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Title: *Drosophila* Embryo Preparation and Microinjection for Live Cell Microscopy Performed Using an Automated High Content Analyzer

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Author Questionnaire

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

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

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Zeiss Stemi 508 doc

   Note: this microscope has a camera port.

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 14
Number of Shots: 31

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ulises Diaz:** This protocol enables the simultaneous acquisition of multiple embryos within a single imaging session.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Ulises Diaz:** While our sample preparation is adapted for a high content analyzer; the samples prepared using this protocol can be imaged on any inverted microscope capable of multipoint acquisition.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Mounting Embryos on Coverslips

2.1. To begin, organize two rows of 15 to 20 embryos on a clean section of grape plate using an egg picker tool **[1-TXT]**. Align the embryos on their ventral side in the same orientation with respect to anterior and posteriors ends **[2]**. *Videographer: This step is important!*

2.1.1. WIDE: Establishing shot of talent at the microscope. **TEXT: Use a stereomicroscope with overhead goose neck lighting**

2.1.2. SCOPE: Talent aligning embryos. Author NOTE: because this step takes 20 minutes to complete. This step was filmed in four 1 minutes shots, shot 1 beginning of first row, shot 2 middle/end of first row, shot 3 start of second row, shot 4 end of both rows. Please feel free to use any combination of shots for post-production

2.2. Adhere a spacer onto a coverslip **[1]** and streak 10 microliters of heptane glue down the exposed coverslip glass inset **[2]**.

2.2.1. Talent adhering the spacer to the coverslip.

2.2.2. Talent streaking glue onto the inset.

2.3. While the glue dries, use a razor blade to cut a rectangle around the two rows of embryos **[1-TXT]**. Cut a second rectangle next to the first **[2]** and remove the second rectangle with the straight edge side of a stainless-steel spatula **[3]**.

2.3.1. Talent cutting the rectangle. **TEXT: < 40 mm x 15 mm**

2.3.2. Talent cutting a second rectangle.

2.3.3. Talent removing the second rectangle.

2.4. Carefully slide the straight edge of the spatula under the first rectangle and remove it **[1]**, then place the cutout with embryos on the external side of a 3.5-centimeter dish **[2]**. Under a stereomicroscope, lower a prepared coverslip over the embryos and gently press the two rows of embryos onto the glue **[3]**.

2.4.1. Talent removing the first rectangle. NOTE: shot 2.4.1 and 2.4.2 were combined/taken as 1 shot

2.4.2. Talent putting the rectangle in the dish.

2.4.3. SCOPE: Talent lowering the coverslip on the embryos.

2.5. If not performing microinjection, fill the silicone spacer halfway to the top with 1 to 1 - halocarbon oil 700 to halocarbon oil 27 **[1]**.

2.5.1. Talent filling the spacer. NOTE: Use 3.2.2. the same shot should be used for shot 2.5.1 and 3.2.2 – as such only shot 3.2.2 was filmed.

2.6. Line the bottom edges of a 4-slide plate adapter with double-sided tape. This will prevent the slide from moving during imaging. Ensure not to place the tape in the path of the objective lens **[1]**. Mount the coverslip on the 4-slide plate adapter **[2].**

2.6.1. Talent lining the edges with the tape. NOTE: this was shot out of order – this was shot last – after shot 3.8 – but order should be maintained, so this is still shot 2.6.1

2.6.2. Talent mounting the coverslip. NOTE: this was shot out of order – this was shot last – but order should be maintained, so this is still shot 2.6.2.

3. Desiccating and Microinjecting Embryos

3.1. If microinjecting the embryos, place the coverslip with embryos over a slide to prevent the bottom of the coverslip from becoming dirty during desiccation and microinjection **[1]**.

3.1.1. Talent placing the coverslip over a slide.

3.2. Desiccate the embryos for 5 to 10 minutes **[1]**, then immediately cover them with 1 to 1 - halocarbon oil 700 to halocarbon oil 27, filling the silicon spacer halfway to the top **[2]**.

3.2.1. Embryos desiccating.

3.2.2. Talent covering the embryos with oil. NOTE: the same shot is used for shot 2.5.1 and 3.2.2.

3.3. Load 2 or 3 microinjection needles with approximately 2 microliters of injectant using an extended loading tip **[1]**. Mount a needle onto the microinjector **[2]** and depress the plunger halfway to the tip, increasing the air pressure inside of the glass capillary **[3]**.

3.3.1. Talent loading a microinjection needle.

3.3.2. Talent mounting a needle onto the microinjector.

3.3.3. Talent depressing the plunger.

3.4. Lower the glass needle onto a glass slide **[1]** and cut the tip of the needle with a new number 9 razor, cutting at a 45-degree angle to produce a sharp open tip. The injectant should flow down to the bottom of the tip without flowing out of the needle **[2]**. *Videographer: This step is important!*

3.4.1. Glass needle lowering onto the glass slide.

3.4.2. SCOPE: Talent cutting the tip of the needle and injectant flowing to the bottom of the tip.

3.5. Carefully add 20 microliters of halocarbon oil over the tip of the needle **[1]** and re-cut it open at a 45-degree angle. The injectant should begin to flow rapidly so remember to adjust pressure by retracting the plunger **[2]**. *Videographer: This step is important!*

3.5.1. ECU: Talent adding halocarbon oil over the tip of the needle.

3.5.2. SCOPE: Talent cutting the needle again and injectant flowing and flow rate being adjusted.

3.6. Use the micromanipulator to position the needle at the center of the field of view **[1]**, then lift the needle and remove the glass slide from under it **[2]**.

3.6.1. SCOPE: Needle coming into the center of the field of view.

3.6.2. Talent lifting the needle and removing the glass slide.

3.7. Place the embryos on the stereomicroscope stage under microinjection needle **[1]** and focus on them at 50 X magnification **[2]**. Lower the needle until it comes into the same focus plane as the embryos **[3]**. Moving the slide with one hand and operating the microinjector with the other **[4]**, inject one row of embryos **[5]**. *Videographer: This step is difficult and important!*

3.7.1. Talent placing the embryos under the needle.

3.7.2. SCOPE: Talent focusing on the embryos.

3.7.3. SCOPE: Needle lowering.

3.7.4. SCOPE: Embryo being injected. NOTE: multiple embryos were injected. For post editing feel free to include the best injected embryo or all embryos.

3.7.5. Added Shot: ECU: shot of injections with one hand operating the injector and one hand moving the plate to move the row of embryos.

3.8. Raise the needle and rotate the slide 180 degrees to expose the second row of embryos to the microinjection needle **[1]**. Lower the needle and inject the second row of embryos **[2]**.

3.8.1. Talent raising the needle and rotating the slide.

3.8.2. SCOPE: Another embryo being injected. NOTE: multiple embryos were injected. For post editing feel free to include the best injected embryo or all embryos.

Results

4. Results: Rtnl1-GFP and ReepB-GFP Enrichment at the Spindle Poles during Mitosis

4.1. This protocol was used to examine the role of microtubules in Endoplasmic Reticulum reorganization during mitosis in the *Drosophila* embryo **[1]**.

4.1.1. LAB MEDIA: Figure 5.

4.2. The effects of several microinjected drug treatments on ER reorganization were quantitatively compared. Colchicine, which prevents new microtubule polymerization, was found to drastically reduce the localization of the ER to spindle poles during mitosis **[1]**.

4.2.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize the colchicine images.*

4.3. Time laps imaging data was acquired for 32 embryos. Mean and max intensity measurements were analyzed from 12,800 regions of interest using a custom MATLAB script. **[1]**.

4.3.1. LAB MEDIA: Figure 6.

Conclusion

5. Conclusion Interview Statements

5.1. **Ulises Diaz:** When attempting this protocol, remember that spending too much time on any step may cause you to miss the developmental stage you want to image. Practice this protocol step by step before performing experiments.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.1 or shots from 3.7 and 3.8*.

5.2. **Ulises Diaz:** Our method for sample preparation facilitates the simultaneous acquisition of multiple embryos within one imaging session, thus generating enough experimental repeats for quantitative analysis.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.3. **Ulises Diaz:** This technique can help developmental embryology transition from qualitative to quantitative imaging experiments. The ability to image multiple developing embryos within one experiment also eliminates experimental noise between replicates.

5.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.