Video Article

A Novel RFP Reporter to Aid in the Visualization of the Eye Imaginal Disc in *Drosophila*

Aamna K. Kaul[1], Joseph M. Bateman[1]

[1]Wolfson Centre for Age-Related Diseases, King's College London

Correspondence to: Joseph M. Bateman at joseph_matthew.bateman@kcl.ac.uk

URL: http://www.jove.com/video/1617
DOI: doi:10.3791/1617

Keywords: Cellular Biology, Issue 34, fluorescence microscopy, Drosophila, eye, RFP, dissection, imaginal disc

Date Published: 12/15/2009

Citation: Kaul, A.K., Bateman, J.M. A Novel RFP Reporter to Aid in the Visualization of the Eye Imaginal Disc in *Drosophila*. *J. Vis. Exp.* (34), e1617, doi:10.3791/1617 (2009).

Abstract

The *Drosophila* eye is a powerful model system for studying areas such as neurogenesis, signal transduction and neurodegeneration. Many of the discoveries made using this system have taken advantage of the spatiotemporal nature of photoreceptor differentiation in the developing eye imaginal disc. To use this system it is first necessary for the researcher to learn to identify and dissect the eye disc. We describe a novel RFP reporter to aid in the identification of the eye disc and the visualization of specific cell types in the developing eye. We detail a methodology for dissection of the eye imaginal disc from third instar larvae and describe how the eye-RFP reporter can aid in this dissection. This eye-RFP reporter is only expressed in the eye and can be visualized using fluorescence microscopy either in live tissue or after fixation without the need for signal amplification. We also show how this reporter can be used to identify specific cells types within the eye disc. This protocol and the use of the eye-RFP reporter will aid researchers using the *Drosophila* eye to address fundamentally important biological questions.

Video Link

The video component of this article can be found at http://www.jove.com/video/1617/

Protocol

1. First place a drop of PBS onto a Sylgard dish under a dissecting microscope.
2. Next remove several wandering third instar larvae from a vial or bottle using a spatula and place them in the PBS.
3. To begin the dissection use your left hand tweezers (if you are right handed) to grasp a larva two thirds of the way towards the posterior and then use the other pair to hold the dark colored mouth hooks at the anterior end.
4. Now gently pull the larva apart by moving both pairs of tweezers away from each other. The mouthparts should come away from the rest of the body with the eye/brain complex attached.
5. To stain the eye discs for immunofluorescence fix the discs at this point by placing them in 4% paraformaldehyde/PBS solution for 30-45 minutes on ice. Then wash the discs four times with PBST and stain then with appropriate antibodies.
6. It is easiest to dissect the eye discs directly on a slide. Place a drop of PBS on a slide and move the discs to the drop of PBS by holding the mouthparts.
7. The imaginal tissues are translucent and so at first the eye discs are difficult to identify. To make it easier to identify them use a dissecting microscope with fluorescence capability. Using the eye-RFP reporter line the eye discs can be clearly seen as red fluorescent tissue overlaying the spherical optic lobes (Figure 1).
8. If you are using a fiber optic cold light source direct the light at an oblique angle to the slide, as this will give the discs greater contrast. The eye/antennal discs are attached to the optic lobes via the optic stalk at the posterior and to the mouthparts via a stalk coming from the antennal disc at the anterior.
9. To dissect the eye discs hold the mouthparts using one pair of tweezers and use the other pair to separate the eye discs from the larval brain. Next separate the eye discs from the mouthparts. Finally remove the mouthparts from the slide as these can prevent the coverslip from lying flat.
10. When you have dissected all the eye discs gently tilt the slide and dry off the excess PBS. Then add a drop of Vectashield and gently place a coverslip on top.
11. The discs are now ready to be imaged by either epifluorescence or confocal microscopy (Figure 2).

Representative Results

When dissected correctly the eye/antennal disc should lie flat on the slide clear of any extraneous tissue such as the brain. The dorsal and ventral poles of the eye disc are curved and so tend to fold, but this can be avoided by taking a Z-series on a confocal microscope and omitting these sections from the projection. Although the disc is a monolayer epithelium the variable positions of the nuclei require a Z-series projection to get an overall picture of the disc. A projection of a typical eye-RFP eye/antennal disc taken on a confocal microscope is shown in Figure 2. We

typically take 1 µm sections through the disc using a 20x or 40x lens. The eye-RFP reporter is nuclear localized and so the pattern seen in Figure 2 represents the nuclei of the cells expressing RFP.

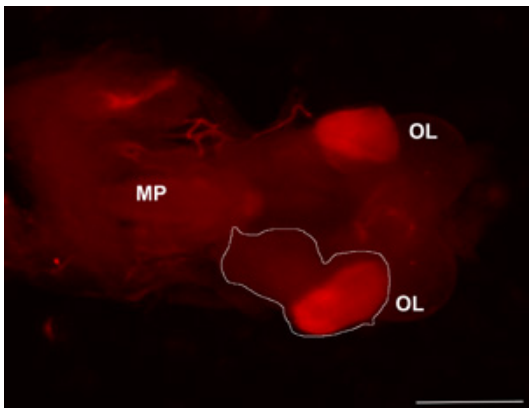

Figure 1. Initial dissection of the eye/brain complex from an eye-RFP third instar Drosophila larva. The eye/antennal imaginal disc (outlined) can be seen attached to the mouthparts (MP) and overlaying the optic lobe (OL) of the brain. The image was taken using a fluorescence dissecting microscope to show the RFP expression in the eye disc. Anterior is to the left. Scale bar: 300 µm.

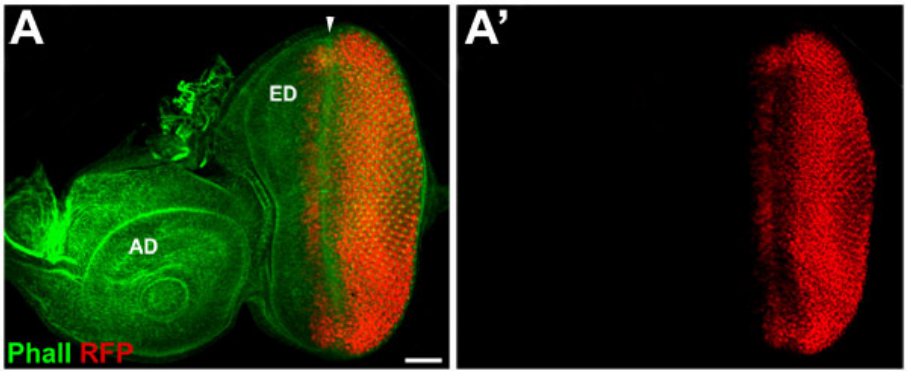

Figure 2. Expression of the eye-RFP reporter in the eye imaginal disc. A third instar eye (ED)/antennal disc (AD) dissected from an eye-RFP larva showing RFP expression (red). The disc was stained with phalloidin (green), which stains apical cell surfaces and shows the morphogenetic furrow (arrowhead). RFP is expressed in precursor cells around the MF, in photoreceptors 1,6,7 and in cone cells. Anterior is to the left. Scale bar: 25 µm.

Discussion

Dissection of the *Drosophila* eye imaginal disc is straightforward, but can take time to master. The critical steps are the identification of the eye disc and its dissection away from the larval brain. Identification of the eye disc is made easier using the eye-RFP line described here. Possible modifications are to remove the antennal disc or the peripodial membrane, or to leave the optic lobe attached to visualize the PR axons entering the brain.

The eye-RFP line was generated by cloning a novel eye-specific enhancer element from the second intron of the *pointed P2* gene [2] into the pRed H-Stinger enhancer-reporter vector [3] and using this to create transgenic flies. The eye-RFP reporter is expressed in a specific subset of PRs and in cone cells (Figure 2.). It can therefore be used to determine whether a gene of interest is expressed in these cell types. The eye-RFP line is available on request.

The developing *Drosophila* eye is a powerful model for studying the genes and molecular pathways controlling PR neurogenesis [1]. The methodology and reporter described here can be used to further the understanding of this process.

Acknowledgements

This work was funded by King's College London and the Royal Society.

References

1. Doroquez, D. B. and Rebay, I., *Crit Rev Biochem Mol Biol* **41** (6), 339 (2006).
2. Kaul, A. K., Craig, G. M., McNeill, H., and Bateman, J. M., *Submitted*.

3. Barolo, S., Castro, B., and Posakony, J. W., *Biotechniques* **36** (3), 436 (2004).

