Lethal Phase - Immunohistochemistry

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Abstract

Experiment was conducted to determine the stage at which mutant embryos arrest their development. All Drosophila melanogaster lines were crossed to balancers with a YFP marker for unambiguous identification of mutant and non-mutant embryos. Embryos were collected on apple juice agar plates with a smear of yeast paste for 1hr and aged for 17 hours at 25°C. Embryos were fixed and stained as described by Fehon et al., 1991 using a modified rotational speed of 150rpm.

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Protocol

Step 1.
Wash embryos and place into wash basket. Dechorionate by pouring bleach directly onto embryos. Let sit for three minutes.

DURATION
00:03:00

Step 2.
Rinse embryos in basket with ddH2O until bleach is completely washed away.

Step 3.
Place embryos into glass vial containing 6ml of Heptane and 6ml of 0.4% Paraformaldehyde. Shake on orbital rotator at 150rpm for 20 minutes.

DURATION
00:20:00

Step 4.
Upon completion of 20 minute fixation, allow heptane and parformaldehyde to seperate. Embyos will be trapped at the interface between the two solutions. Use a glass pipette to remove the bottom (parafomaldehyde) layer be sure to not disrupt the embryos at the interface.