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Induced Pluripotent Stem Cell sorting, culture and differentiation to desired cell lineage

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ABSTRACT

Pluripotent stem cells have the ability to differentiate down different cell fate pathways to form any one of the different cell types in the human body and are an important tool for studying developmental biology and regenerative medicine.[1,2] The discovery of the ability to revert a terminally differentiated cell such as a dermal fibroblast back to a stem cell like state has opened up the possibility of growing subject specific tissue and organs that will originate from the subject's own cells and therefore not be rejected when transplanted.[3,4] Cells that have been reverted back to a pluripotent state in this manner are called induced pluripotent stem cells (iPSC). As advances in induced pluripotent stem cell technology are made, advances in the technology around them are also made, such as pluripotency markers, differentiation techniques, and cell sorting.[4,5,6] Cell sorting is common way of isolating specific cell or cells from a heterogeneous sample. Once isolated these cells can then be grown in culture and then used for multiple scientific purposes.

Currently, what was once a complicated, tedious process requiring expensive instrumentation and dedicated operators is becoming a more approachable technology. Using the Invitrogen™ iSort™ Automated Cell Sorter we show the use of sterile sorting of induced pluripotent stem cells in culture, based on a fluorescent pluripotency indicator and the subsequent culturing and differentiation of these cells to a specific cell fate.

INTRODUCTION

- ✓ Affordable, simple to use benchtop sorter allows ease of use when sorting pluripotent stem cells
- ✓ Minimal training required for setup & analysis for sorting.

MATERIALS AND METHODS

HiPSC were cultured following the Essential 8™ Medium (A1517001) product insert on vitronectin (VTN-N) (A14700) coated plates.

After staining, the hiPSC were harvested and sorted using the Invitrogen™ iSort™ Automated Cell Sorter for Alkaline Phosphate Live (ALP) green fluorescent cells from non fluorescent cells and debris. Prior to sorting a small aliquot of cells were stained using the Propidium Iodide Ready Flow™ Reagent (R37108) and Hoechst 33342 Flow™ Reagent (R37165) kits to assess viability using the Invitrogen™ Attune™ NxT Flow Cytometer. After sorting, a small aliquot of sorted population were removed and stained with PI and Hoechst to assess post sort viability and purity.

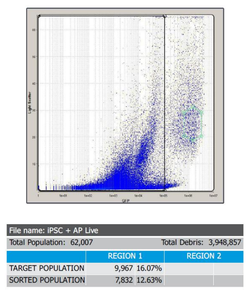
Differentiation was performed following the PSC Cardiomyocyte Differentiation Kit (Gibco™ A29212) product insert. The Fluo-4 NW Calcium Assay (F36205) was used to image the calcium signaling of the differentiated cells. A deviation from the protocol was that cells were placed back in the Cardiac Maintenance media after labeling. Imaging was performed using either the EVOS™ FL Auto 2 (AMAFD2000) or the EVOS™ core. Image quantification was completed using the Celleste™ Image Analysis Software.

RESULTS

Figure 1. Invitrogen™ iSort™ Automated Cell Sorter

Figure 1: The iSort Automated Cell Sorter is an user-friendly, benchtop sorter containing a 488 laser and 530/30BP detector.

Figure 2. iSort plot of sorted hiPSC



File name: iPSC + AP Live		
Total Population: 62,007		Total Debris: 3,948,857
	REGION 1	REGION 2
TARGET POPULATION	9,967 16.07%	
SORTED POPULATION	7,832 12.63%	

Figure 2: Induced Pluripotent Stem Cells, stained with ALP, are sorted using the iSort Automated Cell sorter. Sort was set using the "More Bright" setting, debris gate (black) set to eliminate debris and ALP negative cells. Positive gate (green) set conservatively around ALP+ cells.

Figure 3. Flow cytometric analysis of hiPSC pre and post sort

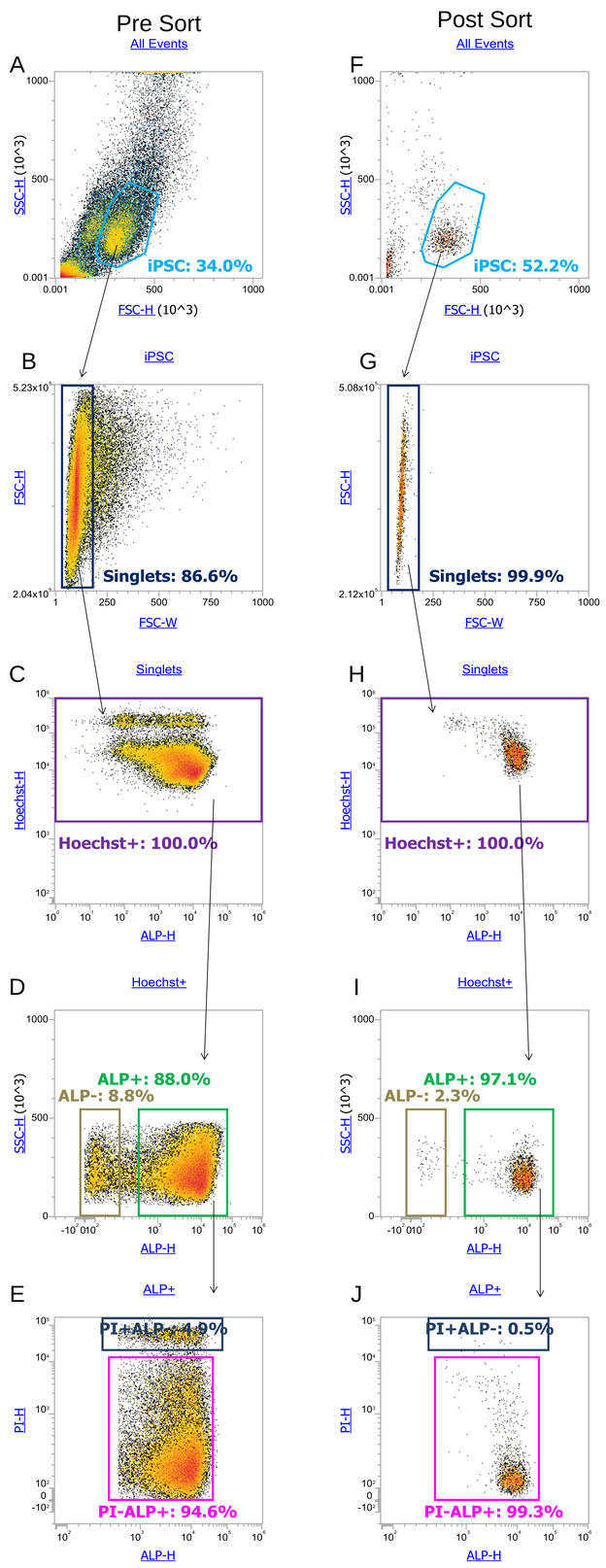
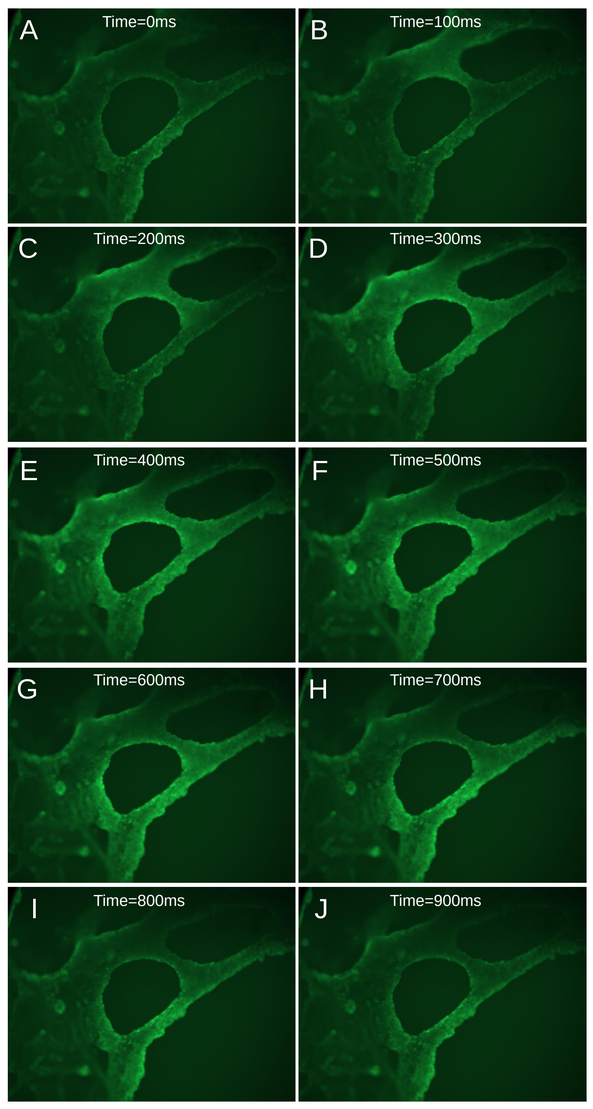


Figure 3: Samples were taken prior to sorting and immediately after sorting, then stained with PI and Hoechst, per the Ready Flow protocols, to assess purity and viability.

- Plots A – E contain data from the presort sample.
- Plots F- J comprises data the post sort sample.
- Plots A & F display the scatter gate around the iPSC population.
- Plots B & G use FSC H vs. FSC W to identify the Singlet population of the iPSC gate.
- Plots C & H are daughter plots of the Singlet gate showing Hoechst staining to identify nucleated cells.
- Plots D & I, gated on Hoechst positive cells, show pre and post purity of 88% & 97% (ALP+), respectively
- Plots E & J, the PI- ALP+ gate in the ALP vs PI evaluate viability of samples prior to and after sorting as 94.5% & 99.3% respectively.

Figure 4. Calcium assay on sorted hiPSCs that are terminally differentiated into cardiomyocytes



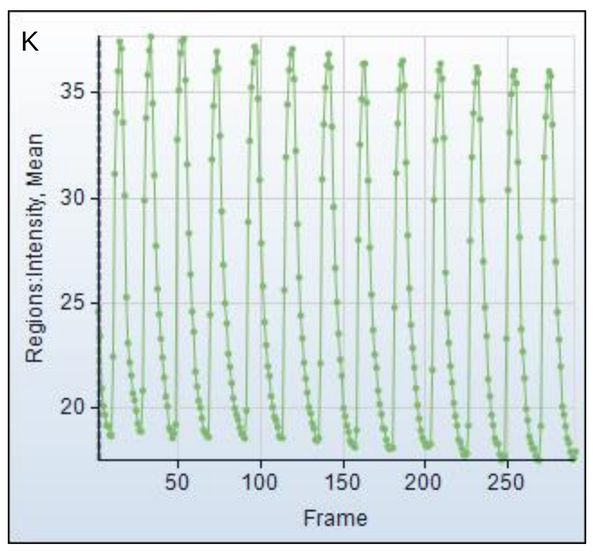
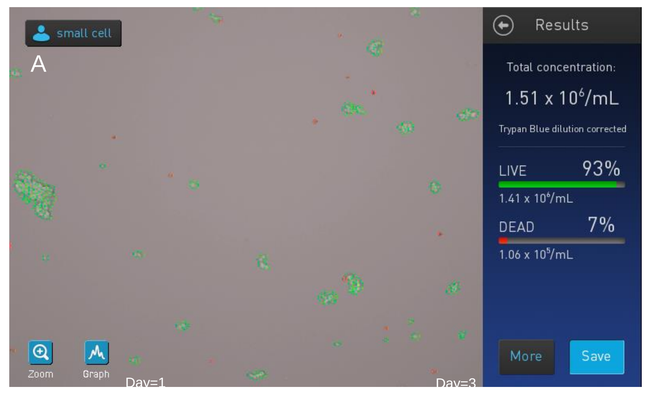


Figure 4: Sorted hiPSCs were cultured until 80% confluent, and then differentiated into cardiomyocytes using Cardiomyocyte Differentiation Kit. Cardiomyocytes were labeled with Fluo-4 NW Calcium Assay Kit. After labeling cells were placed back in cardiomyocyte maintenance media and time lapse imaging was performed on EVOS FL Auto 2 using the video record function at 10x magnification. Images show calcium flux across the cardiomyocytes as they depolarize themselves in order to contract (4A-4J). Quantitative analysis of the time lapse using Celleste Image Analysis Software showed the changes in Fluo-4 NW intensity during contractions and the rate of contractions (4K).

Figure 5. Viability of sorted hiPSCs after freeze and thaw



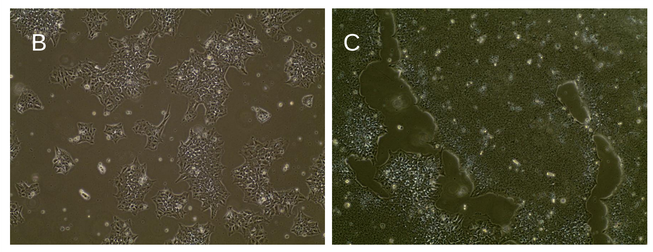


Figure 5: Sorted hiPSCs were expanded then frozen down in PSC Cryopreservation Medium and stored in liquid nitrogen. Cells were thawed and viability was measured using the Countess II FL. Countess II FL measurements showed that 93% of the thawed hiPSCs were viable (5A). Thawed hiPSCs were plated on Vitronectin coated plates and were imaged at day 1 and day 3 on the EVOS Core. Images showed normal hiPSC morphology after thawing (5B and 5C).

CONCLUSIONS

Here we have shown that the sterile sorting of pluripotent stem cells can be effectively achieved using the Invitrogen™ iSort™ Automated Cell Sorter. Once sorted, these cells can be grown, expanded and induced down a designated pathway, in this case cardiomyocytes. Additionally, we show, once frozen and thawed, these sorted cells retain their morphology and pluripotency.

REFERENCES

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