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Laser Microdissection of Fluorescently Stained Drosophila Embryos

PROTOCOL #5

Embryonic Development

The fruit fly *(Drosophila melanogaster)* is a widely used model organism in studies of embryonic development. Spacial and temporal differences in gene expression profiles during the various stages of embryogenesis can be studied using gene reporter constructs. LacZ is commonly used as a reporter gene to localize the expression of a gene of interest. After integrating the LacZ construct into a gene of interest, the gene is introduced into the model organism and the ß–galactosidase expression is monitored during various stages of development with X-Gal staining.

The Veritas™ Microdissection System allows easy visualization, microdissection and collection of very specific segments in a developing drosophila embryo. In this example, the Veritas instrument is used to visualize and capture specific segments displaying LacZ expression. Drosopila containing the *Engrail* gene with the LacZ construct were bred and the embryos were harvested at the seven parasegmental stage during embryogenesis.

Procedure for X-Gal Staining of Whole Drosophila Embryos

Step	Procedure
1.	Dechorionate: 50% bleach for 2 minutes
2.	Wash Water, then PBS + 0.5% TritonX 100 for 5 minutes
3.	Fixation: 2.5% glutaraldehyde / 50% Heptane in PBS for 5 minutes
4.	Several changes of PBS + 0.5% TritonX 100 over 2 hours
5.	X-Gal staining solution for 30-60 minutes at room temperature, monitor color development, stop the reaction by proceeding to step 6.
6.	Several changes of PBS + 0.5% TritonX 100 over 15 minutes
7.	Embed positive stained embryos in OCT

Tissue Preparation and Sectioning

Frozen sections of the OCT embedded embryo(s) were mounted onto PEN membrane slides. The slides were then processed as follows to remove the OCT:

Step	Process	Time
1.	Nuclease Free Water	30 seconds
2.	75% Ethanol	30 seconds
3.	95% Ethanol	30 seconds
4.	100% Ethanol	30 seconds
5.	Xylenes	3 minutes (Minimize time in Xylenes as X-Gal is soluble in this solution)
6.	Air Dry in fume Hood	3-5 minutes
7.	Proceed to microdissection procedure	

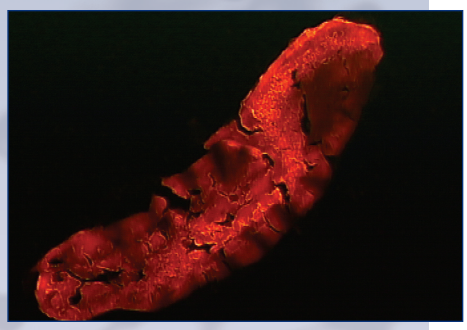

Figure 1

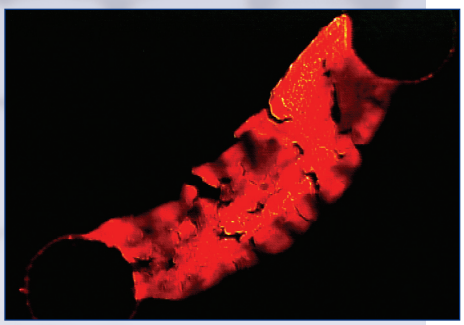

Figure 2

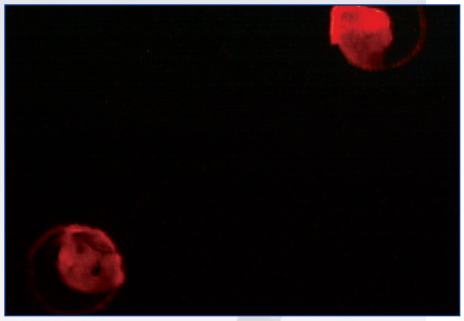

Figure 3

Microdissection

Processed slides were placed into the Veritas™ Microdissection System and the embryo(s) were located using brightfield light. Embryo(s) were placed in the center of the "Live Video Image" and the fluorescence light combined with the red filter cube was used to provide a negative image of the positive X-Gal stained areas (Fig.1). These areas were then collected using standard laser cutting and laser capture methods of the Veritas™ Microdissection system (Figs 2 and 3).

Microdissection Procedure

Step	Procedure
1.	Set Laser Cutting power to lowest possible setting that will still allow full cutting through the PEN membrane.
2.	Set laser cutting properties such that no tabs will be generated during cutting process.
3.	Set capture properties such that no automatic LCM spots are generated after the cutting process.
4.	Locate embryos, activate fluorescence and view samples using red filter cube.
5.	Place a CapSure HS LCM cap onto sample.
6.	Draw around band(s) that will be collected.
7.	Activate the laser cutting process with LCM cap on top of sample.
8.	Manually place the LCM spots to attach the sample to the LCM cap.

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PN 14158-00 Rev A