ThermoFisher SCIENTIFIC

Scorecard™ Report

Client Name: NIH
Quote No: 10617854
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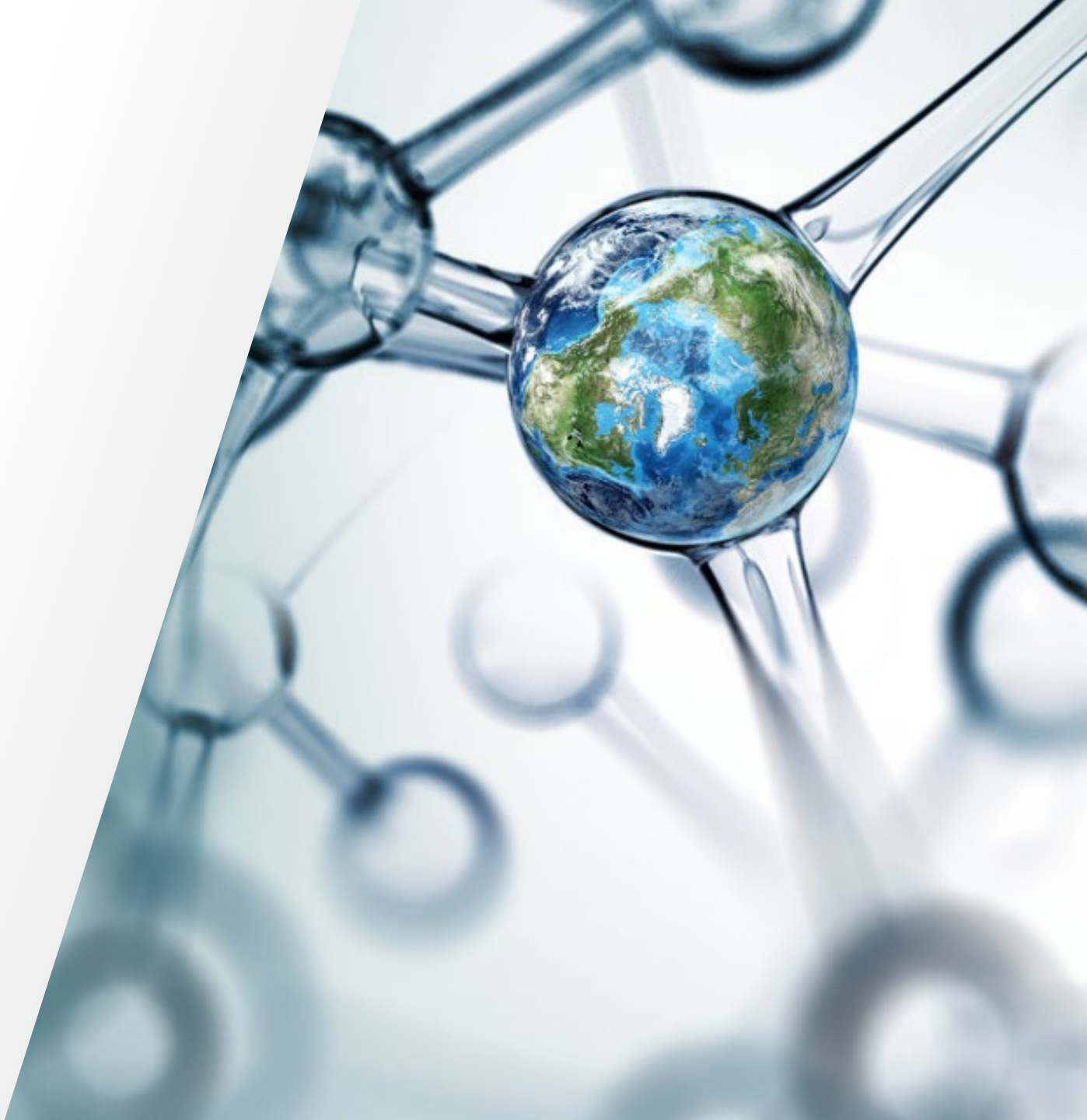
Prepared by: Ian Weiss



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Summary of Services

- **Project Summary**:
  NIH (Client) is interested in Services provided by the Life Technologies Corporation in the analysis of two (2) Client provided samples using the TaqMan® hPSC Scorecard™ Panel.

- **Service Description:**

- Pluripotency consists of two critical characteristics:
  - The ability to self-renew
  - The ability to differentiate into cells representative of all three embryonic germ layers

- Supporting evidence for the first characteristic is obtained by testing undifferentiated cells in the Scorecard assay and confirming expression of the nine genes in the panel associated with self-renewal
  - High quality undifferentiated cultures will also typically lack expression of ectodermal, mesodermal and endodermal genes

- The second characteristic is determined by testing embryoid bodies to verify up-regulation of genes associated with the three embryonic germ layers relative to the basal (undifferentiated) state

Materials and Methods

RNA Extraction

Cell pellets were prepared according to the PureLink™ RNA Mini Kit (Cat.no 12183018A).

Reverse Transcription of Total RNA

DNase-treated RNA were prepared according to the High-capacity cDNA Reverse Transcription kit with RNase Inhibitor (Cat.no. 4374966)

TaqMan® qRT-PCR

cDNA samples were prepared for qRT-PCR using the TaqMan® hPSC Scorecard™ Kit (Cat.no. A14872)

Results: Sample Table

Sample ID	Sample Name	Type
SC-2034	iPSC HT528A P13	Cell Pellet
SC-2035	iPSC HT528A P17 + P 29	Cell Pellet

Table 1: Sample description provided by the client.

Results - Summary

1. Sample SC-2034 scored positive for self renewal markers
2. Sample SC-2035 scored positive for Ectoderm and Mesoderm markers

Figure 1: The samples were analyzed using the TaqMan® hPSC Scorecard™ analysis software. The samples were analyzed for pluripotency and trilineage specific gene expression. If detected, the presence of Sendai virus is indicated by a red flag.

Results - Scores Table

Scores are a statistical comparison of the expression profile of the sample to that of the undifferentiated reference set

Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
SC 2034	-0.32	-0.85	-1.38	-1.66
SC 2035	-1.18	1.45	1.43	-0.10

Gene expression relative to the reference standard

Upregulated						Downregulated
x > 1.5	1.0 < x ≤ 1.5	0.5 < x ≤ 1.0	-0.5 ≤ x ≤ 0.5	-1.0 ≤ x < -0.5	-1.5 ≤ x < -1.0	x < -1.5

Figure 2: Scorecard Values: Algorithm scores for the samples show up regulation or down regulation of the endoderm, mesoderm, ectoderm or pluripotent (self-renewal) markers relative to the reference set of nine undifferentiated pluripotent stem cell lines.

Results - Scores Box Plot

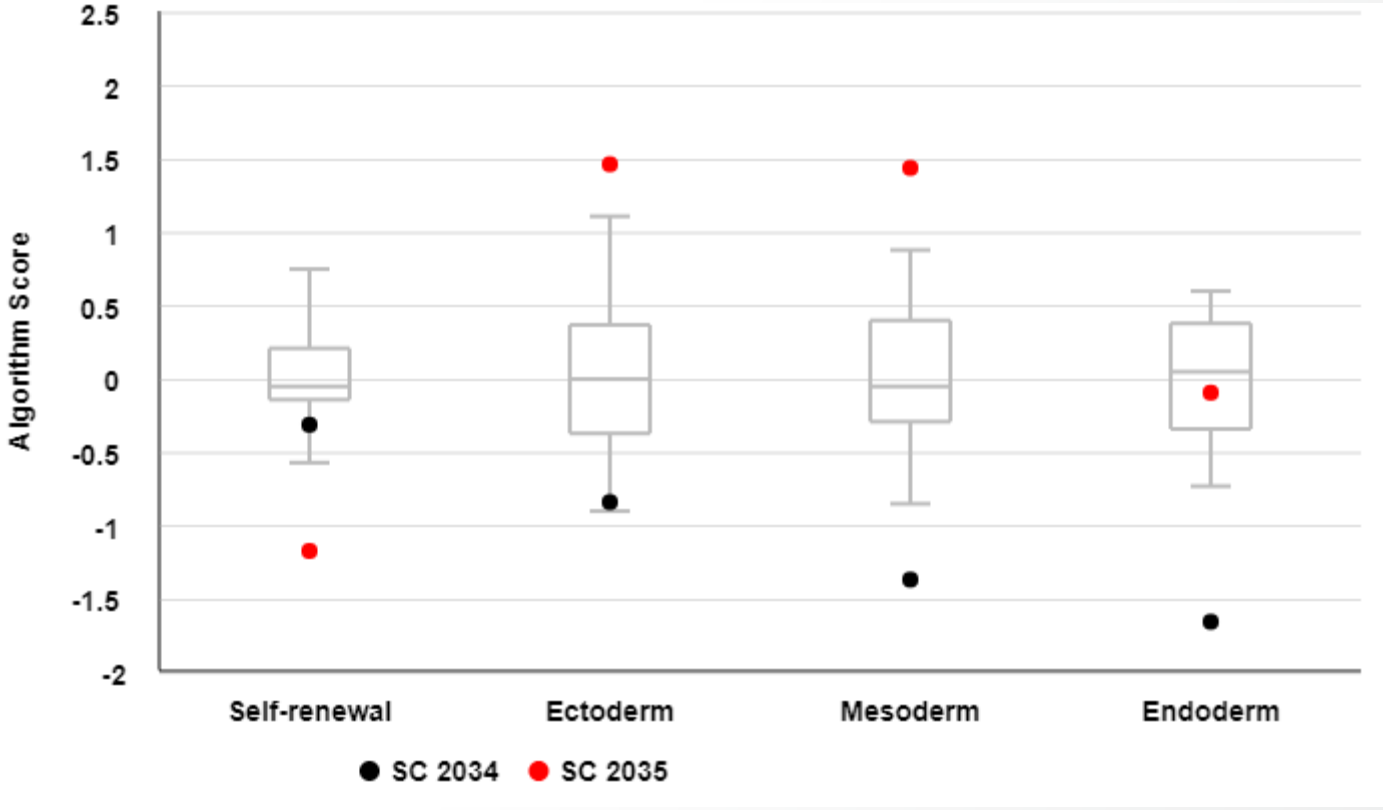

Figure 3: Scores Box plot: Sample scores are plotted in color. The range of scores for the undifferentiated reference set is indicated by the grey box plot.

Disclaimer: This assay was conducted solely for the listed investigator/institution. The results of this assay are for research use only.

Results - Gene Expression Panel

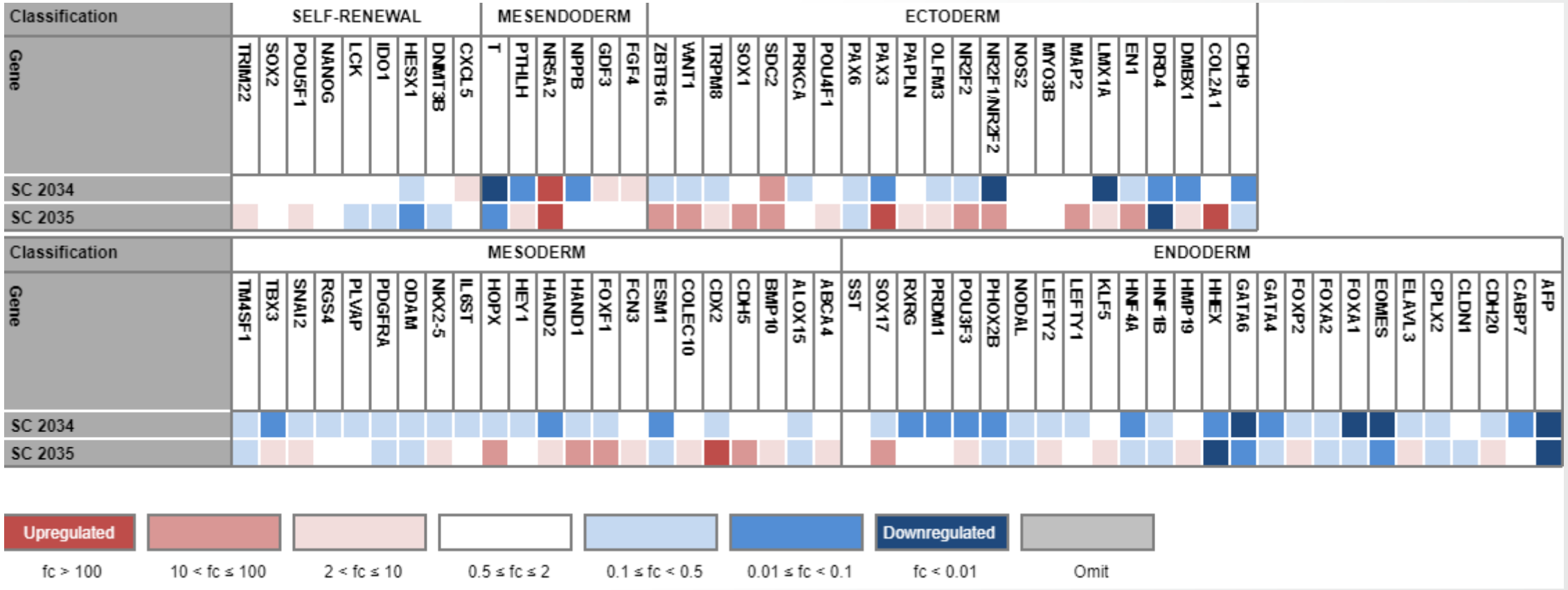

Figure 4: Expression Pattern Plot. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.

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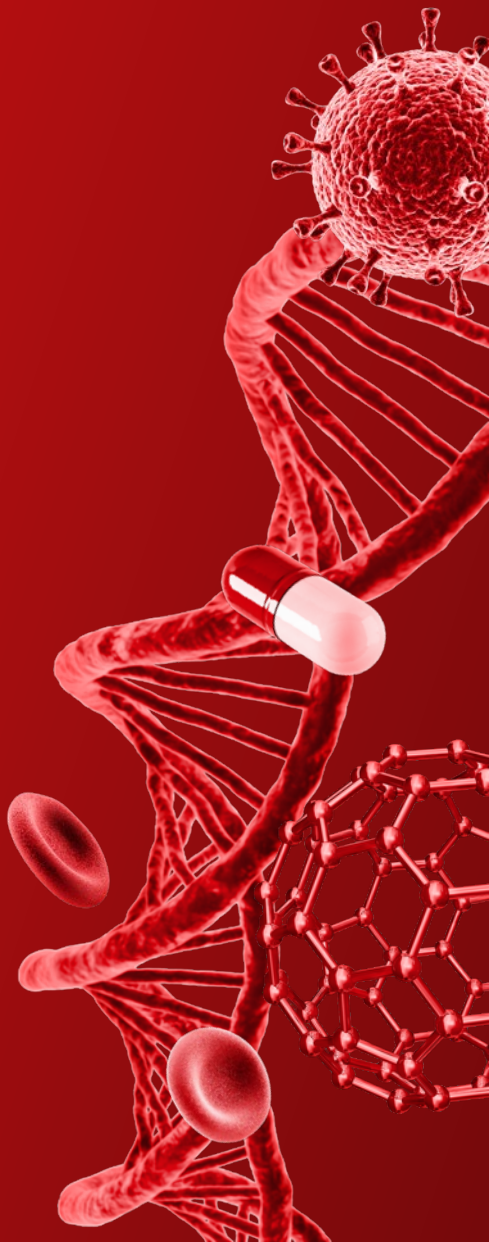
Appendix



Scorecard™ Results Navigation

The gene expression data presented is always shown relative to the gene expression of the 13 iPSC lines that are used as the standard. A sample would be considered positive for pluripotency if:

- The pluripotency value of the sample falls within or above the range of the standards.
- The ectoderm/mesoderm/endoderm value of the sample falls within or below the range of the standards.

The detection of Sendai Virus in the sample is indicated by a red flag

Figure 1: The samples were analyzed using the TaqMan® hPSC Scorecard™ analysis software. The samples were analyzed for pluripotency and trilineage specific gene expression. If detected, the presence of Sendai virus is indicated by a red flag.

Pluripotent samples will show a green circle with a plus sign under the "self" category, which indicates upregulation of self-renewal markers.

Borderline pluripotent samples will show an open green circle which indicates results on the boundary of typical self-renewal markers.

Differentiated samples will be shown as blue, orange, or purple circles with plus signs which shows upregulation of specific trilineage markers.

Image-based assessment of PSC quality during early iPSC establishment poster can be found here

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Results - Scores Table

Scores are a statistical comparison of the expression profile of the sample to that of the undifferentiated reference set

Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
1	0.35	-0.57	-0.26	-0.94
2	-0.47	-0.99	-0.46	-1.50
3	-0.06	-0.86	-0.36	-1.40
4	0.02	-0.70	-0.36	-1.06
5	0.53	-0.75	-0.65	-1.48
6	0.15	-0.53	-0.34	-1.09
7	-2.91	0.99	4.65	0.85
8	-3.49	2.57	2.37	0.58
9	-3.60	2.00	2.56	0.54

Gene expression relative to the reference standard

Upregulated						Downregulated
x > 1.5	1.0 < x ≤ 1.5	0.5 < x ≤ 1.0	-0.5 ≤ x ≤ 0.5	-1.0 ≤ x < -0.5	-1.5 ≤ x < -1.0	x < -1.5

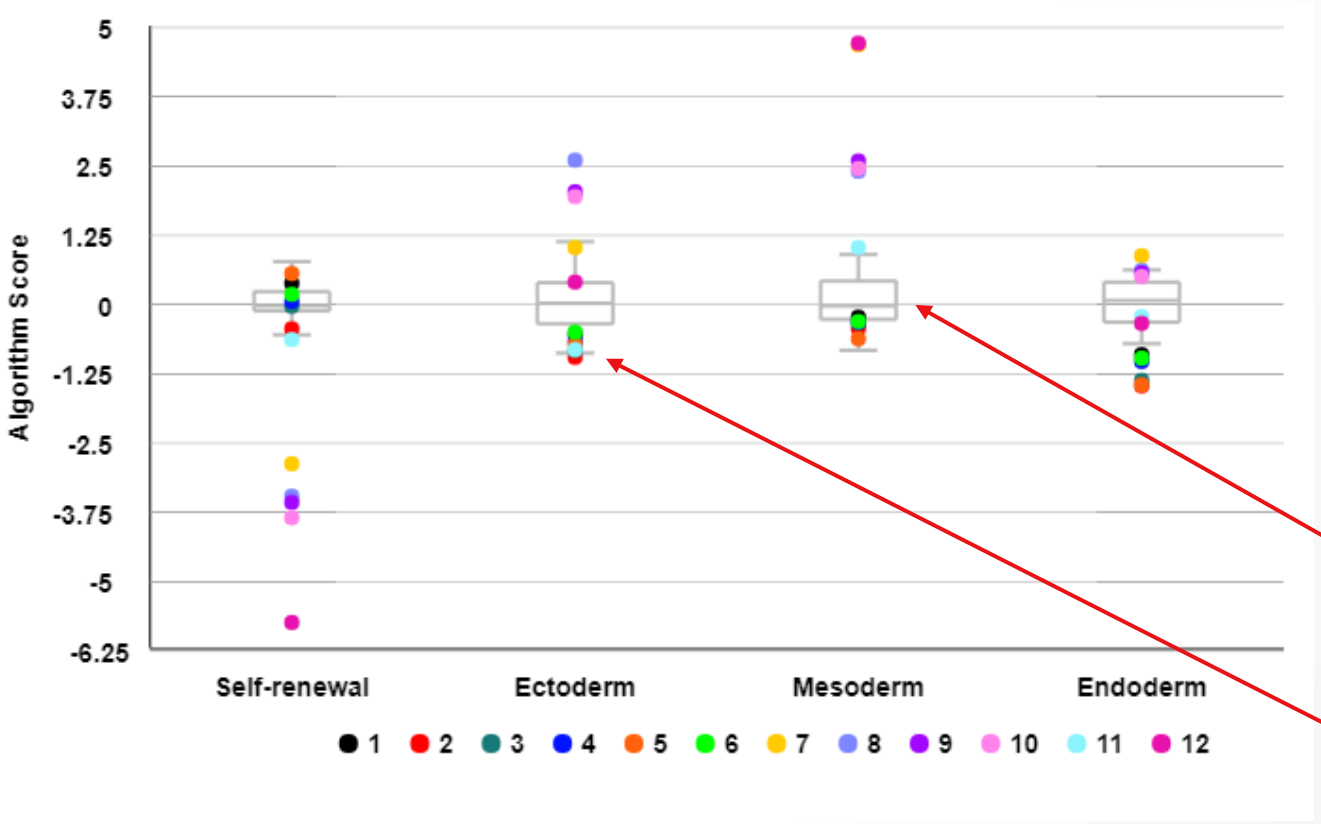
Scores close to 0 represent comparable expression to that of the undifferentiated reference set. These scores have no associated color.

Scores > 1 represent up regulation relative to the undifferentiated reference set. These scores are shown in red, and the color intensity is determined by the fold change amount.

Scores < -1 represent down regulation relative to the undifferentiated reference set. These scores are shown in blue, and the color intensity is determined by the fold change amount.

Figure A1: Scorecard Values: Algorithm scores for the samples show up regulation or down regulation of the endoderm, mesoderm, ectoderm or pluripotent (self-renewal) markers relative to the reference set of nine undifferentiated pluripotent stem cell lines.

Results - Scores Box Plot



The gene expression algorithm compares the sample data to the data in the reference set and assigns a score based on how closely the data align with the reference set of undifferentiated pluripotent stem cell lines.

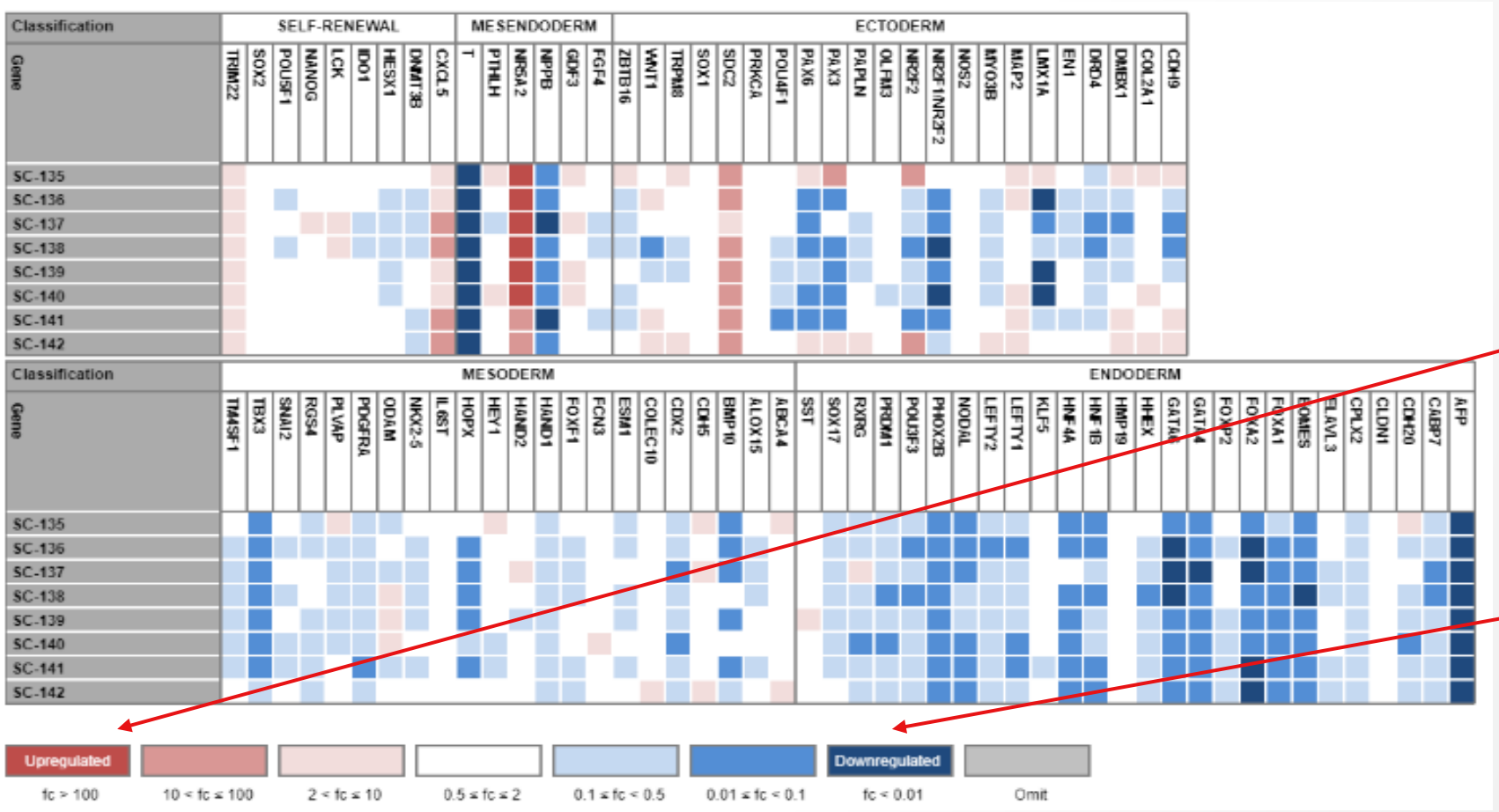
Each box represents the range of values from the median value of the upper 50% of data points (Q1) to the median value of the bottom 50% of data points (Q3).

The horizontal line within the box represents the median value for the entire sample data set.

The whiskers represent the calculated maximum and minimum values of the reference data set.

Figure A2: Scores Box plot: Sample scores are plotted in color. The range of scores for the undifferentiated reference set is indicated by the grey box plot.

Results - Gene Expression Panel



The Gene Expression Panel is a heatmap displaying expression of certain self-renewal and differentiation markers.

Red squares indicate a positive fold change in expression relative to the undifferentiated reference set. Color intensity is determined by the fold change amount.

Blue squares indicate a negative fold change in expression relative to the undifferentiated reference set. Color intensity is determined by the fold change amount.

Figure 3A: Expression Pattern Plot. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.

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Scorecard™ Assay Services Frequently Asked Questions (1 of 3)

- **What is the TaqMan® Scorecard™ Assay and how does it work?**
  - *Scorecard consists of a focused gene expression array of 93 qPCR assays that assesses pluripotency marker expression and differentiation potential. Nine of the assays are for pluripotency associated genes, ten are housekeeping genes and the remainder is devoted to differentiation into ectoderm, mesoderm, endoderm and mesendoderm. The data is then compared to a reference set of 13 well characterized PSC lines.*

- **How many cells are required to run one experiment?**
  - *We require a cell pellet of at least 2 million cells per sample.*

- **How many genes are tested per sample?**
  - *93 genes. There are 9 self-renewal genes, 74 lineage specific genes, and 10 housekeeping and control genes.*

- **What do the values in the scores table mean? / How are the scores calculated?**
  - *A proprietary algorithm compares the Ct values of each marker set to the values in the reference database and calculates the score based on how well the expression correlates. In general, scores close to 0 indicate comparable expression to that of the reference standard using undifferentiated cells. Scores higher than 1 indicates up regulation relative to undifferentiated cells and less than -1 indicate down regulation relative to undifferentiated cells.*

- **What is the earliest passage that I can use when I generate iPSCs?**
  - *We recommend that you culture iPSC clones to at least passage 8–10 until they are stable and homogeneous, prior to analyzing the hPSC Scorecard™ Panel. Early passage iPSC clones may give low self-renewal scores or show higher expression of lineage genes.*

Scorecard™ Assay Services Frequently Asked Questions (2 of 3)

- **Can I analyze somatic non-pluripotent primary cells with the TaqMan® hPSC Scorecard™ Panel?**
  - *Somatic non-pluripotent primary cells, such as the parental lines used for iPSC generation, are not pluripotent in nature and their scores will be low. However, the expression of lineage markers will largely rely on homogeneity of the cells. Note that the markers in the panel are designed to evaluate early germ layer specification and not any particular terminally differentiated state.*

- **When I use Sendai virus (SeV) to generate iPSCs, can I check for the presence of residual virus?**
  - *SEV is included in the panel and it can detect the presence of the Sendai virus backbone. Note that this method will not distinguish between the different reprogramming factors but just the presence or absence of the residual virus in the cells.*

- **Will the presence of Sendai virus affect my results?**
  - *Results indicate that the presence or absence of Sendai virus in established iPSC clones does not have an impact on pluripotency.*

- **What is the minimum time required to form EBs and is there a specific protocol?**
  - *You can differentiate cells using any of the established methods. When using suspension embryoid bodies, we recommend that you allow at least 7 days of differentiation prior to analysis.*

- **I am only interested in directed differentiated cells. Do I need to use a particular method?**
  - *You can perform directed differentiation according to your own methods. However, the time point when expression is noticeable will largely depend on the cells.*

Scorecard™ Assay Services Frequently Asked Questions (3 of 3)

- **How were the scores of reference lines and sample lines normalized?**
  - *The gene expression data presented in the figures are always shown relative to the gene expression of the 13 iPSC lines that are used as the standard. A sample would be considered **positive** for pluripotency if:*
    - *The pluripotency value of the sample falls within or above the range of the standards.*
    - *The ectoderm/mesoderm endoderm value of the sample falls within or below the range of the standards.*
  - *A sample would be considered **negative** for pluripotency if:*
    - *The pluripotency value is below the range of the standards.*
    - *The ectoderm, mesoderm and/or endoderm value of the sample falls above the range of the standards.*

Thank you

Please submit any follow-up questions to
CUBCharacterizationServices@thermofisher.com