Amamoto Lab, 2022

Protocol: Subretinal Injection and Electroporation

Note: Whether to use AAV or electroporation to manipulate cells will depend on many factors. The most important thing to consider is the cell type. Postnatal electroporation is easier and faster than AAV, but it can only target rods, Muller glia, bipolar cells, and some amacrine cells. If you want to target cones, you need to use AAV.

1. Prepare glass needles. Glass needles are created by pulling Wiretrol II capillaries (Drummond Scientific Company, cat. #5-000-2005) using a needle puller (Sutter Instrument, Model P-97). The glass needles are beveled on two edges with a microgrinder (Narishige, cat. #EG- 401). Place beveled needles in a petri dish with a sticky tack (or double sided tape) to prevent anything from touching the needles.
2. Prepare plasmid/AAV. For 5-10 uL of plasmid/AAV, add ~0.5 uL of 2.5% FastGreen.
    a. Note: Keep FastGreen at 4C to avoid contamination.
3. Warm up the heat pad before starting.
4. Set up the injection station. Place a white cloth in the middle, underneath the scope. On the right, place blunt forceps, petri dish with PBS, cotton applicator, and 30G needle. On the left, place a cotton applicator and a petri dish with PBS.
5. Set the electroporator (if electroporating) to these settings: Volts: 80 V, Pulse-On: 50ms, Pulse-Off: 950ms, Number of pulses: 5.
6. Set the Femtojet to these settings: 330 Pa, 3 seconds.
7. Prepare a bucket of ice.
8. Make 3 buckets with the bottom cut out and place on heat pad. One for experimental, one for control, and one for uninjected. Place all pups into the uninjected bucket.
9. Load ~5-10 uL of plasmid/AAV into a needle and place on Femtojet holder.
10. Put one pup on ice to anesthetize.
    a. Note: Cryoanesthesia can be used up to P6.
11. Once the pup is anesthetized, put on the cloth. If the skin around the eye is wet, dab with the cloth to dry.
12. Using a 30G needle, cut the faint eyelid crease of the right eye. Be careful not to cut into the eye.
13. With blunt forceps, pop the eye out. As long as the cut is the right size, the eye will stay out even without force. If the eye readily goes back in, the cut is too big.
14. With the glass needle, pierce through the sclera. Go through the neural retina so that the tip of the needle is in the subretinal space.
    a. Note: If the injection area is too close to the puncture hole, there is a chance that the liquid will come out. So it's easier if the bleb is farther away from the puncture.
15. Press down on the foot pedal to inject. You should see a deep blue color. If it's a faint blue color, you are injecting into either the vitreous or into the retina.
16. Take out the needle.
17. Wet the right side cotton applicator with PBS and push the eye back into the socket. And close eyelids.
18. If electroporating, wet the shock pads with PBS and place the (+) shock pad on top of the eye and the (-) shock pad on the bottom of the head. Press the electroporate button and wait for 5 shocks.
19. Dab the eye with the cloth to dry. Use the left cotton applicator to further dry and make sure the eyelid is fully closed.
    a. Note: Failure to dry the eyelid and ensure closure will lead to eye defects.
20. Put back the pup in the appropriate bucket on the head pad to recover.
21. After all injections, snip the end of the tail from the control group to track the animals.
22. Place all pups back with the mother. Monitor for any abnormalities.