In Vivo Electroporation of Neurons

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Goal

This chapter describes an approach to drive *in vivo* gene expression in neurons through the electroporation of DNA constructs during early development.

Area of application

Most neurons are accessible to ectopic gene expression by electroporation. Examples span the rostro-caudal extent of the central nervous system from olfactory epithelium (Renzi, Wexler and Raper, 2000) to spinal cord neurons (Price et al. 2002). Further, this technique has been used to electroporate neurons in both chick and mouse embryos (Osumi and Inoue, 2001). Gene expression in restricted sub-classes of neurons is also possible with the aid of specific promoter elements. For example, the mouse HB9 promoter drives expression selectively in motor neurons (Arber et al., 1999; William, Tanabe and Jessell, 2003) and the math1, neurogenin1 and neurogenin2 promoters drive expression in distinct overlapping domains of the dorsoventral axis of the spinal cord (Timmer, Johnson and Niswander, 2001). It should be noted, however, that other methods exist for more focal electroporation of DNA constructs (Haas et al., 2002) or for the delivery of DNA constructs by lipofection (Holt, Garlick and Cornel, 1990) but are outside the scope of this text.

Materials

See Table 1 for additional details of suppliers and catalogue numbers.

1. Microcapillary pipets tapered by a micropipette puller.

2. Mouth pipettor.
3. DNA construct with gene to be expressed under the control of a DNA promoter element. The promoter element may be one that drives pan-neuronal expression, for example the β-actin promoter, or one that drives expression in a specific neuronal sub-type. The DNA plasmid should not be linearised and should be dissolved at a concentration of between 1 to 15 mg/ml in PBS (Phosphate buffered Saline (0.1M Sodium phosphate buffer pH 7.4, 0.15M NaCl)) containing 0.1% Fast green dye (SIGMA Cat # F7258).
4. India Ink diluted 1:10 in PBS.
5. Fertilized chicken eggs.
6. Forced draught incubator at a temperature of 39^{o}C and 70% relative humidity.
7. 3mL luer-lok syringe with 21G1½ needle.
8. Blunted forceps (Roboz, Gaithersburg, MD) and electrical tape.
9. Dissecting microscope.
10. Electroporator.
11. Electrodes. We bend the electrodes into an L-shape with the foot of the L around 1 cm in length. Electrode holder may be of any type; we use a homemade apparatus with vertical displacement capability through a screw mechanism.

Protocol and Procedures

1. Turn the fertilized chicken eggs on their sides (with the long axis of the egg horizontal) and incubate in the forced draught incubator until the embryos are at stage 10 to 20 (Hamburger and Hamilton, 1951).
2. Use the mouth pipetter to suck the DNA solution into the capillary. Make a second capillary that contains India Ink.
3. Remove incubated eggs and place under the dissecting microscope.
4. Remove 3ml of albumen from the blunt end of the egg with the syringe.
5. Remove approximately 1 to 4 cm^2 of eggshell from the top of the egg, allowing visualization of the developing chick embryo. Tilt the egg until there is ease of access to the rostro-caudal extent of the embryo.
6. Inject India Ink directly under the embryo (this allows ease of visalisation of the developing embryo and is not necessary if the embryo is older than stage 12/13). Pierce the membranes of the egg with the capillary tube at least 1 to 2 cm away form the embryo and not interfering with the blood vessels.
7. Approaching either the rostral or caudal end of the embryo insert the DNA construct containing microcapillary assembly into the lumen of the central nervous system. At early stages (stage 10-13) the capillary can be inserted directly into the forebrain vesicles. At later stages, when the head has turned, the capillary can be inserted into the cervical spinal cord just caudal to the hindbrain. Push this microcapilary to the rosto-caudal level of the CNS that gene expression is desired. It is possible to push the

capillary throughout the extent of the spinal cord without significant damage resulting to the embryo.

8. Slowly withdraw the microcapillary whilst blowing the DNA solution into the lumen of the CNS. The fast green dye in the DNA solution aids in visualization of the DNA as it is forced into the lumen.
9. Move the egg to the electrode/electroporator assembly. Place the electrodes at the region of the CNS containing the DNA construct, parallel to the rostro-caudal axis of the CNS with each foot of the electrode as close to the embryo as possible but without touching the embryo. The electrodes should not be placed adjacent to the heart from stage 13 onwards, as the electrical pulse will stop the heart and the embryo will die.
10. Pulse the DNA into the embryo using the electroporator. It may be possible to see the fast green dye move from the lumen to one side of the CNS.
11. Remove the electrodes immediately after the last pulse. Clean the electrodes of albumen using water (if the electrodes become coated in albumen it is possible to clean them effectively by pulsing in a PBS solution).
12. Remove an additional 3ml of albumen from the egg. Cover the hole in the egg with electrical tape and return to the incubator until the desired stage (this may be anything from an additional 6 hours to 7 or more days).

Example of application

Figure 1 shows two examples of electroporation in neurons. Figure 1A shows the use of a Chicken β-actin/rabbit β-globin hybrid promoter (pCAGGS, Niwa, Yamamura, and Miyazaki, 1991) to drive expression of nuclearly localized β-galactosidase. Figure 1B shows the use of the mouse HB9 promoter to drive expression of nuclearly localized β-galactosidase specifically in motor neurons of the ventral horn of the spinal cord. (Arber et al., 1999; William, Tanabe and Jessell, 2003) (Figure 1B).

Advantages and limits

The main advantage of this technique is the speed with which the effects of ectopic gene expression may be assessed (Stern, 2002). It has tremendous advantage in time saved over the generation of transgenic mice and other animals. This technique also allows the easy assessment of cell autonomy of effect as the misexpressed genes are generated in a mosaic on one side of the CNS. Those neurons that did not acquire the treatment, either on the electroporated side of the CNS or on the contralateral side provide ideal internal controls for the effect of the misexpression. The main disadvantages are that the technique does not provide a stable line of embryos with the given perturbation; each embryo generated is unique.

Acknowledgements

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Figure Legend

Figure 1A shows the use of a chicken β-actin/rabbit β-globin hybrid promoter to drive expression of nuclearly localized β-galactosidase (NLS lacZ). Figure

1B shows the use of the mouse HB9 promoter to drive expression of NLS LacZ.

Table 1 Details of suppliers and catalogue numbers for materials.

India Ink	Pelikan Fount India Ink (#17, black. Cat # PE 221143)
Microcapillary pipettes	Borosilicate capillary tubing Frederick Haert Co., Bowdoinham, ME. Cat # 27-30-0
Micropipette puller	Sutter Instruments Co., Novato, CA. P-30 Vertical micropipette puller
Mouth pipetter	Aspitator tube assembly for microcapillary pipettes, SIGMA, St. Louis, MO. Cat # A 5177.
Electrodes	Platinum/Iridium (80/20) wire at 250 μm diameter from Frederick Haert Co., Bowdoinham, ME. Cat # UEPMGBVNNNND.
Electroporator	BTX (a division of Genetronics, San Diego, CA) Electrosquareporator ECM 830, 30V pulse 50ms duration 1 sec interval for 5 pulses.