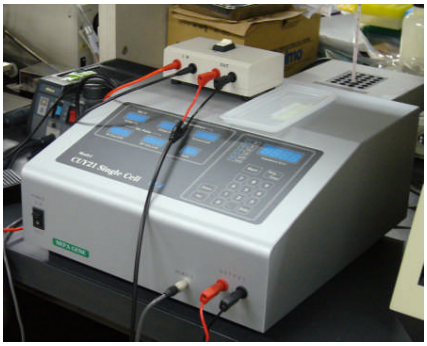
Gene transfection into the brain of an adult mouse

I Required Materials

- Square wave pulse electroporator CUY21EDIT or CUY21SC
- Electrode set　CUY200S (See Fig.1)*[1]
- Brain holder
- Mili-amper meter *[2]
- Electrical hair shaver
- Microsyringe pump, EICOM Corp.
- Hamilton syringe (1cc)
- Hamilton syringe (10ul)
- PVC tube　ID:0.25mm (See Fig. 1 A and B)
- Fluorinert™　FC-77 , 3M
- DNA buffer
- Adult mouse



CUY21SC and polarity changer

*1 Including 2 x Needle electrode (③), electrode holder (②), cleaning wire (①)

*2 If CUY21SC is used, mili-amper meter is not required.

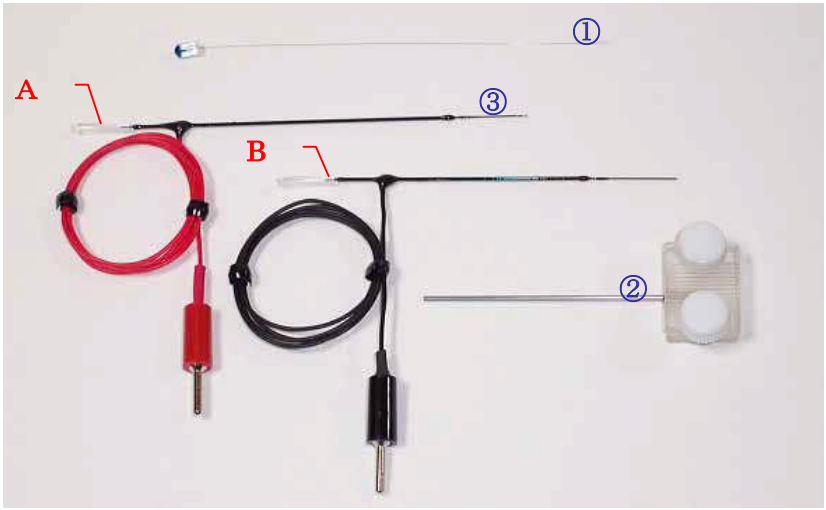
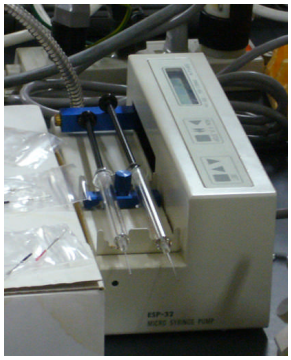


Fig. 1 CUY200S

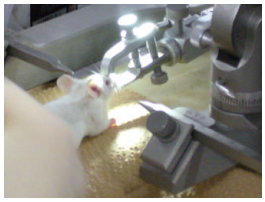


Micro-syringe pump
(EICOM Corp.)

II Electroporation procedure

1. Give an anesthetic (Nembutal®) to the mice

2. Fix mouse's brain by the brain holder

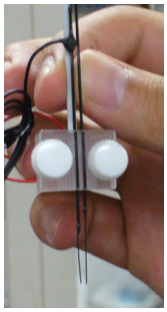

Holding mouse' s brain

3. Set the cathode and anode electrodes to the electrode holder (Fig.1 ②)

4. Connect PVC tubes to both cathode and anode electrodes
(Refer to Fig.1 A and B)

5. Connect Hamilton syringes (1cc) to PVC tubes and inject liquid paraffin.

Note: If the liquid paraffin doses not flow from both electrodes smoothly, please kindly remove the clog by a cleaning wire (Fig.1 ①).


Setting electrodes to
a electrode holder

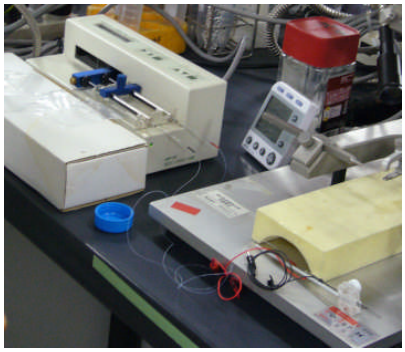
6. Remove Hamilton syringes (1cc) from PVC tubes

7. Connect Hamilton syringes (10 ul) to PVC tubes

8. Injecting Fluorinert™ and aspirating DNA buffer
(Refer to Fig.2)

Note: In order to avoid the contamination, we recommend the aspiration of the redundant volume, 0.2-0.5ul plus required volume.

DNA buffer should be aspirated at very low speed. Otherwise air will be kept inside a syringe.


Connecting syringes to electrodes

9. Shave fur on Mice' head and disinfect the shaved part with 70% ethanol

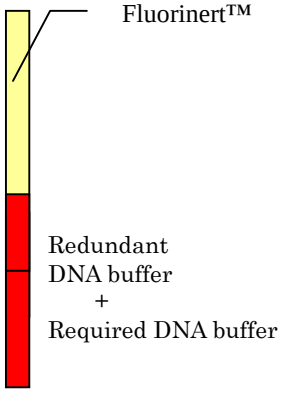
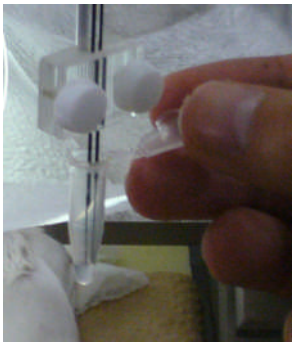
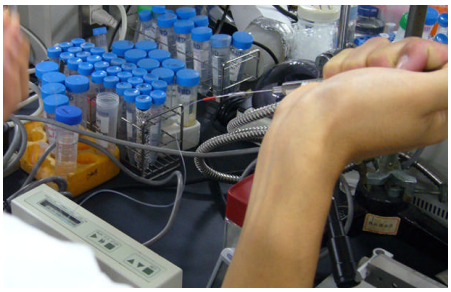


Fig.2 Cross section of electrode needle


Aspirating DNA buffer


Injecting liquid paraffin into an electrode

10. Cut and Open the head

11. Wipe off the tissue and blood on the cranium with a cotton bud

12. Drill the cranium at low rotation speed

13. Insert the electrodes at the speed of 1mm / min until a tip reaches the target part

Note: Please insert electrodes slower than 1mm/min. Otherwise brain may be damaged.

14. Pull out electrodes by 0.3 mm

Note : DNA buffer will be kept in the room.

15. Inject DNA buffer at 0.25 ul/min.

16. Insert the electrodes into the target part again

17. Wait for 20-30 sec.

18. Press the resistance measurement button and measure the actual resistance

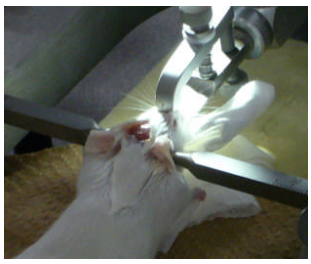
19. Calculate the voltage by the following formula

V = R✕a = R✕6.22 R: Measured resistance a: Constant value (= 6.22)

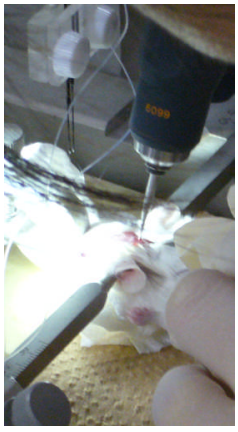
Note: Current will be in the range from 1 to 10mA when the calculated voltage is applied.

Ex) When the actual resistance is 11K ohms, the required voltage V can be calculated as follows.

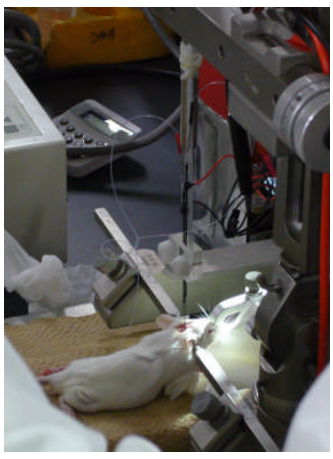
V = 11✕6.22 = 68.42 68 (V)



Cutting open the head



Drilling a hole in cranium



Inserting electrodes into holes on cranium

20. Set the electroporation program as follows.

Voltage*3 : 40V P on: 2ms P off: 98ms No. of cycles: 10 times

*3 Voltage may vary according to the actual resistance. Please kindly refer to the step 20.

21. Apply pluses

Note: Recommend to hold the mouse by hand during electroporation

22. Close the surgical incision by tissue adhesive

23. Clean the electrodes thoroughly. Otherwise electrodes will be clogged by remaining tissue inside a needle.

CUY202
全長　100mm
先端カバー　5mm

CUY207　脳内注入口付シングル電極（陽極）
全長　930mm
電極先端径（下部）　0.1mmφ
電極径（上部）0.3mmφ
電極剥き出し部分　0.5mm
電極剥き出し部分径（上部ケーブル側）　0.3mmφ

CUY208　脳内電極（陰極）
全長　800mm
電極先端径（下部）　0.1mmφ
電極径（上部）0.3mmφ
電極剥き出し部分　0.5mm
電極剥き出し部分径（上部ケーブル側）　0.3mmφ

CUY580　電極固定用ホルダー

電極固定部　縦　10mm
電極固定部　横　18mm
電極固定部　奥行き　15mm
ホルダー軸　92mm