Chapter 8

Electroporation-Mediated Gene Transfer to the Developing Mouse Inner Ear

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Abstract

Key words:

1. Introduction

(1). (2). (3), (4), (5), (6, 7)

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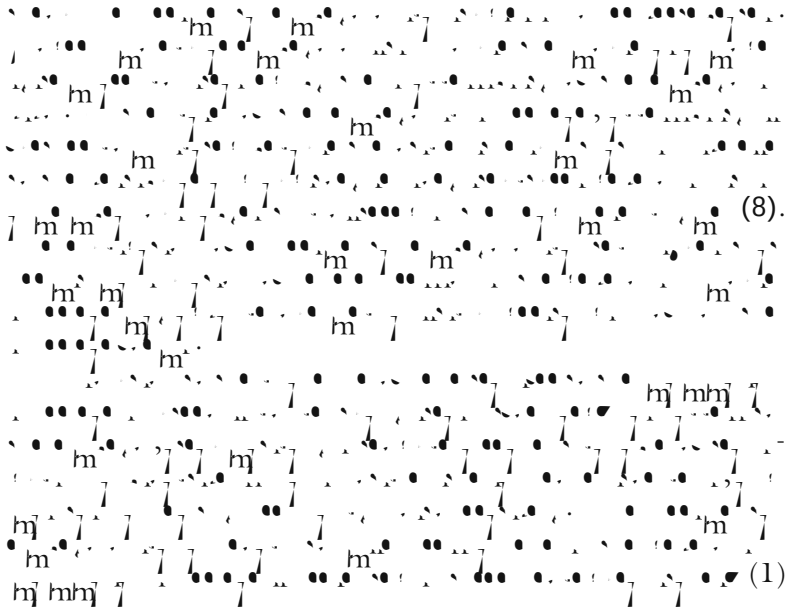

2.2. Ventral Laparotomy

1. [illegible] (12502-12), [illegible] (1410-0), [illegible] (11106-0) (see **Note 2**).
2. [illegible] 6-0 (0.7) [illegible] -1, 30 (5) [illegible] -11, [illegible].
3. [illegible]
4. [illegible] 0% [illegible] 10% [illegible] (10% [illegible]).
5. [illegible] (2323, [illegible]). [illegible], 500 [illegible] 033-011.

12. [illegible].
13. [illegible] 11.5 (11.5). [illegible] 0.5 ([illegible], 11.5 [illegible]).

3. Methods

3.1. General Anesthesia for Mouse Ventral Laparotomy

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(see Note 8).

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3.3. Transuterine Microinjection and In Vivo Electroporation

1. 1.5 0. 6 3 . Conduct the ramp test as indicated in the P-97 instruction manual. = 200, = 3 = 0, = 46, = 110. 14 20 -10

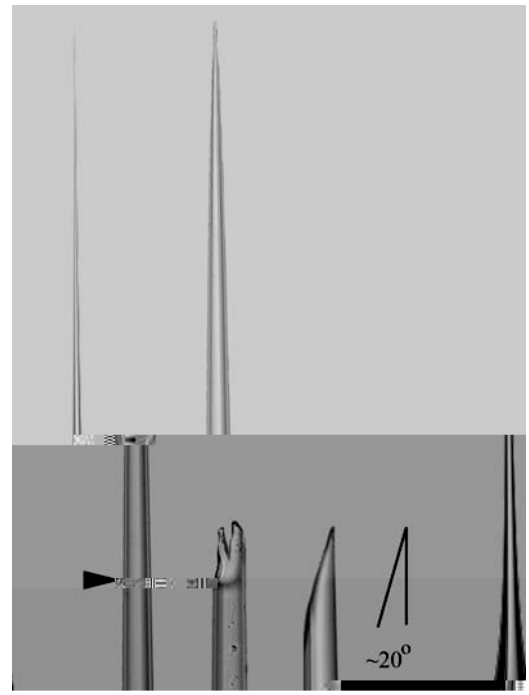

Fig. 8.1. Fabrication of a transuterine microinjection pipette. A pipette was pulled with the P-97 micropipette puller using the pressure:heat:pull:velocity:time settings indicated in **Section 3.3, step 1**. (**A**) The outer diameter of the unpulled shaft is 1.5 mm and the length of the tapered part is □12 mm. The tip of this pipette was imaged in three successive stages of preparation (**B–D**). (**B**) The approximate location of the manual

4. [illegible] 20 [illegible] 15 [illegible]
[illegible]

6.

(**Fig. 8.2A,B**).

1 24 µ 20 10 15 15

(see **Note 13**).

(**Fig. 8.3A**).

(**Fig. 8.3B**).

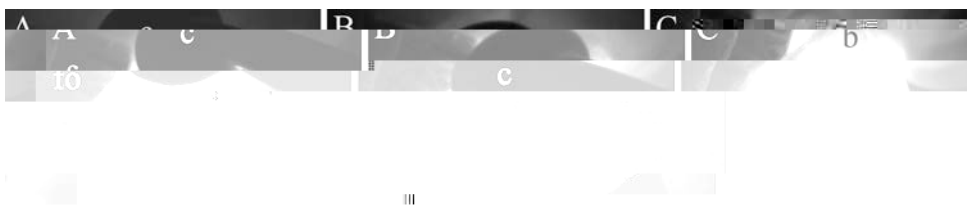

Fig. 8.3. In vivo electroporation of the embryonic day 11.5 mouse otocyst. (**A**) The uterus was transilluminated with light from a fiber optic cable whose output end (fo) was directly in contact with the irrigated uterus. The left otocyst (o) of the E11.5 mouse embryo was injected with fast green solution, and its gross morphology is discernable beneath the nascent 4th ventricle (4th) in the caudal hindbrain. The insulated surface of the cathode (c) and the reflective, platinum surface of the anode (a) were grossly positioned around the uterus to flank the embryo. (**B**) Gentle compression of the uterus with the tweezer-style electrodes forced a counter-clockwise rotation of the embryo, placing the injected otocyst toward the center of the 5 mm cathode–anode field. The goal is to drive negatively charged DNA into ventral progenitors within the otic vesicle. (**C**) Bubbles (b) on the surface of the uterus after execution of the 5-pulse train appear as graininess in the

(**Fig. 8.2B**)

(see **Note 14**).

(see **Note 15**).

(see **Note 16**).

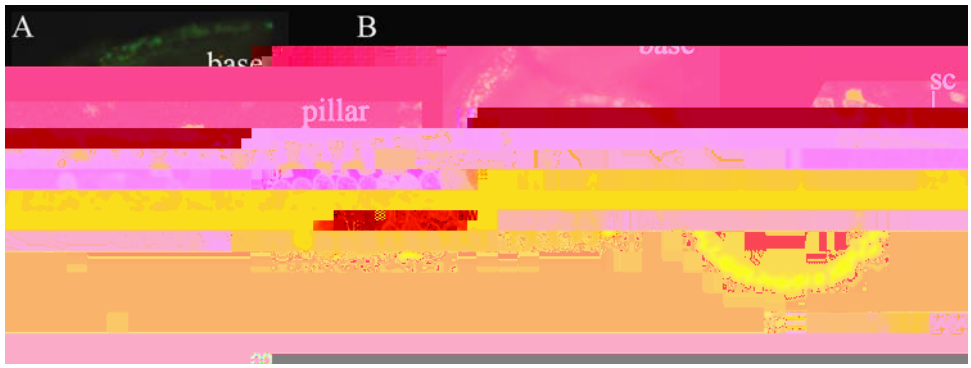

Fig. 8.4. Electroporation-mediated transfer of an expression plasmid encoding green fluorescent protein transfects progenitors that give rise to the organ of Corti. An expression plasmid encoding green fluorescent protein (GFP) driven by the human elongation factor 1-α promoter (, 1-α) was electroporated into the E11.5 mouse otocyst. The inner ear was harvested 6 days later at E17.5 and fixed in 4% paraformaldehyde in PBS for 12 h. The cartilaginous otic capsule and the cochlear lateral wall were removed, and the whole mount preparation was imaged in panel **A**. GFP expression is detectable in the base, midbase, and proximal apex of the cochlea. (**B**) Laser confocal microscopy of a representative 100 μm section of the EF1-α/GFP-transfected cochlea immuostained with an antibody against myosin 7a (*red*) to identify the single row of inner hair cells and three rows of outer hair cells. Several supporting cells (sc), pillar cells (pillar), and outer hair cells (ohc) express GFP. These data indicate that progenitors giving rise to supporting cells and hair cells of the organ of Corti were transfected and expression of the transgene was maintained in differentiated cell types of the maturing cochlea. Scale bar in **A** = 100 μm and in **B** = 10 μm.

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4. Notes

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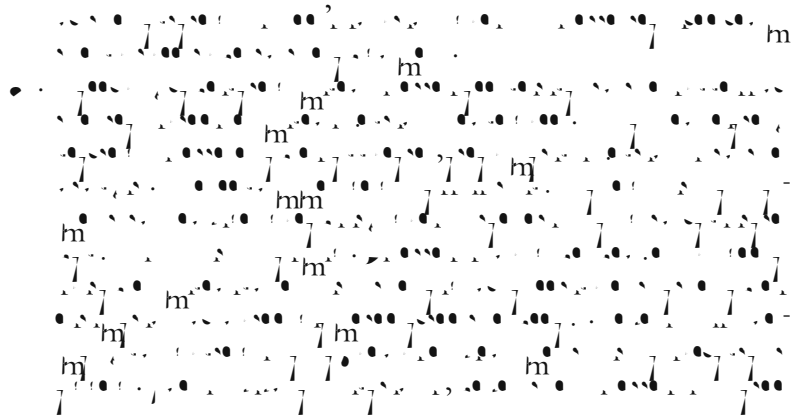
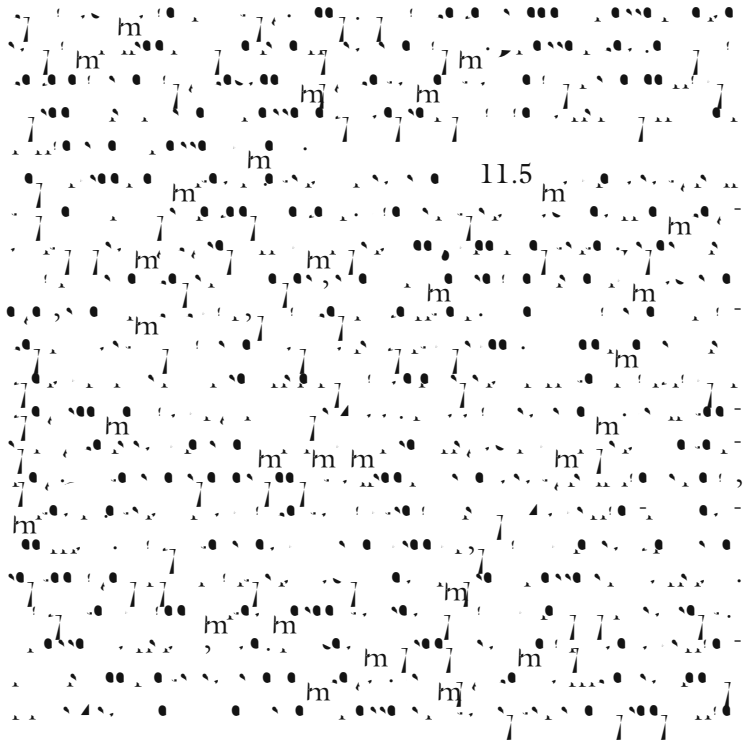
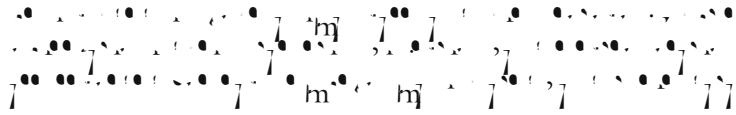




13. 11.5



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