Cell Counting

Cellular Dynamics International
A Fujifilm Company
Company Overview

- Cellular Dynamics International (CDI) is the world’s largest producer of human iPS cells and iPS cell-derived cell types
- Acquired by FUJIFILM in April of 2015
- Headquartered in Madison, WI, with a site in Novato, CA
- Currently employs ~175 total staff
- ~750 yrs human stem cell experience
- >900 patents (owned or licensed) to enable FTO
- Life Sciences and Therapeutics divisions
- Core competencies
  - **Creation and culture of human iPS cells**
    - Normal and disease phenotypes
  - **Genetic engineering of iPS cells**
    - SNP repair, Indels, knock-out, knock-in and more
  - **Development of new differentiation protocols**
    - Differentiated cells from all three germ layers
  - **Manufacture of human iPS cell-derived cell types**
    - Scalable production of highly purified cells
Life Science Research: Current Product Portfolio

- iCell Cardiomyocytes
- iCell Neurons
- iCell Endothelial Cells
- iCell DopapNeurons
- iCell Cardiac Progenitor Cells
- iCell Astrocytes
- iCell Macrophages
- iCell Hepatocytes
- iCell Skeletal Myoblasts
- Additional undisclosed cell types
- iCell Hematopoietic Progenitor Cells

- MyCell Products

- Life Technologies™
  - Essential 8 Medium
  - Episomal Reprogramming Kit
  - Vitronecin

- Quality Management System; Selected guidance from both ISO and GMP
  - ISO – ideal for research phase
  - GMP – required for clinical phase
  - 100% complete batch record and traceability; all material, incubator, operator, refrigerator, freezer, instrument, etc.
  - Material management risk profile for every reagent on BOM, qualified reagents, 2nd vendors, etc.
## Cellular Therapeutics: Active Programs

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<th>Indication</th>
<th>Therapeutic</th>
<th>Discovery</th>
<th>Pre-clinical</th>
<th>IND</th>
<th>Phase I</th>
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<td>Parkinson's Disease</td>
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**Disease Modeling: 250 Donor Panel**

**Flow Diagram**

1. Blood sample collection
   - Cell Count

2. Cell Selection and expansion
   - Cell Count

3. Reprogramming
   - Cell Count

4. Colony picking and expansion
   - Cell Count

5. Nuclease-mediated genome engineering
   - Cell Count

6. iPSC line Cryo and QC tests

7. Cardiomyocytes Cryo and QC tests
   - Cell Count

8. Cardiomyocyte Purification
   - Cell Count

9. Cardiomyocyte differentiation
   - Cell Count

10. Cardiomyocyte Selection and Expansion
    - Cell Count

11. Engineered line Cryo
Basis for Data Quality for Cell Counting

- QC Control Samples
- Method Qualification
- Equipment Qualification

Daily calibration
QC control sample run before each release QC cell counting test

Robustness
Accuracy
Precision
Operator
Device
Product lot
Product vial
Assay reagent

IQ
OQ
PQ
Transition from Cell Counting Device Cedex to ViCell

- Keep using Cedex for existing manufacturing processes and develop new processes with ViCell
- Adjust ViCell settings to match cell count numbers from Cedex from the same sample
- Adjust ViCell settings to reflect the true cell count, understand the cell count differences between Cedex and Vicell from the same samples for a certain process step and adjust process cell number ranges accordingly
Adjust ViCell Settings To Match Cell Count Numbers From Cedex

ViCell Settings
- Min Dia: 7
- Max Dia: 50
- Cell Brightness: 75
- Cell Sharpness: 40
- Viable Cell Spot Brightness: 80
- Viable Cell Spot Area: 20
- Min Circularity: 0.3
Differentiation Process Example I - Day21 Vicell Setting

- Vicell Cell Setting A in-process setting matches the Cedex counts very well (within 8%)
- Vicell Cell Setting A in-process setting matches the reality well enough.
- Recommend this setting for D21 counts
Differentiation Process Example II-
Image comparison: 1018075 s1 d11
Differentiation Process Example II-
Image comparison: 1018136 s1 d11
Adjusting Cell Settings for ViCell

Cell Setting A

- Sample ID: 1014467_D11_S1
- Cell type: In-Process
- Dilution factor: 1.0
- Image 3
- Cell count: 72
- Viable cells: 72
- Viability: 100.0%
- Total cells / ml: $3.65 \times 10^6$
- Viable cells / ml: $3.65 \times 10^5$
- Avg. diam. (microns): 16.96
- Avg. circularity: 0.64
- Avg. background intensity: 208

Cell Setting B

- Sample ID: 1014467_D11_S1
- Cell type: d2-12
- Dilution factor: 1.0
- Image 3
- Cell count: 102
- Viable cells: 99
- Viability: 97.1%
- Total cells / ml: $5.17 \times 10^5$
- Viable cells / ml: $5.92 \times 10^4$
- Avg. diam. (microns): 16.71
- Avg. circularity: 0.52
- Avg. background intensity: 208
Lesson’s Learned

- Choose cell counting platform wisely to avoid changes:
  - Automated
  - Meet sample throughput
  - Reliable and objective results over manual hemacytometer methods
  - Cell Record keeping
  - 21 CFR 11 compliance
  - Audit trail
  - Electronic signature capability
  - Secure user sign-on
  - User level permissions
  - Administrative configuration tools
  - Easy software update
  - Good technical and service support
  - PM
Thank You!