Video Article

Mouse Eye Enucleation for Remote High-throughput Phenotyping

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Abstract

The mouse eye is an important genetic model for the translational study of human ophthalmic disease. Blinding diseases in humans, such as macular degeneration, photoreceptor degeneration, cataract, glaucoma, retinoblastoma, and diabetic retinopathy have been recapitulated in transgenic mice.[1-5] Most transgenic and knockout mice have been generated by laboratories to study non-ophthalmic diseases, but genetic conservation between organ systems suggests that many of the same genes may also play a role in ocular development and disease. Hence, these mice represent an important resource for discovering new genotype-phenotype correlations in the eye. Because these mice are scattered across the globe, it is difficult to acquire, maintain, and phenotype them in an efficient, cost-effective manner. Thus, most high-throughput ophthalmic phenotyping screens are restricted to a few locations that require on-site, ophthalmic expertise to examine eyes in live mice. [6-9] An alternative approach developed by our laboratory is a method for remote tissue-acquisition that can be used in large or small-scale surveys of transgenic mouse eyes. Standardized procedures for video-based surgical skill transfer, tissue fixation, and shipping allow any lab to collect whole eyes from mutant animals and send them for molecular and morphological phenotyping. In this video article, we present techniques to enucleate and transfer both unfixed and perfusion fixed mouse eyes for remote phenotyping analyses.

Video Link

The video component of this article can be found at https://www.jove.com/video/3184/

Protocol

1. Blunt Dissection: Enucleation of the mouse eye in unfixed specimens

1. Pull apart the eyelids to improve exposure and access to the posterior globe (eyeball) surface.
2. Place a curved dressing forcep behind (under) the globe in the orbit (eye socket). The Mahajan Sharptip dressing forcep is a custom instrument with pointed tips to make this step easier (see materials and reagents table).
3. Close the forcep and grasp the orbital connective tissue and optic nerve behind the globe while being careful to avoid squeezing the globe.
4. Gently pull the forcep upward and pluck the eyeball from the orbit. The white thread-like tissue is the optic nerve.
5. Place the eye in PBS. Using a 30-gauge needle, make a single puncture wound immediately posterior to the limbus by inserting the needle 1-2 mm into the eyeball. This allows fixatives, such as glutaraldehyde, that are otherwise unable to penetrate the eye tissue, to directly enter the eye. Creation of this puncture wound may not be necessary if a fixative, such as paraformaldehyde or alcohol, is capable of diffusing into ocular tissues of interest.
6. Place the entire eye immediately into fixative for immersion fixation.

2. Sharp Dissection: Enucleation of the mouse eye following perfusion fixation

1. Use a curved colibri forcep to hold the eyelid away from the globe.
2. Orient curved Westcott dissection scissors parallel to the globe, while aiming towards the back of the orbit.
3. Cut the fixed connective tissue that surrounds the globe from the inferior, medial, superior, and lateral sides.
4. Place the curved forcep behind the globe, grasp the connective tissue without pressing on the globe, and pull forward to enucleate the eye. Since the orbital connective tissue is stiff from perfusion fixation, additional cutting of orbital tissues may be necessary to completely release the globe.

3. Fixation and Packaging

Shipping of fixed tissues must follow your institutional and postal service requirements including appropriate labels and packaging. The following protocol is an example of shipping a non-infectious biological sample at ambient temperature. This requires 3 layers of packaging but no class A/B biological substance or liquid nitrogen labels.

1. Place the eye into a 5-mL glass scintillation vial with at least 3-mL fixative, or post-fixative buffer if fixation is short. Write the tracking number on the lid of the vial and seal it with Parafilm.
2. Place scintillation vials into a biological sample approved shipping container. In our institute, for example, approved containers require three layers: a sealed container in which the sample is placed, an absorbent middle layer, and a protective outer layer.
3. Place the container into a bubble-wrap package and then into an approved laboratory shipping container along with appropriate documentation.

4. Representative Results

Histological examination of eyes can be performed by a variety of methods including Hematoxylin and Eosin staining, enzymatic expression detection, transmission electron microscopy, and immunohistochemistry. Following immersion fixation in 4% paraformaldehyde, tissue was embedded in paraffin and sectioned on a microtome. In high-throughput phenotyping, we analyze pupil-optic nerve sections that sample all tissue types in the eye (Figure 1). Sections of tissue were also examined for *lacZ* expression (Figure 2), transmission electron microscopy of cellular organelles (Figure 3), and expression of specific molecules with antibodies (Figure 4).

In our phenotype screening method, it was not important to maintain orientation of the eye with respect to the nasal (medial canthus) and temporal (lateral canthus) sides. There are at least two options if eye orientation is necessary. After enucleation, we have applied light cautery to the temporal cornea at the three o'clock position with a handheld cautery device. The lesion is obvious on histology and does not get lost during processing like histological inks. The downside is that cautery damages the cornea, which may be a critical tissue for examination. The other option requires a skilled anatomist that can identify the extraocular muscle insertions. Under a stereomicroscope, identification of the inferior and superior oblique muscle insertion marks the inferior and superior and aspect of the globe.[3] With either orientation method, it is important to track the specimen as a right or left eye. Together, this will allow exact globe orientation prior to sectioning.

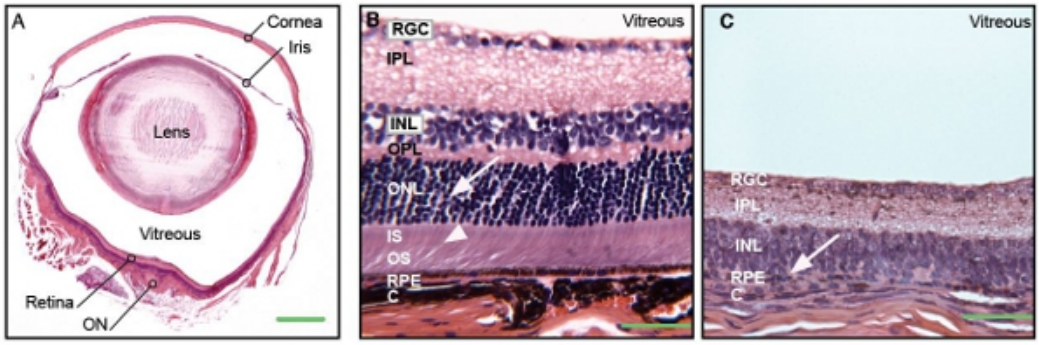

Figure 1. Mouse eye histological images after remote acquisition. Mouse eyes were enucleated by sharp dissection following perfusion fixation with 4% paraformaldehyde. **A.** Pupil-optic nerve section stained with Hematoxylin and Eosin shows preservation of all tissue substructures. ON, optic nerve. (green scale bar = 500 microns) **B.** A higher magnification view of the normal retina shows its laminar structure: RGC, retinal ganglion cells; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer (photoreceptor cell)(arrow); IS, photoreceptor inner segments (arrow head); OS, photoreceptor outer segments; RPE, retinal pigment epithelium; C, choroid. (green scale bar = 50 microns) **C.** Compared to the normal retina, this specimen is thinner due to photoreceptor degeneration. The ONL, IS, and OS are completely absent (arrow). (green scale bar = 50 microns)

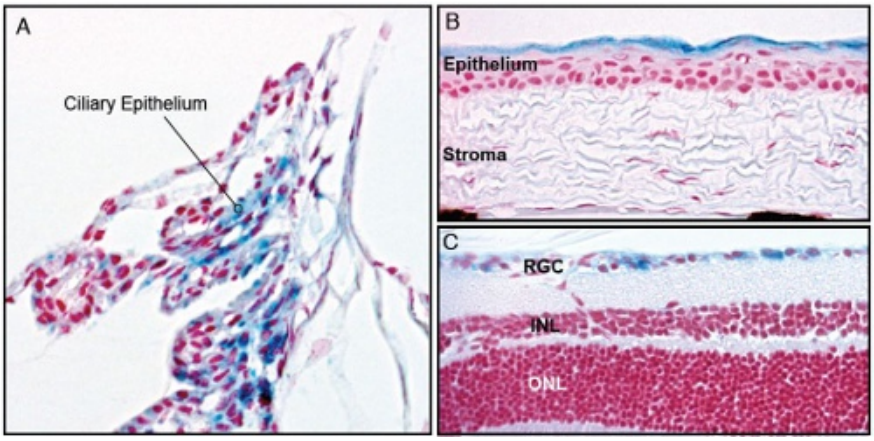

Figure 2. *lacZ* expression in transgenic mice. Eyes were enucleated by blunt dissection and immersed in 1 mg/ml X-gal solution (5 mM K3Fe(CN)6, 5 mM K4Fe(CN)6, 2 mM MgCl2, 0.02% NP40, and 0.1% sodium deoxycholate) overnight at 37°C. Eyes were fixed in 2% formaldehyde/0.2% glutaraldehyde for 30 minutes and sectioned on a microtome. X-gal product (blue) labels: **A.** Ciliary epithelium, **B.** corneal epithelium, and **C.** the retinal ganglion cell (RGC) layer in the retina. INL, inner nuclear layer; ONL, outer nuclear layer.

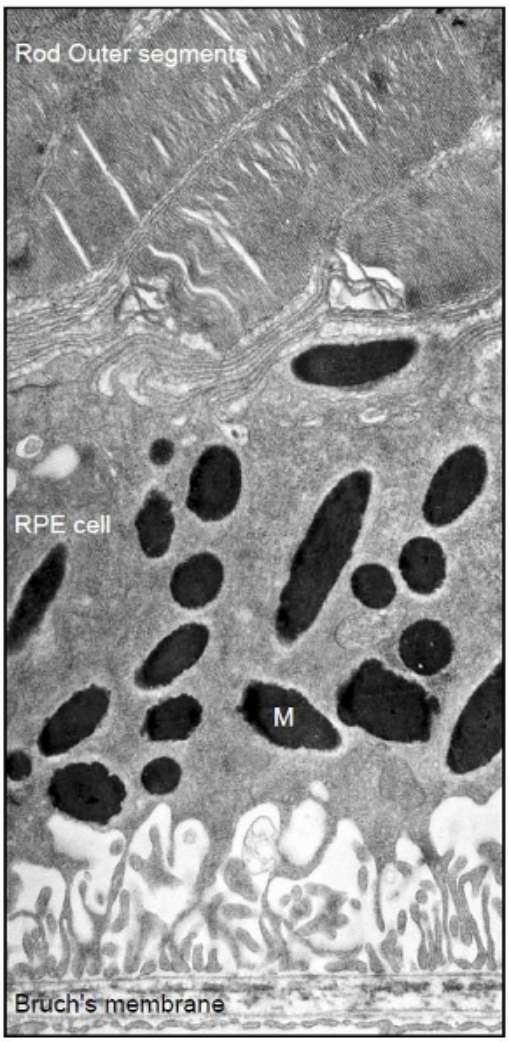

Figure 3. Transmission electron microscopy of retinal pigment epithelium (RPE). After blunt dissection and creation of a puncture wound, eyes were immersion fixed in 2.5% Paraformaldehyde/ 2.5% Glutaraldehyde in 0.1M sodium phosphate buffer. Eyes were secondarily fixed in 1% osmium textroxide, gradually dehydrated, and embedded in Spurr's resin. Tissue was sectioned at 90 nm and placed onto copper slot grids covered in Formvar and imaged using a transmission electron microscope. The micrograph shows, photoreceptor outer segments of the neurosensory retina, melanosomes (M) within the retinal pigment epithelium, and Bruch's membrane.

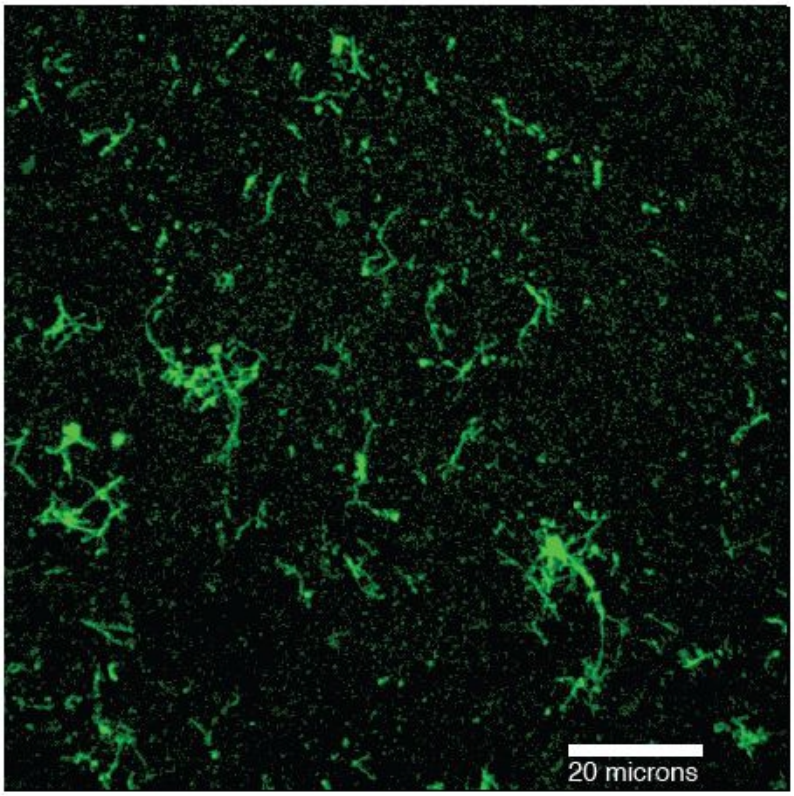

Figure 4. Immunohistochemical labeling of superoxide dismutase-3 (green) in the mouse vitreous. Eyes were enucleated by blunt dissection and underwent immersion fixation in 4% paraformaldehyde. They were embedded in paraffin and sectioned using a microtome. Tissue sections were incubated with rabbit polyclonal SOD3 antiserum diluted 1:50. SOD3 expression was detected using a goat anti-rabbit IgG (H+L)-Alexa Fluor 488 conjugated secondary antibody. Labeling of SOD3 is shown in green.

Discussion

Most transgenic mice exist in laboratories that do not examine the eyes. Our video technique illustrates a simple, standardized method for remote surgical skill transfer to optimize tissue acquisition from laboratories with little experience with the eyes. This video technique helps overcome a major pitfall in high-throughput phenotyping, which is the use of a limited number of expert sites due to non-standardized tissue collection and fixation methods that prevent meaningful comparative phenotypic and molecular studies. To establish and maintain quality control with any new remote lab and technician, it is important to include wild-type eyes at the start and periodically throughout the study. We also found that a tracking system is important when multiple eyes from multiple genotypes were transferred, such as a web-based system to assign a unique identification number to each eye. Although higher quality fixation and subsequent tissue sections are obtained with perfusion-fixed animals, we find that specimens enucleated from unfixed animals are sufficient for most morphological and molecular studies. Moreover, acquisition of eyes from unfixed animals may be significantly easier to process in high-throughput studies from different sources. Transgenic mice are an important instrument for investigating human ophthalmic disease,[10, 11] and remote tissue acquisition for local phenotyping makes the most of this valuable resource. Application of a similar strategy for tissue dissection and sharing may have an important impact on high-throughput phenotyping of non-ophthalmic tissues.

Disclosures

No conflicts of interest declared.

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