

Submission ID #: 69422

Scriptwriter Name: Pallavi Sharma

Project Page Link: https://review.jove.com/files_upload.php?src=21173758

Title: Detection of Global DNA Methylation in the Hearts of Zebrafish Larvae

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

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

Videographer: Please film all the computer screen for all shot labeled as screen

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

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 14

Number of Shots: 24

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Zhao Shoushuang:** In this study, we developed a sensitive immunofluorescence approach to quantify 5 mC in zebrafish embryonic hearts.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.7.1*

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Zhao Shoushuang:** This protocol enables sensitive quantification of 5mC within individual zebrafish embryonic hearts.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Zhao Shoushuang:** Our protocol enables quantitative analysis of DNA methylation at the organ level during early developmental stages.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Zhao Shoushuang:** It is essential to ensure complete DNA denaturation and rigorous washing procedures to avoid weak signal or high background.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Zhao Shoushuang**: This method can be employed in future research to examine the impact of environmental chemicals on alterations in DNA methylation throughout cardiac development.

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

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Protection Institute of the Ethics Committee of Soochow University

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Immunofluorescence Staining and Imaging of the Zebrafish Heart

Demonstrator: Zhao Shoushuang

- 2.1. To begin, take hearts isolated from zebrafish embryos exposed to 5-Azacytidine (*Five-Azacytidine*) [1-TXT]. Using a pipette, transfer 3 hearts into a hydrophobic barrier pen circle on an adhesive slide [2].
 - 2.1.1. WIDE: Talent at the working bench with the heart containing petri dish placed in front of him. **TXT: 5-Azacytidine Concentrations Used: 0 μ M, 25 μ M, and 50 μ M (2–72 hpf)**
 - 2.1.2. Talent transferring 3 isolated hearts with a pipette into the hydrophobic barrier pen circle on the adhesive slide.
- 2.2. Add 45 microliters of 4 percent paraformaldehyde and fix the samples for 30 minutes at room temperature [1]. Wash the samples two times with PBS containing Tween 20, using 50 microliters per wash for 5 minutes each [2].
 - 2.2.1. Talent adding 45 microliters of 4 percent paraformaldehyde to the hearts on the adhesive slide and placing the slide aside for fixation.
 - 2.2.2. Talent washing the samples on the adhesive slide with PBS with Tween 20.
- 2.3. Next, add 45 microliters of 0.3 percent Triton X-100 to permeabilize the samples and incubate for 35 minutes at room temperature [1]. Wash the samples 3 times for 5 minutes each with PBS containing Tween 20 [2].
 - 2.3.1. Talent adding 45 microliters of 0.3 percent Triton X-100 to the samples on the adhesive slide and placing the slide aside for incubation.
 - 2.3.2. Talent washing the samples on the adhesive slide with phosphate buffered saline with Tween 20.
- 2.4. Add 45 microliters of Proteinase K and incubate for 30 minutes. Then wash three times with PBS as demonstrated earlier [1].
 - 2.4.1. Talent adding 45 microliters of Proteinase K at 10 milligrams per milliliter to the samples on the adhesive slide and placing the slide aside for incubation.
- 2.5. Next, treat the samples with 45 microliters of 4 molar hydrochloric acid for 30 minutes to ensure effective DNA denaturation [1]. Neutralize the samples with a Tris-HCl buffer for 20 minutes, then wash with PBS containing Tween-20 three times [2].
 - 2.5.1. Talent adding 45 microliters of 4 molar hydrochloric acid to the samples on the

adhesive slide and placing the slide aside for incubation.

- 2.5.2. Talent adding Tris HCl buffer at pH 8.5 to the samples on the adhesive slide and placing the slide aside.
- 2.6. Then, incubate the samples with 45 microliters of 10 percent goat serum for 1 hour at room temperature to block nonspecific binding [1]. Wash the samples 3 times for 5 minutes each with PBS containing Tween 20 [2].
 - 2.6.1. Talent adding 45 microliters of 10 percent goat serum to the samples on the adhesive slide and placing the slide aside for incubation.
 - 2.6.2. Talent washing the samples on the adhesive slide with PBS with Tween 20.
- 2.7. Now, incubate the samples with anti-5-Methylcytosine antibody overnight at 4 degrees Celsius [1].
 - 2.7.1. Talent adding the anti-5-Methylcytosine antibody solution to the samples on the adhesive slide and placing the slide aside.
- 2.8. On the following day, remove the primary antibody [1]. Wash the samples 3 times for 10 minutes each with PBS containing Tween 20 [2].
 - 2.8.1. Talent removing the primary antibody solution from the samples on the adhesive slide.
 - 2.8.2. Talent washing the samples on the adhesive slide with phosphate buffered saline with Tween 20.
- 2.9. After that, incubate the samples with 45 microliters of Alexa Fluor 488 (Four-Eighty-Eight)-conjugated goat anti-mouse immunoglobulin G for 1 hour at room temperature in the dark [1].
 - 2.9.1. Talent adding 45 microliters of Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin G solution to the samples and placing the slide aside in the dark for incubation.
- 2.10. After washing the sample three times with PBS containing Tween 20 for 10 minutes each, add 10 microliters of DAPI (Dapi) to co-stain the nuclei [1].
 - 2.10.1. Talent adding 10 microliters of DAPI to the samples on the adhesive slide.
- 2.11. Now, cover the samples with a coverslip [1] and keep the slides in the dark for 1 hour [2].
 - 2.11.1. Talent placing a coverslip over the samples on the adhesive slide.
 - 2.11.2. Talent placing the slide in a dark environment for incubation.
- 2.12. After that, acquire images using a standard inverted fluorescence microscope [1]. Use a 20-time objective lens [2] to capture the entire embryonic heart structure [3].
 - 2.12.1. Talent places the sample slide on the microscope stage.

2.12.2. Talent adjusting the microscope to a 20 times objective lens and

SCREEN: 69422_screenshot_1.mp4: 00:00-end NOTE: For 2.12.2. author has filmed a talent shot and uploaded a screen capture as well

Videographer: Please film all the computer screen for all shot labeled as screen

2.13. Image the DAPI channel and Alexa Fluor 488 channel sequentially using standard filter sets to avoid crosstalk [1]. Maintain identical exposure times, gain, and light intensity settings for all samples within the same experiment [2]. Save the images in a lossless format [3].

2.13.1. Selection of DAPI and Alexa Fluor 488 channel.

2.13.2. SCREEN: 69422_screenshot_2.mp4: 00:00-end

2.13.3. SCREEN: 69422_screenshot_3.mp4: 00:00-end

Results

3. Results

- 3.1. 5-Azacytidine at both tested concentrations did not significantly affect the embryo survival rate and hatching rate compared to the DMSO (*D-M-S-O*) control group [1].
 - 3.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the survival and hatching rate chart and show that the bars for DMSO, 5-AzaC 25, and 5-AzaC 50 are at similar heights*
- 3.2. Heartbeat rates were decreased to a similar level in the two 5-Azacytidine treatment groups compared with DMSO controls [1].
 - 3.2.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the heart rate per beat chart and emphasize the 2 shorter bars for 5-AzaC 25 and 5-AzaC 50 compared with DMSO*
- 3.3. 5-Azacytidine exposure increased cardiac malformation rates compared with DMSO controls at 25 micromolar [1] and 50 micromolar [2].
 - 3.3.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the bar labelled 5-AzaC 25 in the heart malformation chart*
 - 3.3.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the bar labelled 5-AzaC 50 in the heart malformation chart*
- 3.4. 5-Azacytidine treatment significantly reduced global deoxyribonucleic acid methylation levels in the hearts of zebrafish larvae [1].
 - 3.4.1. LAB MEDIA: Figure 1C. *Video editor: Highlight the 5-AzaC 25 and 5-AzaC 50 images on the left side and relative 5mC levels chart and emphasize the lower bars for 5-AzaC 25 and 5-AzaC 50 compared with DMSO*

1. Azacytidine

Pronunciation link: <https://www.merriam-webster.com/dictionary/azacytidine>

IPA: /ˌæzəˈsaɪtəˌdiːn/

Phonetic Spelling: az·uh·sai·tuh·deen

2. Hydrophobic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrophobic>
IPA: /ˌhaɪdrəˈfoʊbɪk/
Phonetic Spelling: hai·druh·foh·bik
3. Paraformaldehyde
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraformaldehyde>
IPA: /ˌpærəfɔːrˈmældəˌhaɪd/
Phonetic Spelling: pa·ruh·for·mal·duh·hyde
4. Phosphate
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /ˈfɑːsˌfeɪt/
Phonetic Spelling: faas·fayt
5. Triton
Pronunciation link: <https://www.merriam-webster.com/dictionary/triton>
IPA: /ˈtraɪtɒn/
Phonetic Spelling: trai·ton
6. Permeabilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeabilize>
IPA: /ˌpɜːmiˈæbəˌlaɪz/
Phonetic Spelling: pur·mee·uh·buh·lize
7. Proteinase
Pronunciation link: <https://www.merriam-webster.com/dictionary/proteinase>
IPA: /ˌproʊtiˈneɪs/
Phonetic Spelling: proh·tee·nays
8. Hydrochloric
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrochloric>
IPA: /ˌhaɪdruˈklɔːrɪk/
Phonetic Spelling: hai·droh·klor·ik
9. Denaturation
Pronunciation link: <https://www.merriam-webster.com/dictionary/denaturation>
IPA: /diːˌneɪʃəˈreɪʃən/
Phonetic Spelling: dee·nay·chuh·ray·shuhn
10. Tris-HCl
Pronunciation link: No confirmed link found
IPA: /ˈtrɪsˌeɪtʃˌsiːˈel/
Phonetic Spelling: tris·H·C·L
11. Nonspecific
Pronunciation link: <https://www.merriam-webster.com/dictionary/nonspecific>
IPA: /ˌnɒːnspəˈsɪfɪk/
Phonetic Spelling: non·spuh·si·fik
12. Methylcytosine
Pronunciation link: <https://www.merriam-webster.com/dictionary/methylcytosine>
IPA: /ˌmeθəlˈsaɪtəˌsiːn/
Phonetic Spelling: meth·uhl·sai·tuh·seen

13. Immunoglobulin
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunoglobulin>
IPA: /ɪˌmjuːnoʊˈɡləːbjəlin/
Phonetic Spelling: ih·myoo·noh·glaa·byuh·lin
14. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈresəns/
Phonetic Spelling: floor·eh·suhns
15. Crosstalk
Pronunciation link: <https://www.merriam-webster.com/dictionary/crosstalk>
IPA: /ˈkrɒsˌtɔːk/
Phonetic Spelling: kraws·tawk
16. Deoxyribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonucleic%20acid>
IPA: /diːˌɑːksiˌraɪboʊnuːˈkliːɪk/
Phonetic Spelling: dee·ok·see·rai·boh·noo·klee·ik
17. Methylation
Pronunciation link: <https://www.merriam-webster.com/dictionary/methylation>
IPA: /ˌmɛθəˈleɪʃən/
Phonetic Spelling: meth·uh·lay·shuhn
18. Micromolar
Pronunciation link: <https://www.merriam-webster.com/dictionary/micromolar>
IPA: /ˌmaɪkroʊˈmoʊlər/
Phonetic Spelling: mai·kroh·moh·lur