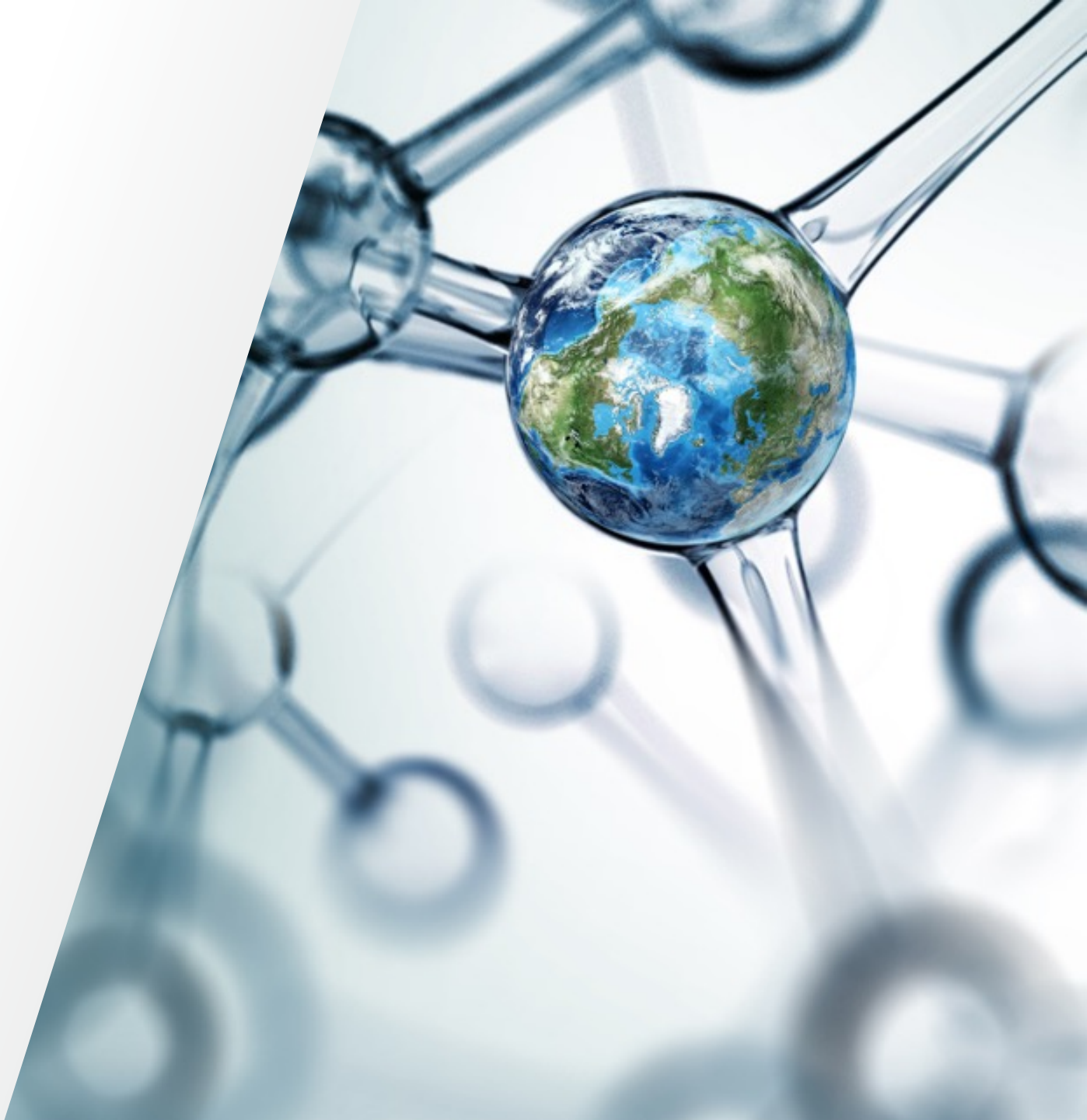
Scorecard™ Report

Client Name: Johns Hopkins University
Quote No: 11947717 SO
Date: 20 May 2024
Prepared by: Katelyn Reyno



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Summary of Services

- **Project Summary**:
  Johns Hopkins University (Client) is interested in Services provided by the Life Technologies Corporation in the analysis of three (3) 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-2254	MCN1-Endoderm	Cell Pellet
SC-2255	MCN1-Mesoderm	Cell Pellet
SC-2256	MCN1-Ectoderm	Cell Pellet

Table 1: Sample description provided by the client.

Results - Summary

1. Sample SC-2254 scored positive for Mesoderm and Endoderm markers
2. Sample SC-2255 scored positive for Mesoderm markers
3. Sample SC-2256 scored positive for Ectoderm 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 2254	-3.63	0.25	1.29	1.45
SC 2255	-5.16	0.28	2.42	-1.27
SC 2256	-3.87	2.05	0.06	-0.84

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.

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

Results - Scores Box Plot

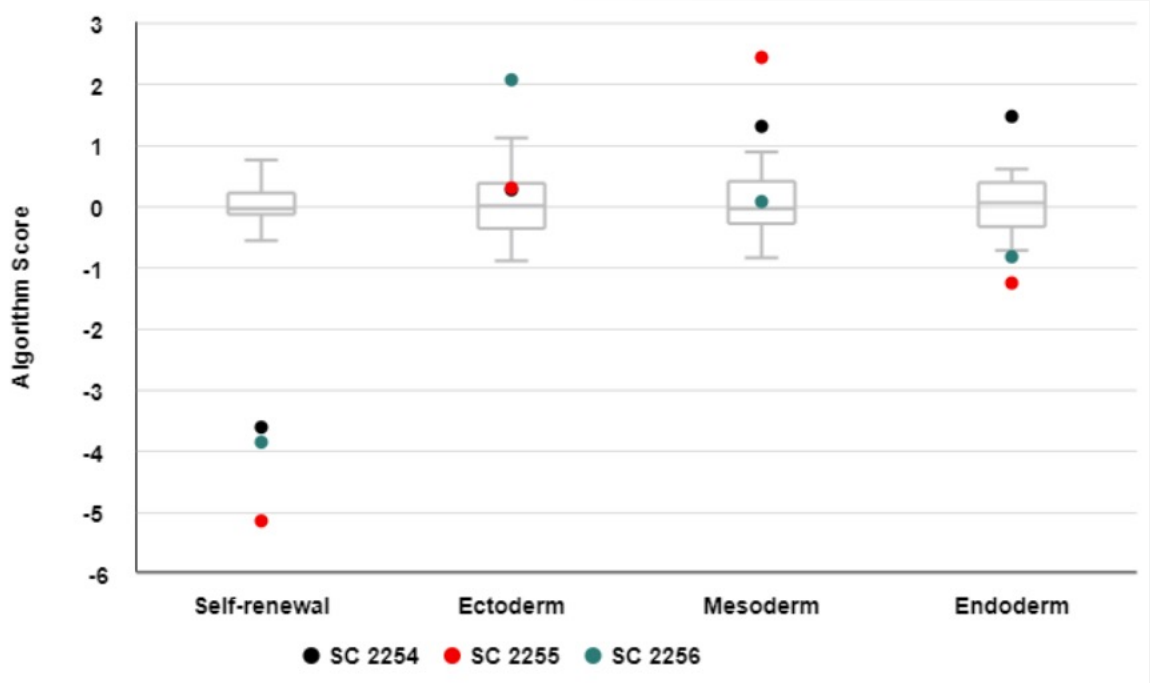

Figure 3: Scores Box plot: Sample scores are plotted in color. The range of scores for the undifferentiated reference set is indicated by the grey box plot.

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Results - Gene Expression Panel

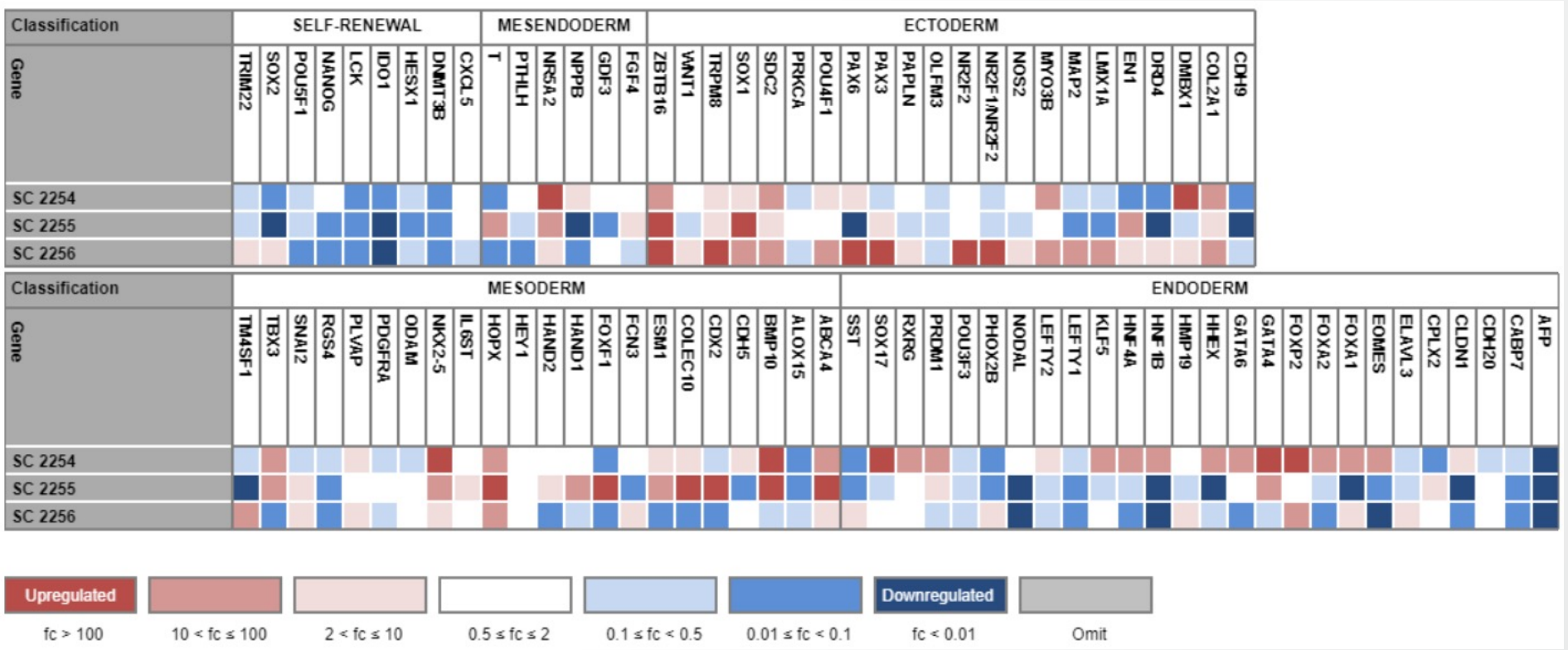

Figure 4: Expression Pattern Plot. Colors correlate to the fold change in expression of the indicated gene relative to the undifferentiated reference set.

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