Creating a Cardiomyocyte Pipeline for Gene Edited Human iPSCs

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**Introduction**

The Allen Institute for Cell Science (AICS) is creating an open source collection of fluorescently tagged human iPSC lines to model cell organization and dynamics of stem cells both in the undifferentiated and differentiated states. Using the WTC human iPSC line and the CRISPR/Cas9 system we have fluorescently tagged 15 target genes representing key cellular organelles, which will be used to characterize the changes in subcellular localization and organization as the stem cell differentiates into cardiomyocytes using live cell fluorescent imaging. Here we present our cardiomyocyte differentiation methods and the quantitative and qualitative assays employed to determine differentiation efficiency including cardiomyocyte protein expression by flow cytometry, immunofluorescent and live cell imaging, and supporting transcriptome profiling by RNAseq. We are currently developing optimized methodologies for scalable and efficient production of cardiomyocytes to support our live cell imaging pipeline. We hope that gaining a better understanding of the organization and activities of cardiomyocytes will lead to advances in the development of better disease models, therapies, and regenerative medicine approaches.

**Allen Institute for Cell Science Workflow**

Quality Control of gene edited clones for healthy colony morphology and pluripotency expression

- **IPSCs**
- **Gene editing**
- **Clonal line generation**
- **Differentiating**
- **Live cell imaging**

Edited cell lines undergo an extensive QC process, including morphological and pluripotency analysis.

**Evaluation of differentiation protocols for robust cardiomyocyte production**

**Protocol 1: small molecule + growth factors**

- **PC1: 39% variance**
- **PC2: 30% variance**

**Protocol 2: small molecule**

- **PC1: 39% variance**
- **PC2: 30% variance**

**Summary of cardiac differentiation experiments**

<table>
<thead>
<tr>
<th>Protein (Gene)</th>
<th>Independent experiments (n)</th>
<th>% cardiac Troponin T</th>
<th>% Experiments with Sealing</th>
<th>Initiation of Sealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unedited</td>
<td>41</td>
<td>75-98</td>
<td>85</td>
<td>d7-01</td>
</tr>
<tr>
<td>Paxillin (PNX)</td>
<td>16</td>
<td>66-98</td>
<td>75</td>
<td>d8-01</td>
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<tr>
<td>Alpha tubulin (TUBA1B)</td>
<td>12</td>
<td>87-96</td>
<td>75</td>
<td>d8-02</td>
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<tr>
<td>Nucleo lamin B1 (LAMB1)</td>
<td>10</td>
<td>39-97</td>
<td>80</td>
<td>d7-04</td>
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<tr>
<td>Tnn1C (TROM1)</td>
<td>13</td>
<td>80-95</td>
<td>75</td>
<td>d7-03</td>
</tr>
<tr>
<td>Desmoplakin (DSP)</td>
<td>10</td>
<td>92-95</td>
<td>50</td>
<td>d7-05</td>
</tr>
<tr>
<td>Beta actin (ACTB)</td>
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<td>72-96</td>
<td>100</td>
<td>d7-08</td>
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<tr>
<td>Scaffold (SECB1)</td>
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<td>80</td>
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<tr>
<td>Fibrillin (FBL)</td>
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<td>64-96</td>
<td>75</td>
<td>d7-09</td>
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<tr>
<td>Mysin heavy chain IIB (MYH10)</td>
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<td>100</td>
<td>d7-14</td>
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<td>Tight junction protein ZO1 (ZJ1P1)</td>
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<td>100</td>
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<tr>
<td>Sarcomeric actinin (ACTN2)</td>
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<td>89-90</td>
<td>100</td>
<td>d7-08</td>
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<tr>
<td>Tropomin (TNNIN)</td>
<td>7</td>
<td>90</td>
<td>100</td>
<td>d7-01</td>
</tr>
</tbody>
</table>

**Establishing a cardiomyocyte imaging pipeline**

In the top and middle panels, edited structures are identified by GFP and cardiomyocytes are labeled with α-actinin. The bottom panel represents live cardiomyocyte images from the ACTN2-GFP tagged cell line.

**Establishing a baseline transcriptional profile of WTC-CMs**

Heatmap of regularized log transformed gene counts. Parental hiPSCs were differentiated using protocol 1 or 2. SECB1, FBL, and ACTC GFP-tagged lines were differentiated using Protocol 1 only. Late samples were collected at day ~50; all other samples were collected at day ~20.

**Integrating transcriptome sequencing into cardiomyocyte QC**

Principal component analysis of hiPSC-cardiomyocytes, evaluating the effect of fluorescent tag and differentiation protocol. Colors indicate cell line; shapes indicate independent differentiation experiments.

**Integration of transcriptome sequencing into cardiomyocyte QC**

Hierarchical clustering of 20 most variable genes among parental CMs (Protocol 1 & 2; day ~20) and edited CMs (Protocol 1; day ~20). Heatmap shows regularized log transformed gene counts scaled by column.

**Acknowledgements**

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**References**


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