the easiest to inject. If it is hard to find injection sites, practice after cutting the uterine wall as described in *ex vivo* electroporation. The dye should be used only for practice, as it may have possible side effects in experimental embryos.

? TROUBLESHOOTING

- (iii) Hold the DNA-injected embryo through the uterus, parallel to the embryonic anteroposterior axis, with forceps-type electrodes (**Fig. 5**) and deliver three to five electric pulses with an electroporator. The electric pulses are delivered every second, with a duration of 50 ms per pulse. Optimal voltages are shown in **Table 1**. **! CAUTION** Do not use extremely high voltages that are higher than enough for *in vivo* electroporation.

The voltages shown in **Table 1** should be used for all types of electrodes. Five pulses are optimal¹⁰. Before and after holding the embryo, the electrodes should be wet with saline by dipping them in a saline-containing Petri dish. To avoid damage to the placenta and blood vessels of the mesometrium, do not hold them with electrodes during electroporation. If the surface of the uterus is dry before delivering electric pulses, drop warm saline between electrodes. DNA can be transfected into both sides of the ventricle, if necessary, by delivering three or four electric pulses and then the same number of pulses in the reverse direction. The direction of current can be easily reversed by a button on the switch box.

▲ CRITICAL STEP Choose appropriate electrodes that cover a region where gene transfer is desired, but not the heart. Electric shocks to the heart should be minimized.

? TROUBLESHOOTING

- (iv) Reposition the uterus carefully into the abdominal cavity.

▲ CRITICAL STEP The uterus should be peeled gently and carefully from the gauze by drenching it with warm saline, to avoid damage to the blood vessels of the mesometrium.

? TROUBLESHOOTING

- (v) Fill the abdominal cavity with warm saline.
- (vi) Close the abdominal wall and then the skin with a needled suture.

▲ CRITICAL STEP The abdominal wall should be sutured closed sufficiently tightly to prevent leakage of saline.
- (vii) Warm the mouse in a metal cage on a slide warmer at 38 °C, until the mouse recovers from the anesthetic. It is usually awake within ~ 1.5 h.

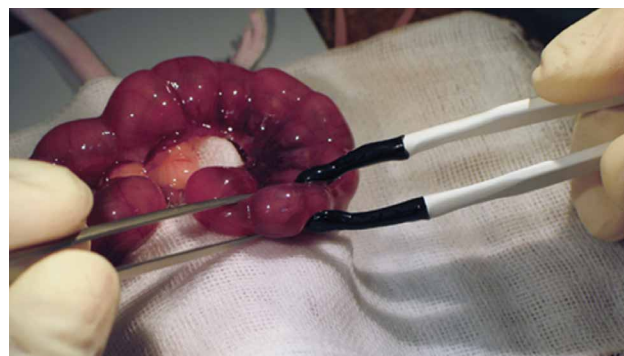


Figure 5 | The *in utero* embryo is held with forceps-type electrodes to deliver electric pulses.

PROTOCOL

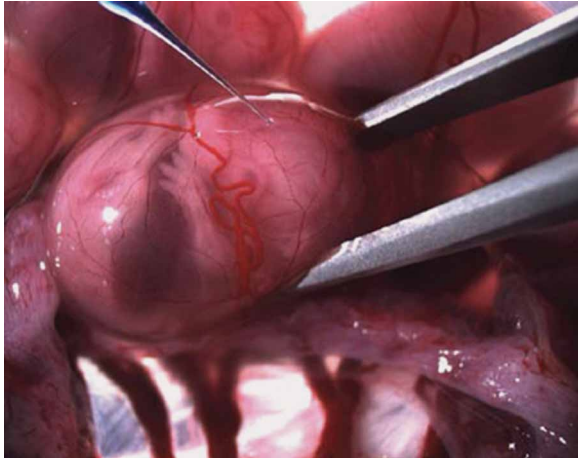


Figure 6 | DNA injection into the *exo utero* embryo.

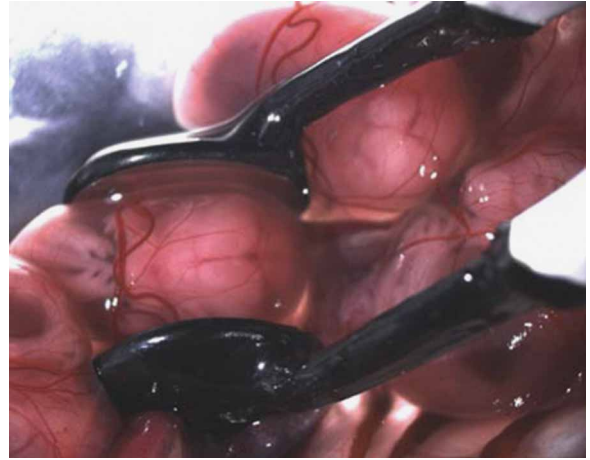


Figure 7 | The *exo utero* embryo is held with forceps-type electrodes.

▲ CRITICAL STEP The bottom of the metal cage must tightly attach to the top of the slide warmer for efficient heat transmission.

(viii) Transfer the mouse to a standard cage.

(B) *Exo utero* electroporation

(i) Pull up the uterus with watchmaker's #5 forceps and cut the uterine wall along the antiplacental side. Be careful not to break the yolk sac.

▲ CRITICAL STEP The basic method of *exo utero* surgery has been described previously²⁶.

? TROUBLESHOOTING

(ii) Inject 1–2 μ l DNA solution into the brain ventricle or the spinal cord central canal (**Fig. 6**).

▲ CRITICAL STEP If a small amount of liquid inside the yolk sac leaks through a hole made by injection, the embryo should still survive. However, avoid making more than one hole or a large hole. It is not necessary to close the yolk sac with a suture.

? TROUBLESHOOTING

(iii) Hold the DNA-injected embryo through the yolk sac, parallel to the embryonic anteroposterior axis, with forceps-type electrodes and deliver electric pulses as in Step 6A(iii) (**Fig. 7**).

? TROUBLESHOOTING

(iv) Reposition the embryo into the abdominal cavity carefully as in Step 6A(iv).

▲ CRITICAL STEP It is not necessary to close the uterine wall with a suture.

? TROUBLESHOOTING

(v) Fill the abdominal cavity with warm saline, close the abdominal wall and skin, and warm the mouse as in Steps 6A(v)–(viii).

▲ CRITICAL STEP If you want to examine gene expression at postnatal stages, pups must be recovered by cesarian section at E19.5 and reared by a foster mother.

7 | Examine expression of transfected genes and analyze their function.

▲ CRITICAL STEP For an initial evaluation of transfection efficiency, analyze embryonic survival and expression of a control gene, such as *EYFP*, 2 days after electroporation, at which stage its expression should be easily detected.

? TROUBLESHOOTING

● TIMING

We usually operate on all embryos and complete the surgery (from incision to closure of the skin) of both *in utero* and *exo utero* electroporation within 30 min. The surgery

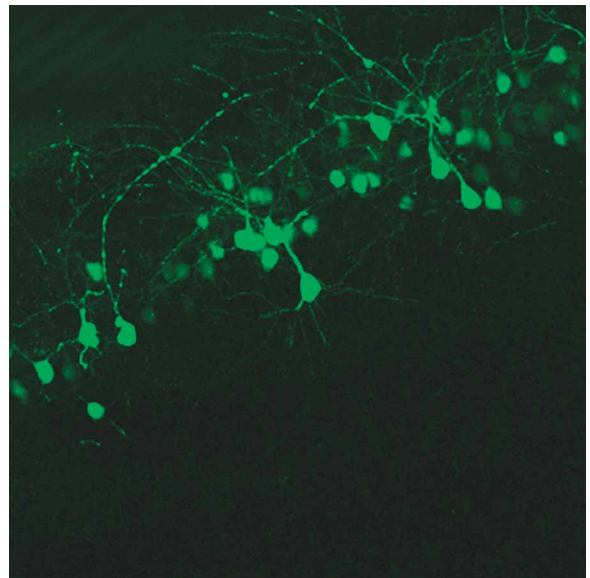


Figure 8 | Neurons labeled with *EYFP*. P15 cortex after electroporation of *EYFP* at E15.5.

should be finished within 45 min. If you cannot do so, the number of operated embryos should be reduced. In most cases, it is not necessary to operate on all embryos in a pregnant mouse.

? TROUBLESHOOTING

Steps 6A(i)–(iv) and 6B(i)–(iv): If survival rate of embryos is very low, make sure that the uterus and embryos are handled properly. If it is low even after omitting the DNA injection and electroporation steps, they must be handled more carefully and gently.

Step 7: If fluorescent signals are not detected in the nervous system even when using a ubiquitous promoter and a fluorescent protein, check the following:

1. Check the embryo from which the brain or the spinal cord was recovered. If fluorescent signals are observed somewhere, such as the skin, it suggests that electroporation worked but DNA injection was not appropriate. Practice injection more.
2. When electric pulses are delivered into saline, air bubbles must appear on the surface of electrodes. If not, check cables or the electroporator.
3. Check the plasmid by transfecting it into cultured cells.

ANTICIPATED RESULTS

Morphology and migration of transfected cells in the nervous system are clearly visualized by the use of a fluorescent protein (Fig. 8). Function of genes at various settings has been examined in transfected cells by coexpressing the genes with a fluorescent protein gene. The activity of transcriptional regulatory elements has also been analyzed in the developing brain¹⁷ and spinal cord¹⁴ after electroporation.

Survival rate of embryos and transfection efficiency depend on how exactly you follow this protocol. Survival rate appears to be improved by decreasing the operation time, as you get more used to the procedures.

When pCAG-EYFP is used, fluorescent signals of EYFP start to be detected 12 h after electroporation and exhibit a higher strength at 2 days after electroporation. They persist for a long time even at postnatal stages, and have been shown to reflect gene expression by *in situ* hybridization analysis¹⁰. Similarly, expression of DsRed is observed for a long time, even about 4 months after electroporation (Fig. 9).

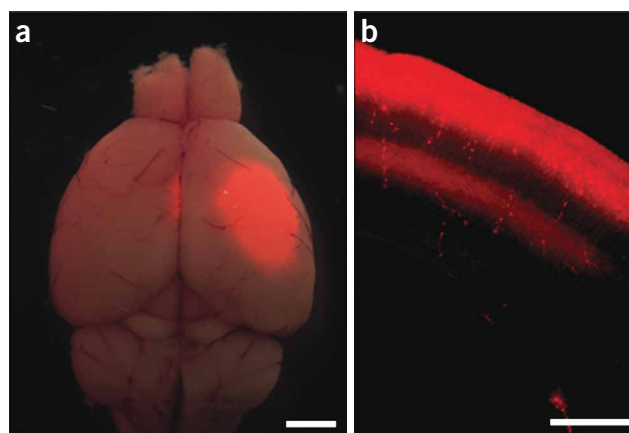


Figure 9 | Long-term expression of the mitochondrial-targeted DsRed protein. (a) P111 brain and (b) its section, after electroporation of its gene at E15.5. Scale bar, a, 2 mm; b, 500 μ m.

ACKNOWLEDGMENTS I thank J. Kutsuna, M. Tanaka and Y. Yamamoto for their help and Dr. D. Kawauchi for critical reading of the manuscript. This work was supported by Brain Science Foundation and grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

COMPETING INTERESTS STATEMENT The authors declare that they have no competing financial interests.

Published online at <http://www.natureprotocols.com>

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>

1. Nagy, A., Gertsenstein, M., Vintersten, K. & Behringer, R. *Manipulating the Mouse Embryo: A Laboratory Manual* 3rd edn. (Cold Spring Harbor laboratory Press, Cold Spring Harbor, NY, USA, 2003).
2. Gaensler, K.M.L. *et al.* Fetal gene transfer by transuterine injection of cationic liposome–DNA complexes. *Nature Biotechnol.* **17**, 1188–1192 (1999).
3. Yang, N.-S. & Sun, W.H. Gene gun and other non-viral approaches for cancer gene therapy. *Nature Med.* **1**, 481–483 (1995).
4. Calvin, N.M. & Hanawalt, P.C. High-efficiency transformation of bacterial cells by electroporation. *J. Bacteriol.* **170**, 2796–2801 (1988).
5. Neumann, E., Schaefer-Ridder, M., Wang, Y. & Hofschneider, P.H. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J.* **1**, 841–845 (1982).
6. Fromm, M., Taylor, L.P. & Walbot, V. Expression of genes transferred into monocot and dicot plant cells by electroporation. *Proc. Natl. Acad. Sci. USA* **82**, 5824–5828 (1985).
7. Aihara, H. & Miyazaki, J. Gene transfer into muscle by electroporation *in vivo*. *Nature Biotechnol.* **16**, 867–870 (1998).
8. Muramatsu, T., Mizutani, Y., Ohmori, Y. & Okumura, J. Comparison of three nonviral transfection methods for foreign gene expression in early chicken embryos *in ovo*. *Biochem. Biophys. Res. Comm.* **230**, 376–380 (1997).
9. Itasaki, N., Bel-Vialar, S. & Krumlauf, R. ‘Shocking’ developments in chick embryology: electroporation and *in ovo* gene expression. *Nature Cell Biol.* **1**, E203–E207 (1999).
10. Saito, T. & Nakatsuji, N. Efficient gene transfer into the embryonic mouse brain using *in vivo* electroporation. *Dev. Biol.* **240**, 237–246 (2001).
11. Saba, R., Nakatsuji, N. & Saito, T. Mammalian *BarH1* confers commissural neuron identity on dorsal cells in the spinal cord. *J. Neurosci.* **23**, 1987–1991 (2003).
12. Ding *et al.* *Lmx1b* controls the differentiation and migration of the superficial dorsal horn neurons of the spinal cord. *Development* **131**, 3693–3703 (2004).
13. Hasegawa, H. *et al.* Lamina patterning in the developing neocortex by temporally coordinated fibroblast growth factor signaling. *J. Neurosci.* **24**, 8711–8719 (2004).
14. Saba, R., Johnson, J.E. & Saito, T. Commissural neuron identity is specified by a homeodomain protein, *Mbh1*, that is directly downstream of *Math1*. *Development* **132**, 2147–2155 (2005).
15. Molyneaux, B.J. *et al.* *Fezl* is required for the birth and specification of corticospinal motor neurons. *Neuron* **47**, 817–831 (2005).

16. Nguyen, L. *et al.* p27^{Kip1} independently promotes neuronal differentiation and migration in the cerebral cortex. *Genes Dev.* **20**, 1511–1524 (2006).
17. Miyagi, S. *et al.* The Sox-2 regulatory regions display their activities in two distinct types of multipotent stem cells. *Mol. Cell. Biol.* **24**, 4207–4220 (2004).
18. Mizutani, K. & Saito, T. Progenitors resume generating neurons after temporary inhibition of neurogenesis by Notch activation in the mammalian cerebral cortex. *Development* **132**, 1295–1304 (2005).
19. Borell, V., Yoshimura, Y. & Callaway, E.M. Targeted gene delivery to telencephalic inhibitory neurons by directional *in utero* electroporation. *J. Neurosci. Methods* **143**, 151–158 (2005).
20. Kawauchi, D. *et al.* Direct visualization of neurogenesis by precerebellar neurons: involvement of ventricle-directed, radial fibre-associated migration. *Development* **133**, 1113–1123 (2006).
21. Pekarik, V. *et al.* Screening for gene function in chicken embryo using RNAi and electroporation. *Nature Biotechnol.* **21**, 93–96 (2003).
22. Bai, J. *et al.* RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nature Neurosci.* **6**, 1277–1283 (2003).
23. Takeuchi, A. & O'Leary, D.M. Radial migration of superficial layer cortical neurons controlled by novel Ig cell adhesion molecule MDGA1. *J. Neurosci.* **26**, 4460–4464 (2006).
24. Huang, Z. *et al.* *In vivo* transfection of testicular germ cells and transgenesis by using the mitochondrially localized jellyfish fluorescent protein gene. *FEBS Lett.* **487**, 248–251 (2000).
25. Matsuda, T. & Cepko, C.L. Electroporation and RNA interference in the rodent retina *in vivo* and *in vitro*. *Proc. Natl. Acad. Sci. USA* **101**, 16–22 (2004).
26. Muneoka, K., Wanek, N., Trevino, C. & Bryant, S.V. *Exo utero* surgery. in *Postimplantation Mammalian Embryos: A Practical Approach* (eds. Copp, A.J. & Cockcroft, D.L.) 41–59 (Oxford University Press, Oxford, UK, 1990).



Corrigendum: *In ovo* electroporation in the embryonic mouse central nervous system

Tetsuichiro Saito

Nat. Protocols 1, 1552–1558 (2006); published online 9 November; corrected online 7 and 29 December 2006.

In the version of this article initially published online, several instances of the phrase *in vivo* were incorrectly substituted with *in ovo*. When the article was first corrected, not all instances were changed appropriately. These errors have been corrected in all versions of the article.

Corrigendum: *In ovo* electroporation in the embryonic mouse central nervous system

Tetsuichiro Saito

Nat. Protocols 1, 1552–1558 (2006); published online 9 November; corrected online 30 November 2006.

In the version of this article initially published online, several instances of the phrase *in vivo* were incorrectly substituted with *in ovo*. This error has been corrected in all versions of the article. In addition, on p. 1557 the first line of the Troubleshooting section should read “Steps 6A(i)-(iv) and 6B(i)-(iv)...” rather than “Steps 6A(i)-(iv) and 6B(ix)-(xii)...”