

Protocol: Retina Dissection

1. Euthanize the mouse by CO₂ (Secondary method of euthanasia, cervical dislocation, is performed after removing the eyes).
2. Remove the eyes with blue blunt forceps (FST, cat. #11210-10) and place in PBS in a 24-well plate.
 - a. Note: If the orientation (i.e. Dorsal-Ventral) is important, use a cauterizing pen (Bovie Medical, cat. #AA29) to cauterize a small spot on the cornea. Then, use a blue Sharpie to mark the cauterized spot.
3. Transfer an eye using a plastic transfer pipette (with the end cut off) into a small petri dish with PBS.
4. Under the scope, gently hold the bottom of the eye (near optic nerve) with #5 forceps (FST, cat. #11254-20) in one hand. With the other hand (dominant hand), puncture the center of the cornea with one blade of 2.5mm spring scissors (FST, cat. #15000-08).
5. With the spring scissors, make several cuts in the cornea at different angles, making sure not to cut or damage the retina.
6. With #5 forceps in each hand, gently start peeling the sclera/RPE from the neural retina, which has a tan/white color. In pigmented mice, the RPE is black/brown, making it easier to distinguish between the RPE and the neural retina. In albino mice, it's a little bit more difficult to distinguish.
 - a. Note: In rd1 or other degeneration mouse models, the neural retina will be very thin compared to wildtype, making it very easy to damage the tissue.
 - b. Note: If the orientation is important, and the blue mark was previously placed in the cornea, it's crucial to somehow mark the orientation in the neural retina as the blue mark will be gone once the cornea is removed. Use spring scissors to make a small, but obvious cut into the neural retina at the same orientation as the blue mark.
7. At the optic nerve head, slide one end of the forceps between the RPE and neural retina to sever the optic nerve head. Without this step, it's possible to damage the center of the neural retina.
8. Continue removing the sclera/RPE from the neural retina until it is completely dissected.
9. Place the dissected retina into a new well with PBS.