

# Chapter 8

## Electroporation-Mediated Gene Transfer to the Developing Mouse Inner Ear

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### Abstract

Electroporation-mediated gene transfer to the developing mouse inner ear was performed using a modified protocol. The protocol involved the use of a modified electroporation buffer containing a high concentration of calcium chloride and a low concentration of calcium acetate. The modified buffer was used to electroporate the developing mouse inner ear. The results showed that the modified buffer significantly increased the efficiency of gene transfer to the developing mouse inner ear compared to the standard buffer. The modified buffer was also used to electroporate the developing mouse inner ear in combination with a modified electroporation protocol. The results showed that the modified buffer and modified protocol significantly increased the efficiency of gene transfer to the developing mouse inner ear compared to the standard buffer and standard protocol.

**Key words:** Electroporation, gene transfer, developing mouse inner ear, calcium chloride, calcium acetate, modified buffer, modified protocol.

### 1. Introduction

The inner ear is a complex organ that is responsible for hearing and balance. The inner ear is composed of the cochlea and the vestibular system. The cochlea is the part of the inner ear that is responsible for hearing. The vestibular system is the part of the inner ear that is responsible for balance. The inner ear is a highly sensitive organ and is easily damaged. Gene transfer to the inner ear can be used to study the function of the inner ear and to develop treatments for inner ear disorders. Electroporation-mediated gene transfer is a technique that can be used to deliver genes to the inner ear. The modified buffer and modified protocol described in this chapter significantly increase the efficiency of gene transfer to the inner ear.

The image displays a complex musical score consisting of approximately 15 staves. The notation is dense, featuring a variety of note values, rests, and dynamic markings. The markings 'm' and 'mh' are prominent throughout the score, often appearing above or below notes. The score is divided into two main sections, labeled (8) and (1) at the right end of the staves. The notation includes many beamed notes and rests, suggesting a fast or intricate piece of music. The overall layout is highly detailed and technical.

**2.2. Ventral Laparotomy**

1. ... (12502-12), ... (1410-0), ... (11106-0) (see Note 2).
2. ... 6-0 (0. ... m<sup>-11</sup>, ... -1, 30 (5. ... m<sup>-11</sup>).
3. ...
4. ... 0% ... 10% ... (10% ... m<sup>-11</sup>).
5. ... (2 2323, ... 500 m<sup>-11</sup> ... 033 -011).

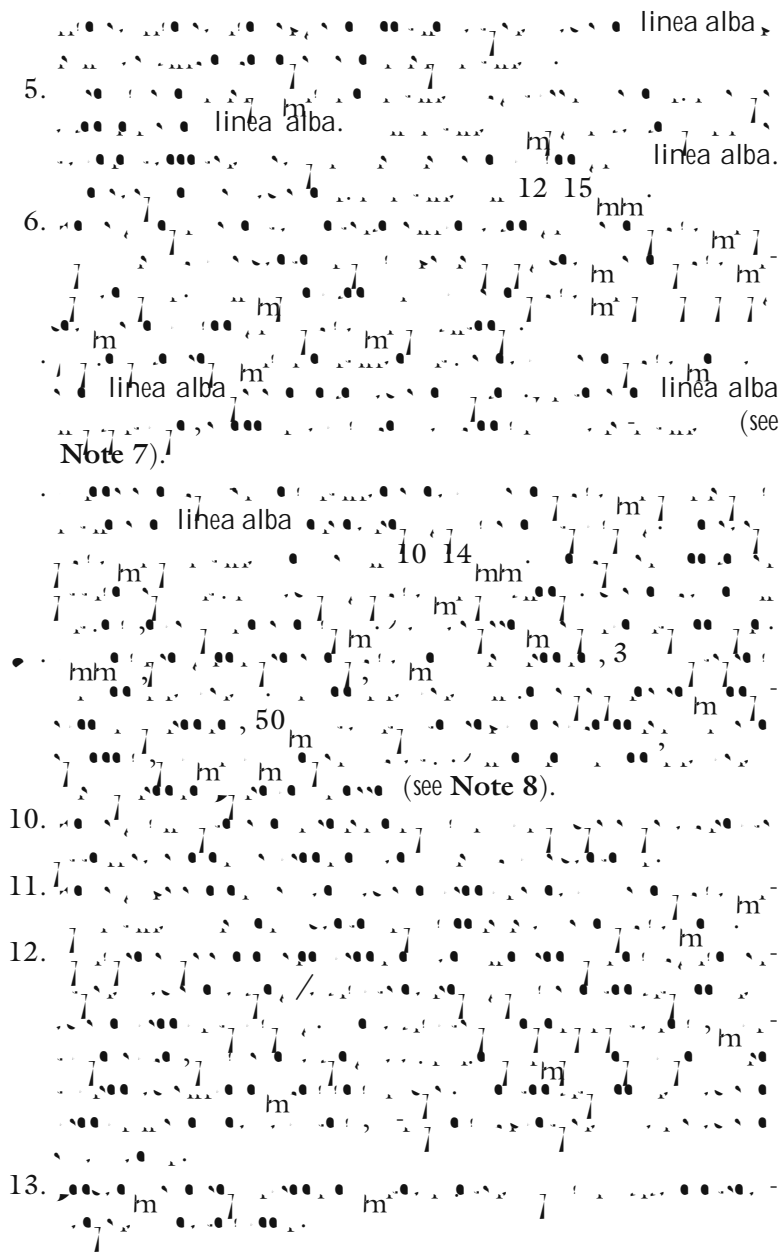
12.  $\frac{1}{m} \left( \frac{1}{m} \right)$ .
13.  $\frac{1}{m} \left( \frac{1}{m} \right)$ .

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### 3. Methods

#### 3.1. General Anesthesia for Mouse Ventral Laparotomy

1.  $\frac{1}{m} \left( \frac{1}{m} \right)$ .



**3.3. Transuterine Microinjection and In Vivo Electroporation**

1. 1.5 mm 0.6 mm  
m 3 mm 3 mm  
Conduct the ramp test as indicated in the P-97 instruction manual.  
= 200, = 10, = 0,  
= 46, = 10,  
14 μ  
20

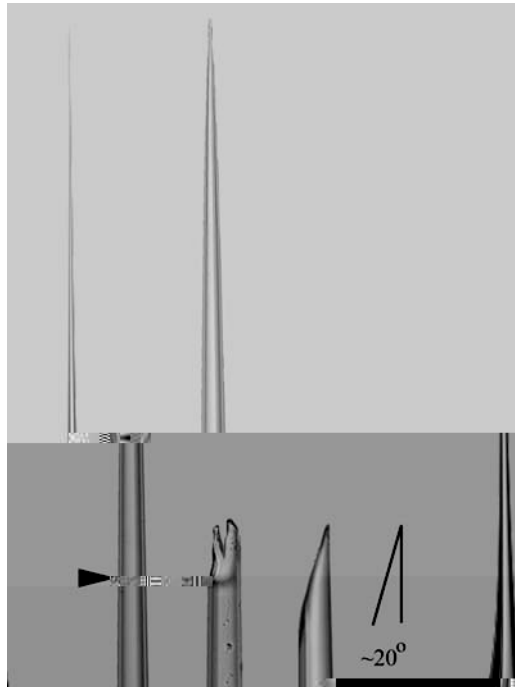
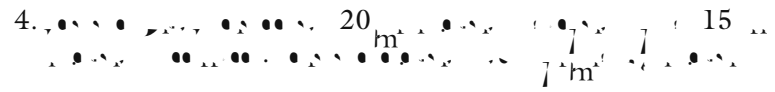
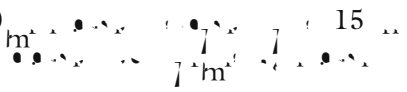
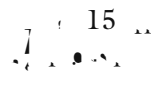


Fig. 8.1. Fabrication of a transuterine microinjection pipette. A pipette was pulled with the P-97 micropipette puller using the pressure:heat:pull:velocity:time settings indicated in **Section 3.3, step 1**. **(A)** The outer diameter of the unpulled shaft is 1.5 mm and the length of the tapered part 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.  20  15 

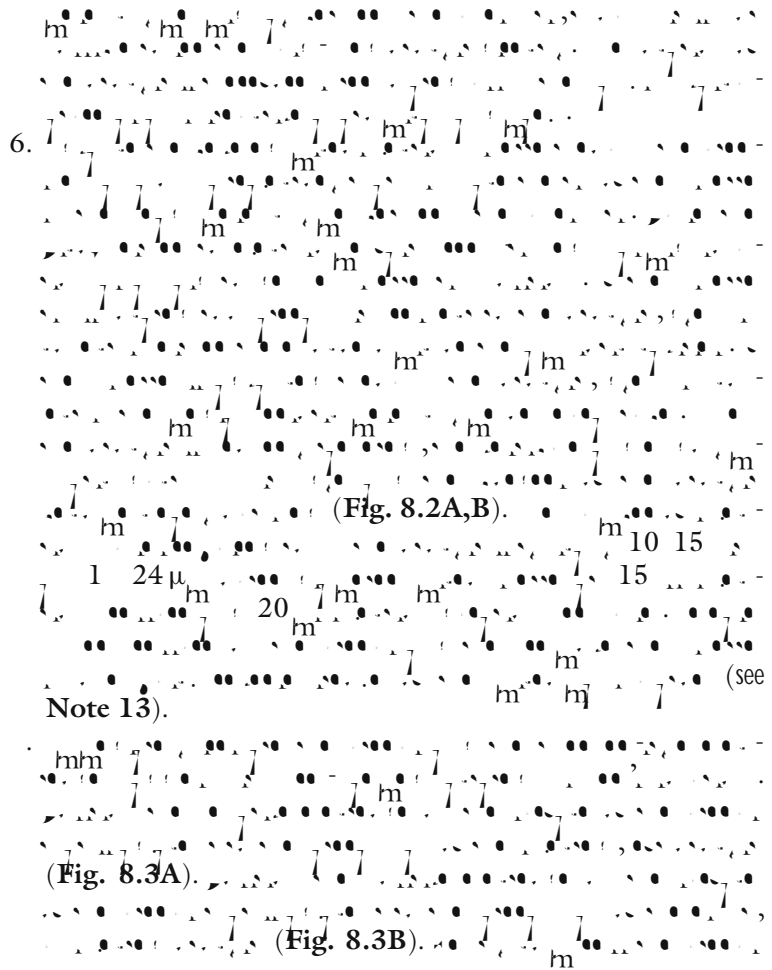
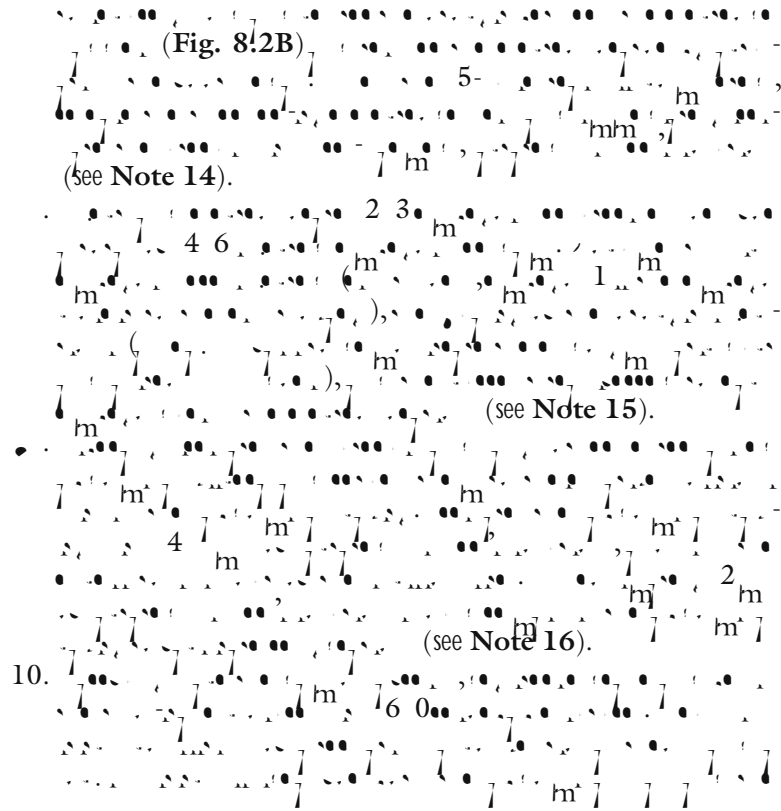


Fig. 8.3. In vivo electroporation of the embryonic day 11.5 mouse otocyst. **(A)** The uterus was transilluminated with light from a fiber optic cable whose output end (fo) was directly in contact with the irrigated uterus. The left otocyst (o) of the E11.5 mouse embryo was injected with fast green solution, 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





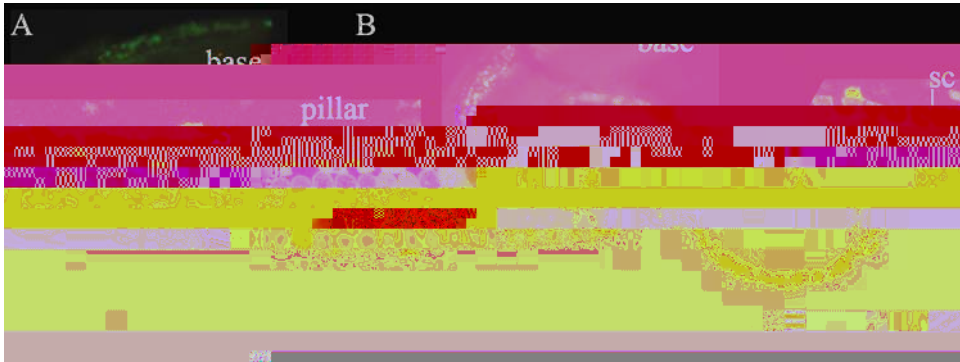


Fig. 8.4. Electroporation-mediated transfer of an expression plasmid encoding green fluorescent protein transfects progenitors that give rise to the organ of Corti. An expression plasmid encoding green fluorescent protein (GFP) driven by the human elongation factor 1- $\alpha$  promoter (EF1- $\alpha$ ) 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  $\mu\text{m}$  section of the EF1- $\alpha$ /GFP-transfected cochlea immunostained 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  $\mu\text{m}$  and in **B** = 10  $\mu\text{m}$ .

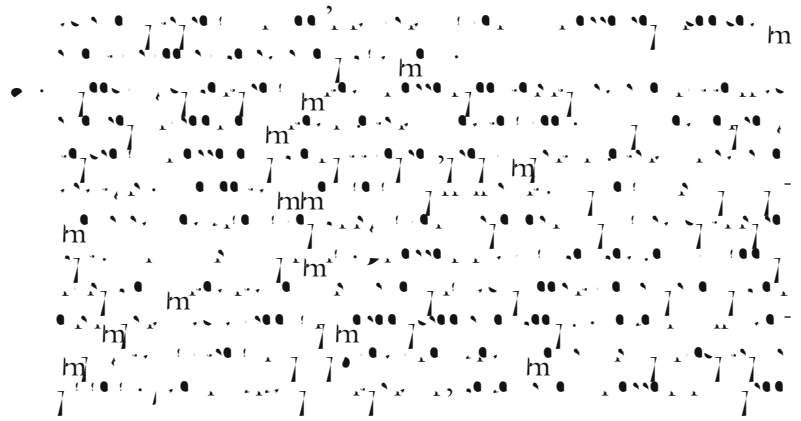


#### 4. Notes

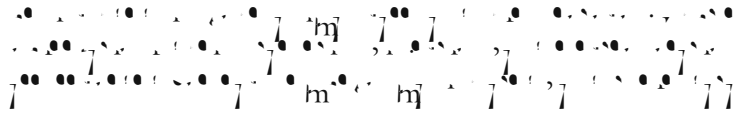


- 1.
- 2.
- 3.











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