Assessing cardiac reprogramming using high content imaging analysis

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Abstract

The goal of this protocol is to describe a method for quantifying induced cardiomyocyte-like cells (iCMs), which are directly reprogrammed *in vitro* by a reprogramming technique. Cardiac reprogramming provides a strategy to generate new cardiomyocytes. By introducing core cardiogenic transcription factors into fibroblasts, fibroblasts can be converted to iCMs without transition through the pluripotent stem cell state. However, the conversion rate of fibroblasts to iCMs still remains low. Accordingly, there have been numerous additional approaches to enhance cardiac reprogramming efficiency. Most of these studies assessed cardiac reprogramming efficiency using flow cytometry, while at the same time performed immunocytochemistry to visualize iCMs. Thus, at least two separate sets of reprogramming experiments have been required to demonstrate the success of iCM reprogramming. In contrast, automated high content imaging analysis will provide both quantification and qualification of iCM reprogramming with a relatively small number of cells. With this method, it is possible to directly assess the quantity and quality of iCMs with a single reprogramming experiment. This approach will be able to facilitate future cardiac reprogramming studies that require large scale reprogramming experiments such as screening genetic or pharmacological factors for enhancing reprogramming efficiency. In addition, the application of high content imaging analysis protocol is not limited to cardiac reprogramming. It can be applied to reprogramming of other cell lineages as well as any immunostaining experiments which need both quantification and visualization of immunostained cells.

SUMMARY:

We present the protocols to quantify directly reprogrammed induced cardiomyocyte-like cells (iCMs) *in vitro* using high content imaging analysis. This method allows us to quantify the efficiency of cardiac reprogramming in an automated manner and to directly visualize iCMs.

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DISCLOSURES

The authors have nothing to disclose.

Keywords

Reprogramming; cardiomyocyte; high content imaging

INTRODUCTION:

Cardiac reprogramming has been developed as an alternative approach to stem cell mediated approaches to generate new cardiomyocytes. Given that it does not transition through stem cell state, it has high potentials to bypass some inherited limitations in stem cell mediated approaches. It has been shown that viral infection of at least three or four cardiogenic transcription factors into fibroblasts can convert fibroblasts toward a cardiac fate by eliminating fibroblast gene programs and rebuilding cardiogenic transcriptional networks in fibroblasts¹⁻¹⁷.

Since the first landmark study demonstrating cardiac reprogramming *in vitro*¹, the cardiac reprogramming protocol has been optimized by numerous studies^{3,5-7,9,11-16,18}. Common technical approaches to assess cardiac phenotypes in fibroblasts following cardiac reprogramming have been flow cytometry analysis for quantifying cells expressing specific cardiomyocyte markers and immunocytochemistry for visualizing those cells in a single cell level. Although both experiments (i.e. flow cytometry and immunocytochemistry) are to demonstrate expression of cardiomyocyte markers using the same antibodies, they have to be performed separately. In addition, flow cytometry needs a relatively larger number of cells, thereby increasing the amount of reagents needed for the experiment. Alternatively, cardiomyocyte marker positive cells can be quantified by manual counting following immunocytochemistry. However, it is very labor intensive and tends to be less accurate.

The purpose of this protocol is to describe the method which can quantify and visualize iCMs by a single immunostaining experiment using automated high content imaging analysis. It requires a relatively small number of starting cells because this protocol is performed in a well of 24 well plate. As many as three different markers can be used at the same time. Single, double, and triple positive cells can be automatically quantified. In addition to quantification of immunostained cells, high content imaging analysis provides high quality 10X objective images. If necessary, the same immunostained cells used in high content imaging analysis can be re-used for further imaging studies, such as confocal microscopy. The main advantage of this protocol is that it provides not only unbiased quantification of iCMs with a much smaller number of cells, but also visualization of iCMs. Furthermore, this protocol can be utilized for assessing non-cardiac lineage reprogramming (e.g. iPSC, neuron, and hepatocyte reprogramming).

PROTOCOL:

NOTE: All animal procedures were performed with the approval of Vanderbilt University Medical Center Institutional Animal Care and Use Committee.

1. Retrovirus generation and *in vitro* cardiac reprogramming

1.1) Culture Platinum E cells in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 1 µg/mL puromycin, and 10 µg/mL blasticidin until Platinum E cell confluency reaches 70-80%.

1.2) On Day 1, seed $\sim 0.55 \times 10^6$ cells (1st well) and $\sim 0.18 \times 10^6$ cells (2nd well) into two separate wells of 12 well plate with 1 mL of DMEM supplemented with 10% FBS and 1% penicillin/streptomycin a day prior to the first transfection.

NOTE: Different sizes of culture dishes can be used depending on the amount of viral media needed for the experiments. Please refer to Table 1 for other scales of experiments.

1.3) On Day 2, transfect the quad-cistronic M-G-T-H retroviral construct encoding Mef2c, Gata4, Tbx5, and Hand2 described in the previous study⁹ or empty vector into Platinum E cells of the first well. 3 µL of transfection reagent is added into 30 µL of reduced serum media. Five minutes later, 1 µg retroviral construct or empty vector is added into the mixture of transfection reagent and reduced serum media. After 20 minutes incubation at room temperature, the mixture is added into Platinum E cells.

1.4) 16-20 hours after transfection on Day 3, suck off media and replenish fresh DMEM supplemented with 10% FBS and 1% penicillin/streptomycin.

1.5) 24 hours after the first transfection on Day 3, perform the second transfection into the second well in which Platinum E cells were plated on Day 1 as described in 1.3).

1.6) On Day 3, seed $\sim 5 \times 10^4$ frozen mouse embryonic fibroblasts (MEFs) isolated from Titin-GFP reporter knock-in mice¹⁹ into a well of 24 well plate. A total of 2 wells of 24 well plate are needed (i.e. uninfected control and M-G-T-H infection).

NOTE: The number of MEFs plated may need to be adjusted, because a recovery rate of frozen MEFs can be varied depending on cell freezing conditions. About 10% confluency a day after seeding cells is adequate for reprogramming. Reprogramming efficiency could be enhanced by using fresh unfrozen MEFs.

1.7) 16-20 hours after the second transfection on Day 4, suck off media and replenish fresh DMEM supplemented with 10% FBS and 1% penicillin/streptomycin.

1.8) 48 hours after the first transfection on Day 4, collect the viral media in the first well of Platinum E cells using 5 mL syringe and filter them through 0.45 µm polyethersulfone (PES) membrane filter. Suck off fibroblast growth media on MEFs, and replace them with the viral media supplemented with polybrene at 6 µg/mL (first infection).

1.9) 48 hours after the second transfection on Day 5, perform the second infection using the viral media in the second well as described in 1.8).

1.10) 24 hours after the second infection on Day 6, the viral media are replaced with cardiac induction media composed of DMEM/199 (4:1), 10% FBS, 5% horse serum, 1% penicillin/streptomycin, 1% non-essential amino acids, 1% essential amino acids, 1% B-27, 1%

insulin-selenium-transferrin, 1% vitamin mixture, and 1% sodium pyruvate, 1 μ M SB431542, and 0.5 μ M A83-01. Change cardiac induction media every 3 days until cells are harvested.

2. Immunostaining

2.1) At 14-15 days post infection, fix the cells on 24 well plate with 2% paraformaldehyde for 15 minutes.

2.2) Permeabilize the fixed cells with permeabilization buffer (0.05% Triton-X in PBS) by washing the cells three times with permeabilization buffer every 5 minutes.

2.3) Incubate the cells with universal blocking buffer for 45 minutes.

2.4) Incubate the cells with mouse α -actinin (1:400 dilution) and chicken GFP antibodies (1:400 dilution) for 1.5 hours at room temperature.

Note: About 150 μ L of antibody solution can cover the whole area of a well of 24 well plate.

2.5) Wash the cells with permeabilization buffer for 5 minutes three times.

2.6) Incubate the cells with anti-mouse Alexa-555 and anti-chicken Alexa-488 secondary antibodies (1:400 dilution) for one hour at room temperature

2.7) Wash the cells with permeabilization buffer for 5 minutes three times.

2.8) Add 2.5 μ L of DAPI solution into 250 μ L of permeabilization buffer.

3. High content imaging

3.1) Turn on the ImageXpress Micro XL system.

3.2) Open the MateXpress software.

3.3) Log in the system.

3.4) On the taskbar, select “Run a Plate”.

3.5) Click “Open Door-Eject Plate” to open the door on the machine and put the plate in. Make sure the plate in the right direction. Click “Close Door-Load Plate” to close the door.

3.6) Click “ Load Plate Settings”. Choose a template protocol, and then click “Load From DB”.

3.7) Click “Acquisition Setup”.

3.8) In “Plate Acquisition Setup” dialog, click “Configure”.

3.9) In “Objective and Camera” tab, select 10x objective.

3.10) In “Plate” tab, select 24 well plate.

3.11) In “Sites to Visit” tab, determine the number of imaging sites by choosing the number in columns and rows. A total of 36 imaging sites are used by selecting “6” in columns and “6” in rows. Choose the distance between each imaging site (i.e. 500 μm).

3.12) In “Acquisition” tab, set the number of wavelengths. “3” is chosen.

3.13) In “Wavelengths” tab, select DAPI, FITC, and Texas Red.

3.14) Click “Run” tab and then click “Calculate” to set up the focus offset for each wavelength. Set up the focus offset for DAPI first. Click “Auto Expose” to set up the exposure time automatically for each wavelength. Then click “Acquire Plate” to start imaging selected sites.

4. Analysis of high content imaging

4.1) Once high content imaging is completed, click “Screening” menu, and choose “Review Plate Data” to select a plate for analysis.

4.2) Click “Select Plate”. In “Select Plate for Review” dialog, open the folder and choose the plate saved in the database and click “Select”.

Note: To display the images, select DAPI, FITC and Texas Red in the “Wavelengths” field. In the “Sites” field, select “All Sites”. Click a site among selected 36 sites to display it in each wavelength image window, and then click “Look-Up Table” to choose a color for the wavelength. Use the “Print Screen” key on the keyboard and paste the image in a photo editing software (e.g. Paint and Photoshop) to save it.

4.3) Click “Run Analysis”, and choose a template setting.

4.4) Click “Configure Settings”, and then “Number of wavelengths”. Choose three wavelengths (i.e. DAPI for nuclei staining, Texas Red for α -actinin, and FITC for Titin-GFP).

4.5) Using “Line” tool in the tool bar, measure the width across the short axis of a cell. Based on the measured widths of cells, set the “Approximate min width” and “Approximate max width” to include most of cells in the selected imaging site.

4.6) Click “Preview” to check if nearly all nuclei are selected. Selected nuclei exhibit white color, while unselected nuclei remain blue (DAPI stained). If necessary, make adjustments for “Approximate min width” and “Approximate max width”.

4.7) To set “Intensity above local background”, place a mouse cursor inside and outside of a cell. The intensity value appears at the bottom of the window. Slightly Reduce the intensity of a dim cell to evenly exhibit the intensity throughout the whole area of each cell. Define this intensity value as “Intensity above local background” value. The intensity above local background for each channel needs to be set separately.

4.8) Click “Screening” menu and choose “Plate Data Utilities”. In “Plate Data Utilities” dialog, click “Run Analysis” to select the plate.

4.9) In the Settings field, select the saved setting for analysis, choose “Add to auto run list”, and then click “OK” to run the analysis.

4.10) After the analysis is completed, click “Screening” menu and choose “Plate Data Utilities”. Then, in “Plate Data Utilities” dialog, click “Export Measurements” to export the analysis results.

4.11) Click “Cell and Image Measurements” and click “OK”.

4.12) On “Export Measurements Wizard - Step 1” page, select the plate and click “Next”.

4.13) On “Export Measurements Wizard - Step 2” page, click “Finish”.

4.14) On “Configure Data Export” page, select the data types (i.e. well name, total cell number, subtotal cell number for DAPI⁺, subtotal cell number for Texas Red⁺, subtotal cell number for FITC⁺, subtotal cell number for DAPI⁺Texas Red⁺, subtotal cell number for DAPI⁺FITC⁺, subtotal cell number for DAPI⁺FITC⁺Texas Red⁺, percentage of DAPI⁺ cells, percentage of DAPI⁺Texas Red⁺ cells, percentage of DAPI⁺FITC⁺ cells, and percentage of DAPI⁺Texas Red⁺FITC⁺ cells) and then click “OK”.

4.16) On “Export as text file” page, select the destination to save the file and click “OK”.

4.17) Open the file in Excel. The results will be presented by each site (Table 2). There are 36 sites per each well. Using the analysis tool “Pivot Table” in Excel, summarize the data of 36 sites (i.e. the sums of cell numbers and the averages of the indicated cell percentage in all 36 sites) (Table 3).

REPRESENTATIVE RESULTS

Following reprogramming experiments, we quantified iCMs using high content imaging analysis as described above. Composite images of 36 imaging sites that were used for high content imaging analysis were shown in Figure 1. iCMs are defined as double positive cells (α -actinin⁺Titin-eGFP⁺) in these experiments. High content imaging analysis showed that ~26% of cells exhibited both cardiac markers following M-G-T-H transduction, while ~1% of empty vector transduced control cells showed double positive cells. Individual iCMs were visualized in 10x images taken by high content imaging system (Figure 2).

DISCUSSION

The previous reprogramming studies assessed reprogramming efficiency using flow cytometry and demonstrated the structural quality of iCMs using immunocytochemistry in two separate experiments. Flow cytometry analysis requires a much larger number of starting cells, thereby increasing the scale of experiments. In contrast, high content imaging analysis can evaluate both quality and quantity of iCM reprogramming by a single experiment with a relatively small number of cells. Therefore, this new method can provide an efficient new technical platform for future reprogramming studies. In particular, this new method will be useful for the reprogramming experiments for screening new genetic or

pharmacological factors. For testing a large number of factors, the format of analysis can be down-scaled to 384 well format.

Transduction efficiency is directly correlated to reprogramming efficiency. In previous studies, we have shown that ensuring the expression of all reprogramming factors into fibroblasts is important to achieve high reprogramming efficiency^{8,9}. Since a relatively small number of fibroblasts expressed all reprogramming factors by transducing individual viral vectors, using a single polycistronic vector harboring all reprogramming factors is beneficial for enhancing reprogramming efficiency^{7,9}. Additionally, to increase transduction efficiency, we performed two sequential transductions using two separate transfections of reprogramming vectors into Platinum E cells. We found that this method is superior to the previous sequential transduction with a single transfection by re-using transfected Platinum E cells for the second transduction.

Success of cardiac reprogramming can be defined by many different ways. The most commonly used method was to quantify the percentage of cells expressing a cardiac structural protein, which is absent in starting fibroblasts. In this study, we used cardiac muscle specific α -actinin as well as striated muscle specific Titin as markers for cardiac structural protein induction during cardiac reprogramming. The reason for using Titin was that we were able to more clearly demonstrate sarcomere formation using fibroblasts isolated from Titin-GFP knock-in mouse line^{8,9}. Regardless, we define iCMs as a double positive cell (actinin⁺Titin⁺) to exclude Titin expressing non-cardiac muscle cells if any. Importantly, any combination of cardiac structural proteins can be used for high content imaging analysis as long as there are specific antibodies against those proteins available. Although the method that we described here was solely based on structural protein expression, it is important to recognize that the cells expressing a cardiac structural protein are not necessarily functional cardiomyocytes. In our previous studies, we have shown that sarcomere assembly is required for iCMs to be functional^{4,8,9}. Importantly, the structural development of iCMs can be directly examined through high content imaging analysis. In addition, further structural evaluation (e.g. confocal microscopy) can be subsequently performed using the same immunostained cells for high content imaging analysis. However, the true functionality of iCMs should be determined by recording spontaneous contraction and electrophysiological properties (i.e. calcium transients and action potentials) of iCMs. In addition, demonstrating whole transcriptomic changes from fibroblast to cardiomyocyte would be necessary for defining successful iCM reprogramming (e.g. single cell RNA-seq). Therefore, the major goal of high content imaging analysis for assessing cardiac reprogramming is not to quantify functional iCMs, but to evaluate overall structural progresses of iCM reprogramming as an initial assessment.

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Figure 1.

Representative composite images of automated high content imaging analysis. MEFs isolated from Titin-GFP knock-in mice were transduced with M-G-T-H construct or empty vector. Empty vector transduced cells were used as control. Fifteen days after transduction, the MEFs were immunostained for GFP and α -actinin. The immunostained cells were analyzed by automated high content imaging system to quantify DAPI⁺GFP⁺, DAPI⁺ α -actinin⁺, or DAPI⁺GFP⁺ α -actinin⁺ cells. The graph showed summary of high content imaging analyses. Three independent experiments are presented as mean $\pm$ s.d. * $P < 0.005$. Scale bar, 2.5mm.

Figure 2.

Representative images of 10X objective pictures used for high content imaging analysis.

These images were chosen from the composite images of 36 imaging sites as shown in Figure 1. Each image represents an individual imaging site per each channel. Scale bar, 400 μ M.

Table 1.

Transfection scale depending on the size of a cell plate

Cell culture dish	Platinum E cells (million/plate)	Media (ml)	Fugene 6 (μl)	Opti-MEM (μl)	DNA (μg)
15 cm	13.75	25	75	750	25
10 cm	5.5	10	30	300	10
6 cm	2.2	4	12	120	4
35 mm (6 well)	1.1	2	6	60	2
12 well	0.55	1	3	30	1
24 well	0.275	0.5	1.5	15	0.5

Table 2.

Raw data set analyzed by high content imaging system

Well Name	Total Cells (MultiWaveScoring)	Subtotal Profile Ixxxxxx (MultiWaveScoring)	Subtotal Profile I2xxxxx (MultiWaveScoring)	Subtotal Profile Ix3xxxx (MultiWaveScoring)	Subtotal Profile I23xxxx (MultiWaveScoring)	Percentage Subtotal Profile Ixxxxxx (MultiWaveScoring)	Percentage Subtotal Profile I2xxxxx (MultiWaveScoring)	Percentage Subtotal Profile Ix3xxxx (MultiWaveScoring)	Percentage Subtotal Profile I23xxxx (MultiWaveScoring)
A02	128	128		10	9	3	7.8125	7.03125	2.34375
A02	37	37		7	1	1	18.918919	2.702703	2.702703
A02	89	89		16	18	12	17.977528	20.22472	13.483146
A02	336	336		74	138	66	22.023809	41.07143	19.642857
A02	789	789		173	333	159	21.926489	42.205322	20.152092
A02	1224	1224		669	911	636	54.656864	74.428108	51.960785
A02	51	51		14	3	3	27.450981	5.882353	5.882353
A02	95	95		22	26	17	23.157894	27.368422	17.894737
A02	488	488		135	278	134	27.663935	56.967213	27.459017
A02	1765	1765		845	1171	788	47.875355	66.345612	44.645893
A02	1713	1713		1069	1350	1019	62.405136	78.809105	59.486282
A02	1801	1801		1389	1373	1257	77.123817	76.235428	69.794556
A02	32	32		7	2	2	21.875	6.25	6.25
A02	187	187		66	76	57	35.294117	40.641712	30.481283
A02	1046	1046		496	661	447	47.418739	63.193115	42.734226
A02	1120	1120		451	728	418	40.267857	65	37.32143
A02	1709	1709		1042	1261	961	60.971329	73.785843	56.231716
A02	1904	1904		1378	1341	1171	72.373947	70.430672	61.502102
A02	8	8		1	0	0	12.5	0	0
A02	101	101		26	23	15	25.742575	22.772278	14.851485
A02	1134	1134		461	597	393	40.652557	52.645504	34.656086
A02	731	731		226	470	205	30.916553	64.295486	28.043776
A02	1017	1017		523	754	505	51.425762	74.139626	49.655849
A02	1585	1585		1038	1057	871	65.48896	66.687698	54.952682

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Well Name	Total Cells (MultiWaveScoring)	Subtotal Profile Ixxxxxx (MultiWaveScoring)	Subtotal Profile I2xxxxx (MultiWaveScoring)	Subtotal Profile Ix3xxxx (MultiWaveScoring)	Subtotal Profile I23xxxx (MultiWaveScoring)	Percentage Subtotal Profile Ixxxxxx (MultiWaveScoring)	Percentage Subtotal Profile I2xxxxx (MultiWaveScoring)	Percentage Subtotal Profile Ix3xxxx (MultiWaveScoring)	Percentage Subtotal Profile I23xxxx (MultiWaveScoring)
A02	14	14	0	0	0	100	0	0	0
A02	67	67	7	5	2	100	10.447762	7.462687	2.985075
A02	393	393	158	193	144	100	40.203564	49.109413	36.64122
A02	1042	1042	591	713	554	100	56.71785	68.426102	53.166988
A02	725	725	275	431	253	100	37.931034	59.448277	34.896553
A02	1618	1618	806	920	740	100	49.814587	56.860321	45.735477
A02	22	22	11	7	7	100	50	31.818182	31.818182
A02	35	35	10	7	5	100	28.571428	20	14.285714
A02	160	160	29	34	22	100	18.125	21.25	13.75
A02	728	728	332	381	299	100	45.604397	52.335163	41.07143
A02	846	846	464	572	419	100	54.846336	67.612289	49.527187
A02	852	852	539	649	498	100	63.262909	76.173706	58.450703
D04	753	753	0	0	0	100	0	0	0
D04	813	813	0	0	0	100	0	0	0
D04	1363	1363	3	0	0	100	0.220103	0	0
D04	1168	1168	1	2	1	100	8.56E-02	0.171233	8.56E-02
D04	746	746	0	0	0	100	0	0	0
D04	780	780	0	0	0	100	0	0	0
D04	719	719	3	0	0	100	0.417246	0	0
D04	854	854	10	0	0	100	1.17096	0	0
D04	886	886	8	0	0	100	0.902935	0	0
D04	966	966	28	10	10	100	2.898551	1.035197	1.035197
D04	775	775	14	4	4	100	1.806452	0.516129	0.516129
D04	632	632	1	0	0	100	0.158228	0	0
D04	782	782	0	0	0	100	0	0	0
D04	631	631	0	0	0	100	0	0	0
D04	863	863	0	0	0	100	0	0	0

Well Name	Total Cells (MultiWaveScoring)	Subtotal Profile Ixxxxxx (MultiWaveScoring)	Subtotal Profile I2xxxxx (MultiWaveScoring)	Subtotal Profile Ix3xxxx (MultiWaveScoring)	Subtotal Profile I23xxxx (MultiWaveScoring)	Subtotal Profile Ixxxxxx (MultiWaveScoring)	Percentage Subtotal Profile Ixxxxxx (MultiWaveScoring)	Percentage Subtotal Profile I2xxxxx (MultiWaveScoring)	Percentage Subtotal Profile Ix3xxxx (MultiWaveScoring)	Percentage Subtotal Profile I23xxxxx (MultiWaveScoring)
D04	652	652	39	6	6	39	100	5.981595	0.920245	0.920245
D04	1080	1080	37	13	12	37	100	3.425926	1.203704	1.111111
D04	888	888	0	1	0	0	100	0	0.112613	0
D04	926	926	0	0	0	0	100	0	0	0
D04	1138	1138	3	0	0	3	100	0.26362	0	0
D04	1498	1498	0	0	0	0	100	0	0	0
D04	1262	1262	0	0	0	0	100	0	0	0
D04	1030	1030	0	0	0	0	100	0	0	0
D04	1022	1022	90	35	35	90	100	8.806262	3.424658	3.424658
D04	916	916	0	0	0	0	100	0	0	0
D04	1556	1556	0	0	0	0	100	0	0	0
D04	1788	1788	0	0	0	0	100	0	0	0
D04	1651	1651	8	5	5	8	100	0.484555	0.302847	0.302847
D04	902	902	3	0	0	3	100	0.332594	0	0
D04	993	993	34	13	13	34	100	3.423968	1.309164	1.309164
D04	839	839	3	0	0	3	100	0.357569	0	0
D04	936	936	0	0	0	0	100	0	0	0
D04	942	942	46	0	0	46	100	4.883227	0	0
D04	790	790	0	0	0	0	100	0	0	0
D04	467	467	4	0	0	4	100	0.856531	0	0
D04	1000	1000	0	0	0	0	100	0	0	0

Table 3.

Summarized data set

Well Name	Total Cells (MultiWaveScoring)	DAPI+	DAPI+/Titin -GFP+	DAPI+/actinin +/Titin -GFP+	Percentage DAPI+	Percentage DAPI+/Titin -GFP+	Percentage DAPI+/actinin+	Percentage DAPI+/actinin +/Titin -GFP+
		Subtotal Profile 1xxxxx (MultiWaveScoring)	Subtotal Profile 12xxxxx (MultiWaveScoring)	Subtotal Profile 123xxxx (MultiWaveScoring)	Percentage Subtotal Profile 1xxxxx (MultiWaveScoring)	Percentage Subtotal Profile 12xxxxx (MultiWaveScoring)	Percentage Subtotal Profile 1x3xxxx (MultiWaveScoring)	Percentage Subtotal Profile 123xxxx (MultiWaveScoring)
A02	25592	25592	25592	12083	100	38.0401525	44.71138167	31.51270375
D04	35007	35007	335	89	100	1.0132205	0.249883056	0.241804639

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Platinum E cells	Cell Biolabs	RV-101	
Fugene 6	Promega	E2691	
Opti-MEM	Gibco	31905-070	
SB431542	Sigma	S4317	
A83-01	Tocris	2939	
Universal blocking buffer	BiogeneX	HK083-50K	
anti- α -actinin antibody	Sigma	A7811	
anti-GFP antibody	Invitrogen	A10262	
anti-mouse Alexa 555	ThermoFisher	A21422	
anti-chicken Alexa 488	ThermoFisher	A11039	
DAPI solution	Vector labs	H1200	
Polybrene	Sigma	H9268	
PES filter (0.45 μ m)	Thomas scientific	1159T84	
Medium 199	Invitrogen	11150059	
MEM vitamin solution	Invitrogen	11120-052	
Insulin-Transferrin-SeleniumG supplement	Invitrogen	41400-045	
Minimal essential amino acid solution	Sigma	M7145	
Micro XL automated cell imaging system	Molecular device		
MetaXpress software	Molecular device		