Kelley Lab Electroporation Protocol (developed by J. Jones, M. Montcouquiol and A. Dabdoub)

This protocol summarizes our current protocol for electorporation in cochlear tissue. We predominantly do electroporations using E13 to E14 cochleae, however this same technique can also be used on older tissue. Typically, approximately 50 cells will be transfected within the sensory epithelium, however this number is highly variable and the number of transfected cells within the sensory epithelium goes down with age.

Another important consideration is the promoter that will be used to drive expression of the transgene. We have experimented with several promoters and have found the hybrid CMV/b-actin promoters or just straight CMV promoters to work well.

So, following dissection, each cochlea or piece of cochlea should be transferred to a sterile sylgard dish and placed in a 10µl drop of water or saline containing 1-2µg/µl of plasmid DNA. Regardless of the age, the piece of cochlea to be transfected should be no more than one turn, such that it can be treated as a nearly a flattened disk. The disk should be oriented roughly perpendicular to the surface of the dish with the sensory epithelium facing the negative electrode and the disk tipped with slight backward angle. The two electrodes are then placed on opposite sides of the disk. The electrodes need to be in the solution but should not be touching the tissue. We use a T830 square wave electroporator from BTX (San Diego, California) for the electroporation. The electrodes are either genetrodes or gene paddles, also from BTX. The following parameters are used: 27 volts, 30 ms duration, 9-10 pulses per cochlea. After electroporation, individual cochleae are left in the drop of DNA for an additional 5 minutes. Following the 5 minute incubation, each cochlea can be moved to a culture dish containing culture media. Some

transfected cells should be evident within 12 – 18 hours with more developing over the next day or two.