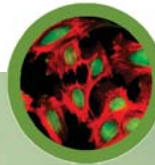
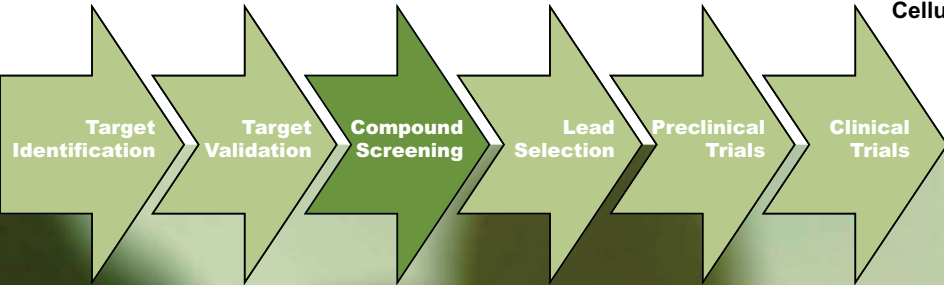


# Characterization of Induced Pluripotent Stem Cell-derived Cardiomyocytes and Their Industrialized Production for use in Drug Discovery and Toxicity Testing.

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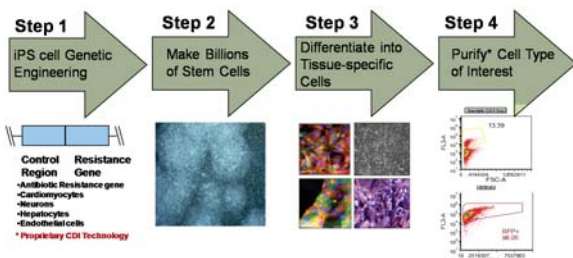


## Abstract

Industrialized quantities of purified human cells are needed for drug discovery, toxicity testing, and therapeutics. Using induced pluripotent stem (iPS) cell technology, Cellular Dynamics International (CDI) has developed a manufacturing pipeline to meet this need and is currently generating high quality, pure human cardiomyocytes for large-scale commercial use. iCell Cardiomyocytes show expected electrophysiological and biochemical responses to a variety of stimuli across a number of different platforms and are therefore an excellent source of human material for use drug discovery and toxicity testing.

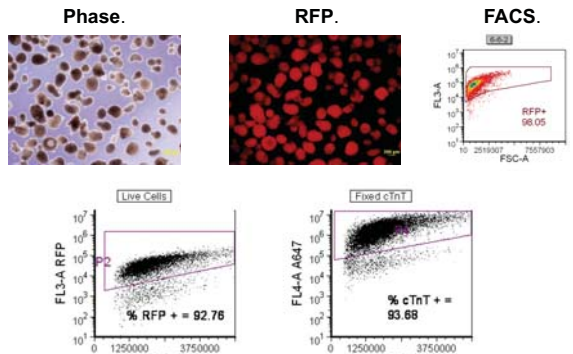
## iCell™ Cardiomyocytes: Production and Purification

### A. Differentiation Strategy



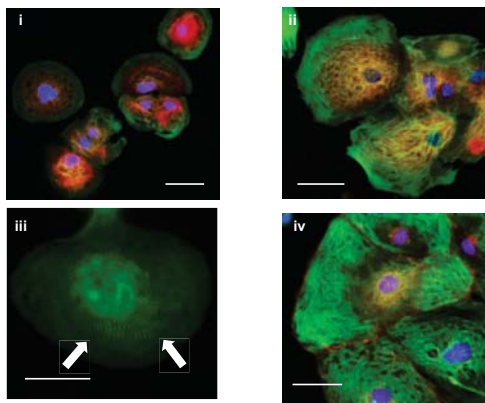
iCell Cardiomyocyte Differentiation System. Human iPS stem cells are differentiated into cardiomyocytes

### B. Purification



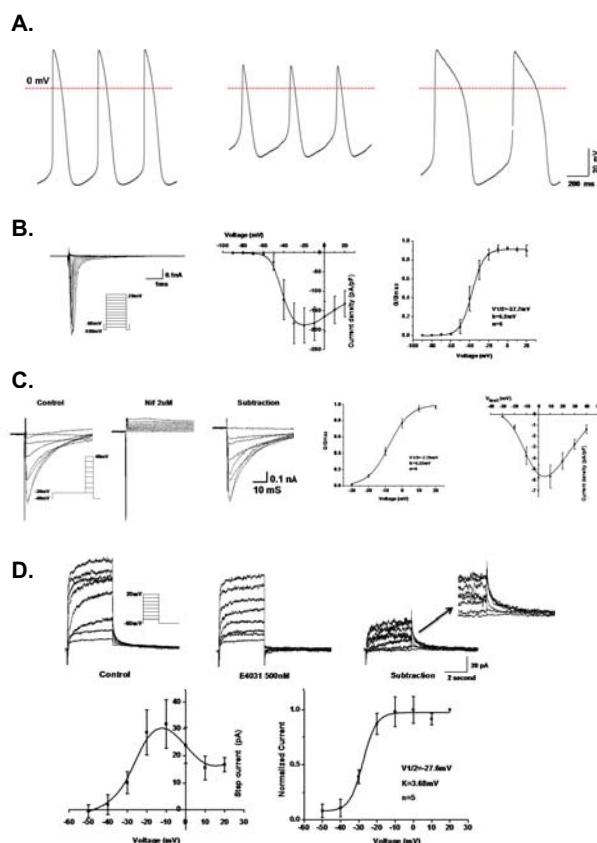
iCell Cardiomyocyte Purification. Antibiotic selection results in a pure cardiomyocyte population as shown by red fluorescent protein (RFP) expression and FACS analysis. The upper row illustrates cardiomyocyte aggregates post antibiotic selection, while the bottom row illustrates the concordance of the genetically engineered (RFP) and non-engineered (cTnT) markers.

### C. Immunocytochemical Characterization

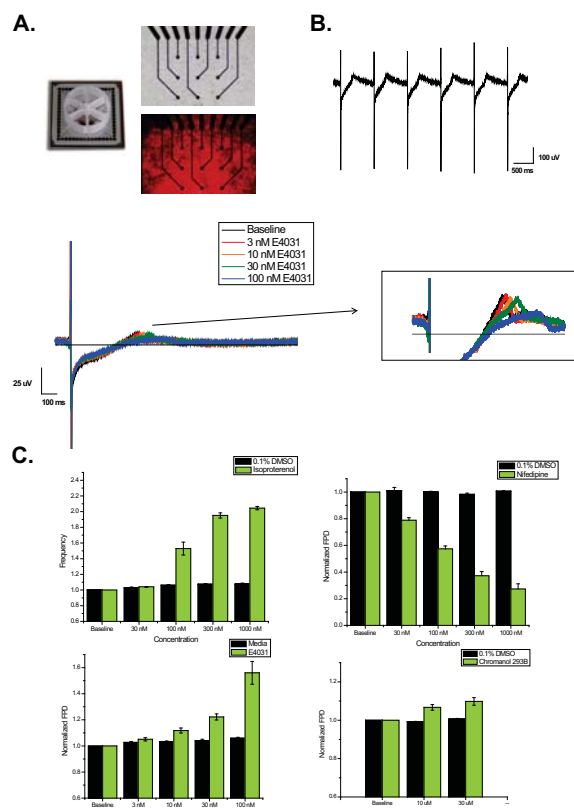


iCell Cardiomyocyte Protein Expression i: cTnT (green)/MLC2a (red), ii: cTnT (green)/MHC (red), iii: alpha-actinin, and, iv: Cx43. Note the presence of poly-nucleation in (i) and sarcomeric organization in (iv). Scale bars 50 (i) and 25µm (ii - iv)

## iCell™ Cardiomyocyte Electrophysiology

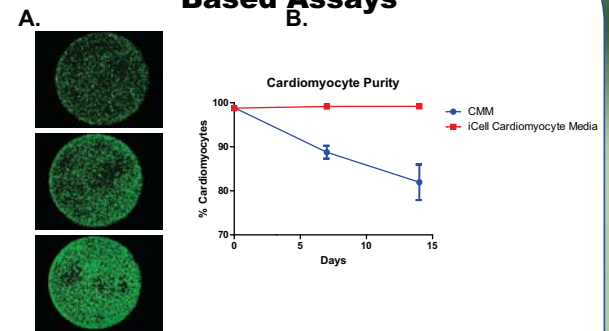


Conventional Patch Clamp Recordings. iCell Cardiomyocytes were plated as single cells and conventional perforated patch techniques were used to record action potentials at 37°C. A. Action potential phenotypes resembled (from left to right) Atrial-, Nodal-, and Ventricular-like waveforms. B, C, and D.,  $I_{Na}$ ,  $I_{CaL}$ , and hERG (respectively) currents were recorded with each panel showing the current waveform, stimulation protocol, and I/V and/or conductance relations.

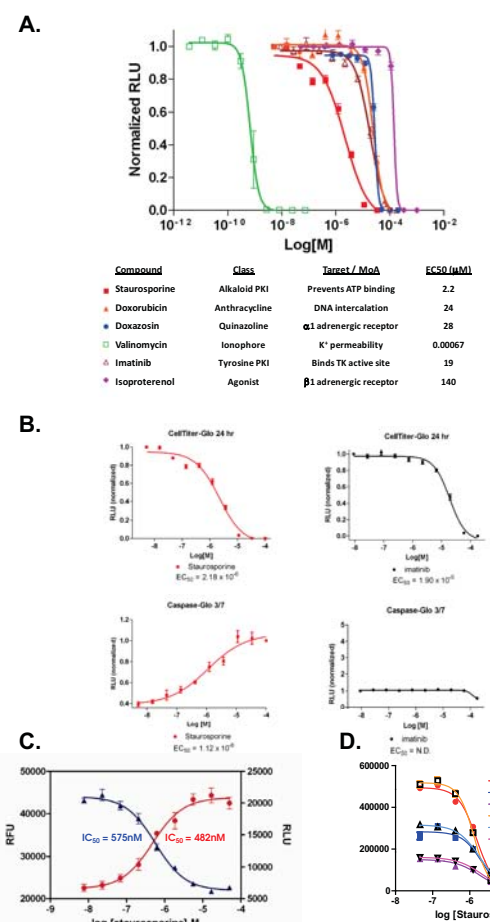


Microelectrode Array Recordings. A. Images of the recording dish (6-array configuration), single array overlaid with cells, and representative field potential tracings. B. Expanded field potential from a single 'beat' each overlay illustrates the effect of increasing concentrations of E-4031. C. Effects of beta-adrenergic stimulation (Isoproterenol),  $Ca^{++}$  channel (Nifedipine), hERG channel (E4031), and KCNQ channel (Chromanol 293B) block.

## iCell Cardiomyocytes in Cell-Based Assays



iCell Cardiomyocytes in Cell-based Assay format A. iCell Cardiomyocytes were plated at ~5, 10, and 15K cells/well (top to bottom) in a 96-well plate (>95% cardiomyocyte purity), and labeled with Calcein Green AM to illustrate the viability and uniform coverage. B. Purity was monitored over two weeks of culture in a 96-well plate in standard cell culture media (CMM) and iCell Cardiomyocyte Media. Complete purity was maintained in iCell Cardiomyocyte Media.



iCell Cardiomyocytes Perform in Cell-Based Assays iCell Cardiomyocytes were plated at 15K cell/well in a 96-well plate (>95% cardiomyocyte purity), exposed toxic agents, and assayed for a variety of endpoints. A. Cell-Titer Glo (Promega) was used to illustrate the wide sensitivity of iCell Cardiomyocytes to toxicants. B. Mechanisms of toxicity can be elucidated; Stausporine-induced decreased viability is through the Caspase pathway whereas Gleevec (imatinib) is not. C. Multiple parameters can be measured simultaneously as evidenced through the use of the Multitox-Fluor kit (Promega). D. Assays can be miniaturized for use in 384-well plates as shown by the dose-dependent loss of viability (Cell-Titer Glo) when seeded at the listed densities. Panels A and B courtesy of Chad Zimprich (Promega).

## Summary

### iCell Cardiomyocytes:

- > Are generated from human iPS Stem Cells.
- > Are produced in Industrialized quantities at near 100% purity.
- > Show expected normal and patho-electrophysiological responses.
- > Are responsive to multiple end-point analyses to a variety of toxicants.

**Are therefore a suitable human-based cardiac test system for use in drug discovery and toxicity testing**