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Title: Examining Muscle regeneration in Zebrafish Models of Muscle Disease

Authors and Affiliations: Margo Montandon[1], Peter D. Currie[1], and Avnika A. Ruparelia[1]

[1]Australian Regenerative Medicine Institute, Monash University

Corresponding Author:
Avnika A. Ruparelia
avnika.ruparelia@monash.edu

Co-authors:
margo.montandon@monash.edu
peter.currie@monash.edu

Author Questionnaire

1. **Microscopy**: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y**, ZEISS SteREO Discovery.V8

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

   Videographer: All screen capture files provided, do not film

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Protocol Length
Number of Shots: **28**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Margo Montandon**: The overall goal of this protocol is to examine and quantify muscle regeneration in zebrafish models of muscle disease **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested b-roll: 4.1.2 and 4.2.1*

REQUIRED:

1.2. **Margo Montandon**: This method provides a high-throughput pipeline that is not only cost-effective, but also highly reproducible, in scoring the regenerative potential of a diseased muscle in an in vivo setting **[1]**.

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

Introduction of Demonstrator on Camera

1.3. **Margo Montandon**: Demonstrating the procedure with me will be Avnika Ruparelia, a research fellow from the Australian Regenerative Medicine Institute **[1][2]**.

1.3.1. INTERVIEW: Author saying the above

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Protocol

2. Live Embryo Genotyping

2.1. For live embryo genotyping, peel the clear protective film from the top surface of a 24-chamber DNA extraction chip **[1]** and use a 20-microliter filter tip with a cut tip to transfer a single, anesthetized embryo in 13 microliters of embryo medium into each chamber of the chip **[2-TXT]**.

2.1.1. WIDE: Talent peeling film

2.1.2. Embryo being added to chamber *Videographer: Important step* **TEXT: Anesthesia: 0.016% tricaine methanesulfonate**

2.2. When all of the embryos have been loaded, gently mount the chip onto the zebrafish embryo genotyping platform by placing one side in first, followed by the rest of the chip **[1]**.

2.2.1. Chip being mounted onto platform

2.3. Affix the magnetic platform lid over the chip to prevent the evaporation of embryo medium during the DNA extraction protocol **[1]** and close the lid **[2]**.

2.3.1. Talent placing lid over chip
2.3.2. Talent closing lid

2.4. Set the base unit to 2.4 volts, 0.051 amps, and 0.12 watts **[1]** and start the DNA extraction protocol **[2]**. The platform should start vibrating, which can be assessed by gently touching the lid **[3]**. NOTE: 2.4.1, 2.4.2 and 2.4.3 are all combined in single shot

2.4.1. Talent setting extraction parameters *Videographer: Important step*
2.4.2. Talent pressing ON/OFF *Videographer: Important step*
2.4.3. Talent checking lid *Videographer: Important step*

2.5. While the program is running, prepare a 24-well plate by adding 1 milliliter of embryo medium to each well **[1]** and label 8-well strip tubes to be used for the collection of DNA material from each embryo **[2]**.

2.5.1. Talent adding medium to well(s), with medium container visible in frame
2.5.2. Talent labelling strip

2.6. After 8 minutes of extraction, press the ON/OFF button to stop the vibration of the platform **[1]**, gently remove the magnetic lid **[2]**, and lift the chip from the platform **[3]**.
NOTE: 2.6.1, 2.6.2 and 2.6.3 are all combined in single shot
2.6.1. Talent pressing button
2.6.2. Talent opening and/or removing lid
2.6.3. Talent removing chip

2.7. Transfer 10 microliters of embryo medium from one chamber into the appropriate well of the 8-well strip tube **[1]** and immediately add two drops of fresh embryo medium to the chambers **[2]**.
NOTE: Shots 2.7.2 and 2.8.1 were combined as it made more sense logically.
2.7.1. Medium being removed/added to tube *Videographer: Important step*
2.7.2. Medium being added to chamber *Videographer: Important step* NOTE: An extra shot of 2.7.2 was made with a different view if needed.

2.8. Transfer the embryo from the chamber to an appropriate well of the previously prepared 24-well plate **[1].** When all of the embryos have been transferred, place the plate in a 28-degree Celsius incubator **[2]**.

2.8.1. Talent adding embryo to well *Videographer: Important step* NOTE: Shots 2.7.2 and 2.8.1 were combined.
2.8.2. Talent placing plate into incubator *Videographer: Important step*

2.9. Perform the appropriate downstream genotyping assays on the genetic material collected in the 8-well strip tubes to determine the genotype of each embryo **[1]**.

2.9.1. Talent adding sample to thermocycle or similar

2.10. Once the genotype of each embryo has been identified, transfer the embryos to 90-millimeter petri dishes containing 25 milliliters of medium water **[1-TXT]** and incubate the plates at 28 degrees Celsius until muscle injury **[2]**.

2.10.1. Talent adding embryo to dish, with medium water container visible in frame
TEXT: Place each genotype in different dish
2.10.2. Talent placing plate(s) at 28 °C

3. Muscle Injury

3.1. At 4 days post fertilization, use a pipette to transfer one anesthetized larva into a new Petri dish **[1]** and carefully remove any excess medium from the dish under a dissecting microscope **[2]**.

3.1.1. WIDE: Talent placing larva into dish, with microscope visible in frame
3.1.2. SCOPE: Medium being removed

3.2. Orient the fish such that the head is on the left, the tail is on the right, the dorsal region is up, and the ventral region is down **[1]** and use a 30-gauge needle to make a quick, precise stab into 1-2 somites in the epaxial muscle above the anal pore and horizontal to the myoseptum **[2-TXT]**.

3.2.1. SCREEN: Shot3.2.1.mov.00:00-00:12 *Videographer: Important step; Video Editor: please emphasize head, tail, dorsal region, and ventral region when mentioned if appropriate*
3.2.2. SCREEN: Shot3.2.2.mov.00:00-00:09 *Videographer: Important/difficult step* **TEXT: Caution: Avoid stabbing neural tube and notochord**

3.3. Apply a drop of embryo medium to the injured zebrafish **[1]** and carefully transfer the larva into one well of 24-well plate containing 1 milliliter of fresh embryo medium per well **[2]**.

3.3.1. SCREEN: Shot3.3.1.mov.00:00-00:17
3.3.2. Talent placing larva into well, with medium container visible in frame

3.4. When all of the larvae have been injured, place the plate in the 28-degree Celsius incubator until the zebrafish are imaged **[1]**.

3.4.1. Talent placing plate into incubator

4. Muscle Regeneration Quantification

4.1. To quantify the muscle regeneration, open a 1-day post injury image in an appropriate image analysis software program **[1-TXT]** and use the polygon tool to draw a shape around the wound site **[2]**.

4.1.1. WIDE: Talent opening image, with monitor visible in frame **TEXT: Image injured larvae at d1 and d3 post injury by standard polarizing micrscopy**
4.1.2. SCREEN: screenshot_1: 00:15-00:30

4.2. Use the software to measure the area and mean birefringence intensity of the region and copy these values to cells D3 and E3 of the provided template **[1]**.

4.2.1. SCREEN: screenshot_2: 00:00-00:22 *Video Editor: please speed up*

4.3. Draw two additional regions, each spanning 1-2 uninjured somites, and measure the area and mean birefringence intensities of each of these regions. Copy these values to

cells D4 and D5 and E4 and E5 in the template **[1]**.

4.3.1. SCREEN: screenshot_3: 00:02-00:38 *Video Editor: please speed up*

4.4. Then repeat the measurement for same regions in the 3 days post injury image **[1-TXT]**.

4.4.1. SCREEN: screenshot_4: 00:07-00:33 **TEXT: If same sites not selectable, select two different unaffected areas at each timepoint**

4.5. When the birefringence has been measured in the same manner in all of the images, calculate the normalized birefringence for each region by dividing the mean birefringence intensity of each region by its area **[1]**.

4.5.1. SCREEN: screenshot_5: 00:03-00:10 region *Video Editor: please emphasize column F values*

4.6. For each time point, calculate the average normalized birefringence of the two uninjured regions to provide a reference point for the uninjured muscle **[1]**.

4.6.1. SCREEN: screenshot_6: 00:03-00:08

4.7. To determine the extent of muscle injury at day 1 post injury, divide the normalized birefringence of the injury region by the average normalized birefringence of the uninjured regions **[1]**.

4.7.1. SCREEN: screenshot_7: 00:03-00:14

4.8. To determine the extent of muscle regeneration, divide the normalized birefringence of the injury region in the 3 days post injury image by the average normalized intensity of the uninjured regions at this stage **[1]**.

4.8.1. SCREEN: screenshot_8: 00:03-00:12

4.9. Finally, calculate the regenerative index by dividing the value for the extent of the muscle regeneration at day 3 post injury by the value for the extent of the muscle injury at day 1 post injury **[1]**.

4.9.1. SCREEN: screenshot_9: 00:03-00:14

Results

5. Results: Representative Muscle Regeneration Analysis in Wildtype and *Lama2* Deficient Zebrafish Larvae

5.1. In this representative analysis **[1]**, a clutch of embryos from a cross between two *lama2* (lah-mah-two) heterozygous zebrafish was transferred to a DNA extraction chip and subsequently genotyped **[2-TXT]**.

5.1.1. LAB MEDIA: Figure 1A
5.1.2. LAB MEDIA: Figure 1A *Video Editor: please emphasize embryos* **TEXT: *lama2*: laminin subunit alpha-2**

5.2. While muscle injury results in a reduction in birefringence intensity at day 1 post injury **[1]**, the successful regeneration of muscle results in an increased birefringence within the same region **[2]**.

5.2.1. LAB MEDIA: Figures 1Ci and 1Di *Video Editor: please emphasize white and red outlined regions*
5.2.2. LAB MEDIA: Figures 1C and 1D *Video Editor: please emphasize white and red outlined regions in Figures 1Cii and 1Dii*

5.3. It is also noteworthy that while wildtype larvae display a uniform birefringence intensity due to a normal muscle patterning **[1]**, the birefringence intensity in the muscle of *lama2* knockout larvae is uneven and highly sporadic, likely due to a reduced muscle integrity **[2]**.

5.3.1. LAB MEDIA: Figures 1C and 1D *Video Editor: please emphasize muscle in 1Ci or 1Cii image*
5.3.2. LAB MEDIA: Figures 1C and 1D *Video Editor: please emphasize muscle in 1Di or 1Dii image*

5.4. As observed **[1]**, both wildtype **[2]** and lama2-deficient larvae **[3]** demonstrate a significantly increased birefringence intensity in the wound site at 3 days post injury **[4]** compared to at 1 day post injury, indicating that the muscle has regenerated **[5]**.

5.4.1. LAB MEDIA: Figures 2A-2C
5.4.2. LAB MEDIA: Figures 2A-2C *Video Editor: please emphasize lama2+/+ 3 dpi image*
5.4.3. LAB MEDIA: Figures 2A-2C *Video Editor: please emphasize lama2-/- 3 dpi image*
5.4.4. LAB MEDIA: Figures 2A-2C *Video Editor: please emphasize red 3 dpi data points*

5.4.5. LAB MEDIA: Figures 2A-2C *Video Editor: please emphasize blue 1 dpi data points*

5.5. Determination of the regenerative index **[1]** reveals that lama2-deficient larvae **[2]** display a striking increase in muscle regeneration compared to wildtype animals **[3]**.

5.5.1. LAB MEDIA: Figure 2D
5.5.2. LAB MEDIA: Figure 2D *Video Editor: please emphasize red data points*
5.5.3. LAB MEDIA: Figure 2D *Video Editor: please emphasize blue data points*

Conclusion

6. Conclusion Interview Statements

6.1. **Avnika Ruparelia**: While this protocol can reveal alterations in the ability of muscle to regenerate in models of muscle disease, downstream cellular and molecular analyses need to be performed to identify the mechanisms responsible for the observed changes **[1]**.

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

6.2. **Avnika Ruparelia**: This method has allowed us to examine how impaired muscle stem cell function and the associated niche elements contribute to muscle disease pathogenesis, facilitating the identification of novel disease mechanisms **[1]**.

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