**Submission ID #: 61252**
**Scriptwriter Name: Bridget Colvin**
**Project Page Link:** http://www.jove.com/files_upload.php?src=18681908

# Title: A *Drosophila* model to study wound-induced polyploidization

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# Author Questionnaire

**1. Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Y**

If **Yes**, can you record movies/images using your own microscope camera?

**Y**

**2. Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

**3. Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

# Introduction

**1. Introductory Interview Statements**

**REQUIRED:**

1.1 **Erin Bailey**: Our protocol can be used to study wound-induced polyploidization, a conserved tissue repair process in which cells grow instead of divide, in the adult fruit fly **[1]**.

1.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera Note to Video Editor: original statements provided by authors do not need publication criteria, new files will be submitted

**REQUIRED:**

1.2. **Erin Bailey**: A simple puncture wound can be made to induce polyploidy in the fly epithelium within just two days. We can then easily assess the epithelial cell size, ploidy, and organization **[1]**.

1.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera Note to Video Editor: original statements provided by authors do not need publication criteria, new files will be submitted

**Introduction of Demonstrator on Camera**

1.3. **Erin Bailey**: Vicki Losick, Assistant Professor and Principle Investigator of the Losick Lab at Boston College and who first developed this method, will be demonstrating the procedure **[1][2]**.

1.3.1. LAB MEDIA: To be provided by Authors: Author saying the above

1.3.2. Named demonstrator(s) looks up from workbench or desk or microscope and acknowledges camera Note to Video Editor: original statements provided by authors do not need publication criteria, new files will be submitted

# Protocol

## 2. Adult Fruit Fly Staging and Wounding

2.1. Begin by collecting two vials containing 10-15 newly enclosed female fruit flies of the strain of interest **[1-TXT]** and aging the flies in fresh food vials with approximately 5 male flies per vial at 25 degrees Celsius until 3-5 days old **[2]**.

2.1.1. WIDE: Talent placing vial(s) onto bench **TEXT: *e.g.*, epi-Gal4/ UAS strain**

2.1.2. Talent adding flies to new vial

2.2. For abdominal wounding, at the end of the incubation, use a single 0.1-millimeter, stainless steel pin to assemble several pin holders **[1-TXT]** with the sharp end of each pin facing out **[2]**.

2.2.1. Talent wearing safety goggles to assemble pin holder **TEXT: Discard hooked or damaged pins**

2.2.2. Shot of pin ends facing out

2.3. Anesthetize the aged female fruit flies on a carbon dioxide-fly pad under a stereomicroscope **[1]** and use a paint brush to arrange the files into a row **[2]**.

2.3.1. Talent placing fly pad under microscope

2.3.2. LAB MEDIA: 2.3

2.4. Wearing safety glasses, with the pin holder one hand and forceps in the other **[1]**, use the forceps to position the flies with their ventral abdomens facing up **[3]**.

2.4.1. Talent wearing safety goggles, picking up pin holder and/or forceps

2.4.2. LAB MEDIA: 2.4

2.5. Then puncture the adult female flies within the epithelial pleurite region of tergite A4 on either side of the ventral midline sternites **[1]** and return the wounded flies to the food vial until the desired experimental time point post injury **[2-TXT]**.

2.5.1. LAB MEDIA: 2.5: 00:00-00:10

2.5.2. Talent adding flies to vial **TEXT: *i.e.*, epithelial wound healing starts at 1 dpi and ends by 3 dpi; endoreplication peaks at 2 dpi**

## 3. Fly Abdomen Dissection

3.1. At the experimental endpoint, check for the presence of the wound scar in each anesthetized fly under the stereomicroscope **[1-TXT]** and fill one well of a 9-well glass dissection dish with Grace's solution **[2-TXT]**.

3.1.1. WIDE: Talent at microscope, checking flies **TEXT: Discard unsuccessfully wounded flies**
3.1.2. Talent filling well, with solution container visible in frame **TEXT: See text for all solution preparation details**

3.2. Using a pair of forceps, grasp a wounded female fly by the dorsal side of the thorax **[1]** and submerge the fly in the well of solution **[2]**.

3.2.1. LAB MEDIA: 3.2: 00:00-00:09
3.2.2. LAB MEDIA: 3.2: 00:13-00:32

3.3. With the other hand, use a second pair of forceps to puncture and remove the dorsal cuticle below tergite A6 the rear of the fruit fly **[2]**.

3.3.1. LAB MEDIA: 3.3

3.4. If the internal organs are not extracted, apply pressure to the dorsal side of the abdomen to squeeze out the remaining organs **[1-TXT]**.

3.4.1. LAB MEDIA: 3.4 **TEXT: Discard tissues in empty well**

3.5. Use the forceps to snap off the full abdomen at the thorax junction above tergite A2 **[1-TXT]** and transfer the abdomen to a well containing approximately 100 microliters of Grace's solution **[2-TXT]**.

3.5.1. LAB MEDIA: 3.5 **TEXT: Avoid touching ventral abdominal tissue with dissection tools**
3.5.2. Talent placing abdomen into well **TEXT: Repeat for each abdomen**

3.6. When all of the abdomens have been collected, reduce the volume of Grace's solution in the collection well to 30 microliters **[1]**.

3.6.1. LAB MEDIA: 3.6: 00:05-00:15

3.7. Use forceps in one hand to hold the abdomens dorsal side-down, use the other hand to insert the bottom blade of Vanna's spring scissors into the abdominal cavity of each abdomen and cut along the dorsal midline until the abdomens are fully opened **[2]**.

3.7.1. LAB MEDIA: 3.7

3.8. Add 30 microliters of Grace's solution into each mounting area of a dry dissecting plate with four, 0.1-millimeter pins per abdominal mounting area **[1]** and place the filleted abdomen onto the droplet of solution **[2]**.

3.8.1. Solution being added
3.8.2. Abdomen being placed

3.9. When all of the abdomens have been filleted and placed, pin the abdomens to the dish on the four dorsal corners, taking care that the tissues lie flat without tearing or overstretching the abdominal tissue **[1]**.

3.9.1. LAB MEDIA: 3.9

3.10. Then replace the Grace's solution with 30 microliters of fixative solution per mounting area **[1]** and place a tape label on the bottom of each dish to mark each control and experimental group **[2-TXT]**.

3.10.1. Talent adding fixative to area(s), with fixative solution container visible in frame
3.10.2. Talent placing label(s) onto dish **TEXT: Fix 30-60 min RT**

3.11. **Ari Dehn**: Be sure to always wear a lab coat and goggles to prevent insect pin contact with eyes and clothing and wear gloves when handling toxic fixative solutions **[1]**.

3.11.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera Note to Video Editor: original statements provided by authors do not need publication criteria, new files will be submitted

## 4. Stained Tissue Mounting

4.1. To mount the fly tissue samples, after staining, use forceps to unpin the abdomens from the dissecting plate under the stereomicroscope **[1-TXT]** and use forceps to transfer each sample by its dorsal flank into approximately 30 microliters of mounting medium on individual glass coverslips **[2]**. Note to Video editor: Authors added video to be used as replacement or in addition to videographer shot

4.1.1. WIDE: Talent unpinning abdomen(s) **TEXT: See text for tissue staining details**
Added shot: LAB MEDIA: 4.1: 00:00-00:06
4.1.2. Abdomen being placed onto coverslip

4.2. Under the stereomicroscope, orient the abdominal tissue so that the inside is facing down toward the coverslip and use forceps to pull the oriented abdomens to the edge of each media droplet **[1]**.

4.2.1. LAB MEDIA: 4.2: 00:00-00:23

4.3. Place each mounted coverslip onto a labeled glass slide **[1]** and use a lab tissue to remove any excess mounting medium **[2]**.

4.3.1. Coverslip being placed
4.3.2. Medium being wiped

4.4. Then seal the edges of the coverslip with clear nail polish **[1]** and store the slides in a slide box at 4 degrees Celsius until their imaging **[2]**.

4.4.1. Edge(s) being sealed

# Protocol Script Questions

**A.** Which steps from the protocol are the most important for viewers to see?

3.9.

**B.** What is the single most difficult aspect of this procedure and what do you do to ensure success?

The single most difficult aspect is to perform the dissection without touching the abdominal epithelium. Dissect carefully to avoid scratching your sample. This difficulty and advice is applicable to the entire portion of the protocol being filmed, not just 1 or 2 individual steps.

# Results

**5. Results: Representative Re-Epithelization and Endoreplication Detection**

5.1. The septate junction protein Fas-three, which labels cell-cell junctions, provides an indicator for whether any processing perturbations occurred during the preparation **[1]**.

5.1.1. LAB MEDIA: Figures 1G and 1H

5.2. Abdomens with large scratches of unstained area that perturb the wound area must be discarded and not included in the analysis **[1]**.

5.2.1. L AB MEDIA: Figures 1G and 1H *Video Editor: please add/emphasize asterisk/emphasize area indicated by asterisk in Figure 1H*

5.3. Wound repair is complete when a central, large, multinucleated cell covers the wound scab **[1]**.

5.3.1. LAB MEDIA: Figures 3A *Video Editor: please emphasize cell in center of wound image(s)*

5.4. Gaps of greater than 10 micrometers in the epithelial sheet are considered defects in wound closure and re-epithelialization **[1]**.

5.4.1. LAB MEDIA: Figure 3B *Video Editor: please add red arrowhead and/or emphasize black area in center of right Figure 3B image as indicated by the red arrowhead in the original Figure 3B*

5.5. In this representative analysis using flies with inhibited mitotic cycle activation **[1]**, 52% of the wounds were unable to form a continuous epithelial sheet over the wound scab **[2]**.

5.5.1. LAB MEDIA: Figures 3B and 3C
5.5.2. LAB MEDIA: Figures 3B and 3C *Video Editor: please emphasize red data bar in Figure 3C*

5.6. This membrane wound healing assay provides more information on the extent of the wound repair defect **[1]**, allowing re-epithelialization defects to be grouped as either completely open **[2]**, partially closed **[3]**, or completely closed **[4]**.

5.6.1. LAB MEDIA: Figures 3D and 3E
5.6.2. LAB MEDIA: Figures 3D and 3E *Video Editor: please emphasize red data line in middle image*
5.6.3. LAB MEDIA: Figures 3D and 3E *Video Editor: please emphasize red data line in right image*
5.6.4. LAB MEDIA: Figures 3D and 3E *Video Editor: please emphasize yellow data line in left image*

5.7. For example, in this experiment, the inhibition of wound-induced polyploidization by the simultaneous blocking of endoreplication and cell fusion **[1]** caused 92% of the epithelial wounds to remain completely open **[2]**, while activation of the mitotic cell cycle resulted in an epithelial wound closure defect **[3]**.

5.7.1. LAB MEDIA: Figures 3D and 3E
5.7.2. LAB MEDIA: Figures 3D and 3E *Video Editor: please emphasize middle image and white portion of middle data bar*
5.7.3. LAB MEDIA: Figures 3D and 3E *Video Editor: please emphasize right image and grey portion of right data bar*

5.8. In addition, cell cycle activity was detected by incorporation of the thymidine analog EdU (E-D-U) **[1]** and epithelial ploidy was determined by directly measuring the nuclear DNA content **[2]**.

5.8.1. LAB MEDIA: Figures 4B and 4C *Video Editor: please emphasize Figure 4C graph*
5.8.2. LAB MEDIA: Figures 4E and 4F *Video Editor: please emphasize Figure 4F graph*

# Conclusion

**6. Conclusion Interview Statements**

6.1. **Ari Dehn**: When dissecting out the abdominal epithelium, take care to not touch the tissue with any of the dissecting tools, as this will scratch the sample **[1]**.

6.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera Note to Video Editor: original statements provided by authors do not need publication criteria, new files will be submitted

6.2. **Ari Dehn**: After dissection, cell proliferation, cell death, cell or syncytium size, or nuclear DNA content assessments can be performed to better understand wound-induced polyploidization or other processes within the epithelium **[1]**.

6.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera Note to Video Editor: original statements provided by authors do not need publication criteria, new files will be submitted