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Title: An Approach to Study Shape-Dependent Transcriptomics at a Single Cell Level

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Author Questionnaire

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

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

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

Protocol Length
Number of Shots: **37**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Payam Haftbaradaran Esfahani**: We propose a novel platform for investigating the effects of cell shape on gene expression using methods for growing and sorting adherent cells with different morphologies at the single-cell level **[1]**.

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

REQUIRED:

1.2. **Payam Haftbaradaran Esfahani**: The main advantage of this technique is that it facilitates the high-throughput study of cell shapes in vitro, as comparing cells with different shapes in vivo is technically demanding **[1]**.

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

Protocol

2. Cardiomyocyte (CM) Patterning

2.1. To set up a patterned cardiomyocyte culture, transfer a cardiomyocyte suspension at the aspect ratio of interest to a 15-milliliter tube for counting **[1]** and dilute the cells to a 1 x 10^5 cells/milliliter of appropriate plating medium concentration **[2-TXT]**.

2.1.1. WIDE: Talent adding cells to tube, with hemocytometer visible in frame

2.1.2. Talent adding medium to tube, with medium container **TEXT: See text for all medium and solution preparation details**

2.2. Add 2 milliliters of cells onto a fibronectin-coated micropatterned chip submerged in 2 milliliters of warm plating medium inside a 35-milliliter Greiner Petri dish **[1-TXT]** and place the dish in a 37-degree Celsius and 5% carbon dioxide incubator for 18 hours **[2]**.

2.2.1. Talent add cells to dish **TEXT: See text for chip preparation details**

2.2.2. Talent placing dish into incubator

2.3. The next day, check the chip by light microscopy to confirm that most of the cells have attached **[1]**.

2.3.1. Talent at microscope, checking chip

2.4. Remove the medium from the dish to remove any debris and/or dead cells **[1]** and, starting from the center and moving toward the sides in a dropwise fashion, gently add PBS to the cells **[2]**.

2.4.1. Medium being removed *Videographer: Important step*

2.4.2. PBS being added, with PBS container visible in frame *Videographer: Important step*

2.5. After the third wash, replace the PBS with 4-milliliter maintenance medium **[1]** and return the cells to the cell culture incubator **[2]**.

2.5.1. Talent adding medium to dish, with medium container visible in frame

2.5.2. Talent placing plate into incubator

3. Adherent CM Picking

3.1. After 72 hours, gently flush the chip surface two times with two milliliters of warm Dulbecco's PBS per wash as demonstrated **[1]** and use forceps to immediately transfer the washed chip to a new, sterile 35-milliliter Greiner Petri dish **[2]**.

3.1.1. WIDE: Talent adding DPBS to chip, with DPBS container visible in frame *Videographer: Important step*

3.1.2. Talent placing chip into dish *Videographer: Important step*

3.2. Quickly add 1.5 microliters of Vibrant Dye Cycle green, diluted 1000-fold in DPBS, to the chip **[1]**.

3.2.1. Talent adding 1.5 milliliters of Dye:DPBS (1:1000) to the chip NOTE: Used to be 2 shots but combined into 1

3.3. Fix a chamber over the chip **[1]** and place the dish onto the dish holder of the cell picker stage **[2]**.

3.3.1. Talent placing chamber over chip *Videographer: Important step*

3.3.2. Talent placing dish onto dish holder *Videographer: Important step*

3.4. Insert the magnetic cap **[1]** and locate the crosshair in the Live View window **[2]**.

3.4.1. Talent inserting cap

3.4.2. Talent at computer, locating crosshair, with monitor visible in frame

3.5. Focus on the crosshair **[1]** and, in the **Scanning and sorting** window, select the **Calibration for automated injection** and **Calibrate [2]**.

3.5.1. SCREEN: screenshot_1: 00:00-00:12

3.5.2. SCREEN: screenshot_1: 00:13-00:23

3.6. Replace the dish supernatant with 1.5 milliliters a 1:1 TryplE **(*Pronounce "triple E")*** in DPBS solution to loosen the cells from the fibronectin **[1]** and open the **Scanning** tab **[2]**.

3.6.1. TryplE-DPBS being added to dish, with TryplE-DPBS container visible in frame *Videographer: Important step*

3.6.2. SCREEN: screenshot_2

3.7. To scan the entire chip, focus on the top left corner of the chip in the field of view **[1]** and click **Get current microscope position [2]**.

3.7.1. SCREEN: screenshot_3: 00:00-00:11

3.7.2. SCREEN: screenshot_3: 00:12-00:15

3.8. Next, focus on the bottom right corner of the chip **[1]** and click **Get current microscope position [2]**.

3.8.1. SCREEN: screenshot_4: 00:00-00:17

3.8.2. SCREEN: screenshot_4: 00:18-00:20

3.9. In the pop-up window, click **Set sharpest plane** and click **Go to the top right** and **Go to the bottom left corner** buttons. Then click **Finish** to start scanning **[1]**.

3.9.1. SCREEN: screenshot_5

3.10. When the scanning is complete, open the **Analyzing** tab and click **Show Map** to select the single cells that pass the study criteria **[1]**.

3.10.1. SCREEN: screenshot_6 *Video Editor: can speed up*

3.11. Center the glass microcapillary in the middle of the microscope live view **[1]** and open the **Pump** tab **[2]**.

3.11.1. SCREEN: screenshot_7: 00:00-00:06

3.11.2. SCREEN: screenshot_7: 00:07-00:13

3.12. To create a vacuum, retract the plunger from a 50-milliliter number-1 syringe 4 milliliters **[1]** and open the **Sorting** tab **[2]**.

3.12.1. Plunger being retracted

3.12.2. SCREEN: screenshot_8

3.13. To allow a single cell to be picked, set the valve 2 to be opened for 120 milliseconds and valve 1 to be opened for 20 milliseconds after a time lapse of 200 milliseconds **[1]**.

3.13.1. SCREEN: screenshot_9

3.14. To allow the picked cell to be delivered successfully to its PCR tube containing lysis buffer, set the valve 1 to be opened for 20 milliseconds and valve 2 to be opened for 10 milliseconds after a time lapse of 10 milliseconds **[1]**.

3.14.1. SCREEN: screenshot_10

3.15. When the valves settings have been adjusted, click **Compute the path**. The software will compute the fastest path from cell to cell for picking up and injecting the selected cells throughout the chip. Then focus the microscope on a pattern on the chip surface **[1]**.

3.15.1. SCREEN: screenshot_11

3.16. Use the joystick to move the microcapillary down carefully, so that the sharpest image of the tip of the microcapillary can be obtained without touching the chip surface **[1]** and click **Set**. A new window will open, showing the microcapillary cross section **[2]**.

3.16.1. Talent using joystick, with monitor and joystick visible in frame *Videographer: Important/difficult step*

3.16.2. SCREEN: screenshot_12

3.17. To have the software record the tip offset of the capillary in the x, y, and z coordinates, click on the exact center of the capillary. Then click **Start sorting** to launch the sorting **[1]**.

3.17.1. SCREEN: screenshot_13

Protocol Script Questions

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

2.4., 3.1., 3.3., 3.4., 3.6.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.17.

Results

4. Results: Representative Shape-Dependent Transcriptomic Analyses

4.1. In this representative analysis of pre-amplified cDNA (C-D-N-A) from a picked single cell **[1-TXT]**, a clear band in the gel-like densitometry plot was observed **[2]**, corresponding to the peak at 1852 base pairs in the electropherogram **[3]**.

4.1.1. LAB MEDIA: Figure 4 **TEXT: cDNA: complementary DNA**
4.1.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize grey bad in gel on right of image*
4.1.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize peak at 1852 bp*

4.2. The average size of fragments was 1588 base pairs **[1]** with a small number of fragments that were shorter than 300 base pairs **[2]**, indicating the generation of a good cDNA library **[3]**.

4.2.1. LAB MEDIA: Figure 4 *Video Editor: please emphasize Average Size [bp] column*
4.2.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize data lines to left of 300 bp dotted vertical line*
4.2.3. LAB MEDIA: Figure 4

4.3. Immunofluorescent staining and analysis can also be performed to evaluate the sarcomere structure within the patterned cardiomyocytes **[1]**.

4.3.1. LAB MEDIA: Figure 5 *Video Editor: please add/emphasize images from AR1 to AR7 to AR11*

Conclusion

5. Conclusion Interview Statements

5.1. **Payam Haftbaradaran Esfahani**: This platform paves the way for high-throughput studies and drug screenings of different types of heart failure **[1]**.

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