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Title: In Vitro Assessment of Cardiac Reprogramming by Measuring Cardiac Specific Calcium Flux with a GCaMP3 Reporter

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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**

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.**



Interview Statements are read by JoVE's voiceover talent.

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

Current Protocol Length

Number of Steps: 11

Number of Shots: 22

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. The methods described in this protocol will provide a valuable platform to assess the functional maturation of iCMs for cardiac reprogramming studies.
 - 1.1.1. [4.1.1.](#)
- 1.2. The calcium flux is exclusively observed in iCMs and provides a way to evaluate iCM functional maturation. This mouse strain simplifies the evaluation procedures and enhances the reproducibility of results.
 - 1.2.1. [3.4.2.](#)

OPTIONAL:

- 1.3. This protocol is a new method to evaluate iCM functional maturation in cardiac reprogramming field. It can also be applied to evaluate cardiomyocyte differentiation and cardiac function.
 - 1.3.1. [4.4.1.](#)

Introduction of Demonstrator on Camera

- 1.4. Demonstrating the procedure will be Liu Liu, a Research Investigator from Dr. Wang's laboratory.
 - 1.4.1. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

- 1.5. All experimental procedures and practices involving animals were approved by Institutional Animal Care and Use Committee at the University of Michigan.

Protocol

2. Isolation and Selection of Neonatal Myh6-Cre/Rosa26A-Flox-Stop-Flox-GCaMP3 Mice Hearts.

- 2.1. Begin by obtaining 8 to 10 pups P0 to P2 pups to isolate 10 million neonatal cardiac fibroblasts, or NCFs [1].

2.1.1. WIDE: Establishing shot of the talent acquiring the pups. Use LAB MEDIA: 2.1.1.mp4

- 2.2. Observe the hearts under the fluorescence microscope to ensure that the heart with the desired genotype shows calcium flux, indicated by GCaMP3 (*pronounce 'G-Camp-Three'*) with the hearts beating. The other genotypes will not show fluorescence [1-TXT]. *Videographer: This step is important!*

2.2.1. LAB MEDIA: Zhaokai Li - Video 1 GCaMP3+ heart.mp4 and Zhaokai Li - Video 2 control heart.mp4

Play the 2 videos side-by-side

Video editor:

Label Video 1 (fluorescent heart) using text overlay: Myh6-Cre/Rosa26A-Flox-Stop-Flox-GCaMP3

Label Video 2 (control heart) using text overlay: Control

3. Isolation of neonatal cardiac fibroblasts (NCFs)

- 3.1. After isolation of the hearts, cut them into four pieces such that they are loosely connected [1]. Wash them thoroughly several times in ice-cold Dulbecco's phosphate-buffered saline or DPBS in a 6-centimeter plate to limit the blood cell pollution in the isolated cells [2]. *Videographer: This step is important!*

3.1.1. Talent cutting the hearts into four pieces.

3.1.2. Talent washing the hearts using DPBS.

- 3.2. Digest the hearts with 8 milliliters of warm 0.25 percent Trypsin-EDTA at 37 degrees Celsius for 10 minutes [1]. Discard the trypsin supernatant [2] and add 5 milliliters of 0.5 milligrams per milliliter warm type-II (*'type-2'*) collagenase in Hanks' balanced salt solution [3]. *Videographer: This step is important!*

- 3.2.1. Talent digesting the hearts.
- 3.2.2. Talent discarding the supernatant.
- 3.2.3. Talent adding the collagenase.
- 3.3. Vortex the mixture thoroughly and incubate it at 37 degrees Celsius for 5 minutes [1]. After the incubation, again vortex the mixture thoroughly and let the undigested tissue settle down by gravity [2]. *Videographer: This step is important!*
 - 3.3.1. Talent vortexing the mixture.
 - 3.3.2. ECU: A shot of the tube with the undigested tissue settling down.
- 3.4. Collect the supernatant in a 15-milliliter conical tube with 5 milliliters of cold fibroblast medium [1-TXT]. Dilute the cells with fibroblast medium such that the seeding density is around $2 \text{ to } 2.5 \times 10^4$ cells per cubic centimeter [2], then seed the cells in dishes or plates [3].
 - 3.4.1. Talent collecting the supernatant in the conical tube. **TEXT: IMDM with 20% FBS and 1% penicillin/streptomycin**
 - 3.4.2. Talent diluting the cells.
 - 3.4.3. Talent seeding the cells onto plates.
- 3.5. The attached fibroblasts should have an oval to round shape on the second day after seeding [1]. *Videographer: This step is important!*
 - 3.5.1. Talent checking the shape of the attached fibroblasts.

4. Reprogramming NCFs to iCMs with MGT Encoding Retrovirus Infection

- 4.1. On day 0, seed the NCFs to the density of around $1 \text{ to } 2 \times 10^4$ cells per cubic centimeter in fibroblast medium [1]. On day 1, replace the culture medium with a virus-containing medium for each well as desired [2].
 - 4.1.1. Talent seeding the cells in fibroblast medium.
 - 4.1.2. Talent replacing the culture medium.
- ~~4.2. Titer the virus to determine the optimal virus concentration [1]. Use the virus from one well Plat-E in a 6-well plate to infect two wells in a 24-well plate [2].~~

~~4.2.1. Talent titrating the virus.~~

~~4.2.2. Talent infecting two wells in the 24-well plate.~~

- 4.3. On day 2, 24 hours after the virus infection, replace the virus-containing medium with a regular iCM medium [1].

4.3.1. Talent replacing the virus-containing medium.

- 4.4. Assess the calcium ion flux with an inverted fluorescence microscope at room temperature [1]. Observe the GCaMP3+ cells under 10x objective, ensuring that it shows spontaneous cells beating in the bright field channel [2]. *Videographer: This step is important!*

4.4.1. Talent assessing the calcium ion flux.

4.4.2. Talent observing the GCaMP3+ cells.

Results

5. Results: In Vitro Assessment of Cardiac Reprogramming

- 5.1. Hearts with the correct genotype showed calcium ion flux synchronized with beating, visualized as GCaMP3 (*pronounce 'G-Camp-Three'*) fluorescence [1], while no fluorescence was observed in control hearts [2].
 - 5.1.1. LAB MEDIA: Figure 2. *Video editor only include the two images labeled GCaMP3+ heart.*
 - 5.1.2. LAB MEDIA: Figure 2. *Video editor only include the two images labeled control heart.*
- 5.2. Isolated NCFs attached to the well within 2 hours [1] and showed an oval to round shape 1 day after the seeding [2].
 - 5.2.1. LAB MEDIA: Figure 3A.
 - 5.2.2. LAB MEDIA: Figure 3B.
- 5.3. The calcium ion oscillation showed GCaMP3 fluorescence change between the maximum [1] and minimum [2], and the calcium ion oscillation of such cells changed periodically [3].
 - 5.3.1. LAB MEDIA: Figure 4A. *Video editor only include the image labeled Ca²⁺ max.*
 - 5.3.2. LAB MEDIA: Figure 4A. *Video editor only include the image labeled Ca²⁺ min.*
 - 5.3.3. LAB MEDIA: Figure 4B.
- 5.4. After introducing IMAP, the number of beating clusters was significantly higher than that in the control group, as the number of GCaMP3-positive cells with calcium ion flux per high-power field was increased in the IMAP-medium-treated group [1].
 - 5.4.1. LAB MEDIA: Figure 5.

Conclusion

6. Conclusion Interview Statements

- 6.1. NCFs should be freshly prepared and healthy after isolation. A rapid procedure of heart isolation, cutting, and proper digestion time can help to avoid reduction in cell viability and condition.

6.1.1. [3.1.1.](#) , [3.2.1.](#)

- 6.2. This method provides a way to identify functional matured iCMs for cardiac reprogramming researchers. With multiple further evaluation methods, one can get a more concise understanding about functional matured iCMs.

6.2.1. [4.3.1.](#)