Utility of TaqMan® hPSC Scorecard™ Assay in the Assessment of Functional Pluripotency of hPSCs

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Abstract

Improvements in induced pluripotent stem cell (iPSC) reprogramming technologies have led to the generation of patient-derived stem cells from various sources and conditions, creating valuable tools in drug discovery and future cell therapies. The steep challenge of characterizing these resulting iPSCs is minimally addressed by current methods that rely on a combination of *in vitro* and *in vivo* cellular methods. Molecular analysis methods offer an appealing solution for rapid, quantitative, and comprehensive characterization.

We earlier reported the development of a TaqMan® hPSC Scorecard™ Panel comprising a 94-gene panel. The accompanying cloud-based analysis software computes the signature for self-renewal and lineage markers for test samples and compares the signature against a pluripotent reference standard to generate scores. Over two hundred samples were analyzed using the TaqMan® hPSC Scorecard™ Panel to determine pluripotency along several stages of the iPSC workflow. Established clones were subjected to spontaneous embryoid bodies to assess for trilineage differentiation potential. Established clones were further used for directed differentiation into specific lineages and the optimal combination of cytokines and length of differentiation was determined using the TaqMan® hPSC Scorecard™ Panel. To further simplify the process and minimize sample size, methods were developed for direct use of cell lysate in the assay without compromising the quality of the results.

These results collectively demonstrate the simplicity, ease and consistency of this method to predict functionality, thus offering a much needed uniform standardization and qualification of pluripotent cell lines.

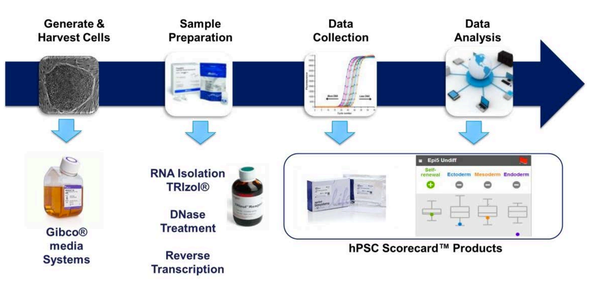
Introduction

Human pluripotent stem cells (hPSCs) are traditionally monitored based on morphology and characterized using a panel of tests assessing differential marker expression to test pluripotency and differentiation potential[1]. The ability of iPSC to form teratomas, in vivo, is often considered the most stringent assay for human cells and is based on histological and immunohistochemical detection of all three germ layers[2,3]. Although definitive, this method requires 1-2 months to complete, is difficult to standardize, expensive, and highly variable[4]. There is therefore a need for a cost-effective, animal-free alternative for assessing functional pluripotency[5].

In order to address this problem, we developed the TaqMan® hPSC Scorecard™ Panel, in collaboration with Alex Miessner. This assay is comprised of 94 predefined markers for self-renewal, Ectoderm, Mesoderm, and Endoderm and a cloud-based analysis software that compares the input sample's expression pattern to a functionally validated reference standard generated from a combination of ESC and iPSC lines. This method is simple, easy and flexible and allows for analysis of cells cultured under varying conditions and preparation of RNA and cDNA using different methods. Both undifferentiating and cells spontaneously differentiating via embryoid body formation are analyzed to confirm self-renewal marker expression and expression of trilineage differentiation markers, thus confirming functional pluripotency.

Workflow

The TaqMan® hPSC Scorecard™ Panel simplifies the characterization workflow by providing a quick and easy way to confirm pluripotency and differentiation potential.



Acknowledgements

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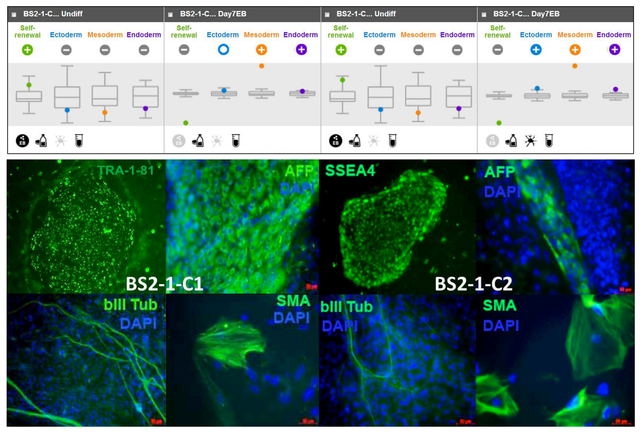
The gene content for the TaqMan® hPSC Scorecard™ Panel and reference data was a collaborative effort with team at Harvard led by Alex Meissner.

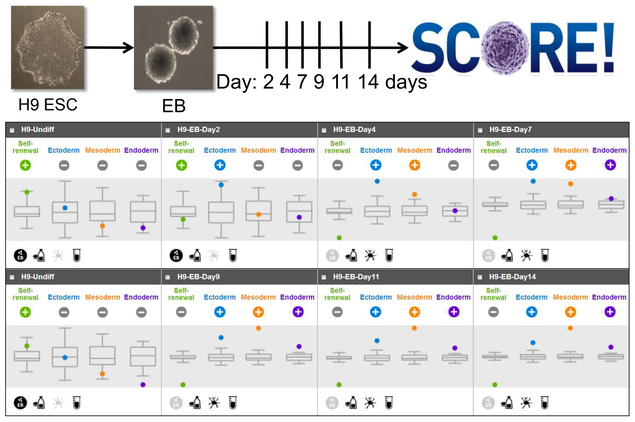
Results

Figure 1: Validating the TaqMan® hPSC Scorecard™ Panel



BS2-1-C1 and BS2-1-C2 iPSC clones were derived from human fibroblasts using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Undifferentiated and spontaneously differentiated iPSCs were stained with appropriate markers and analyzed with the TaqMan® hPSC Scorecard™ Panel to confirm functional pluripotency. The TaqMan® hPSC Scorecard™ Panel effectively recapitulates data generated with immunocytochemistry and only requires 7 days of differentiation instead of 21 days of differentiation.

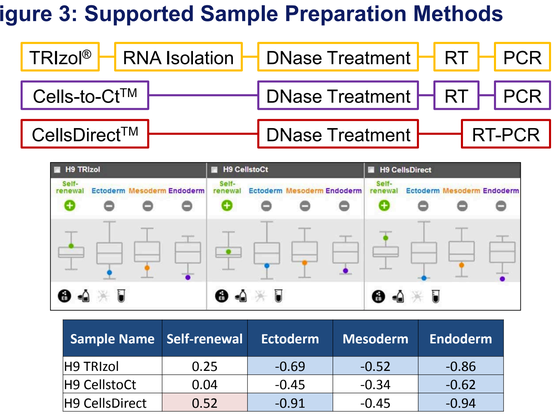
Figure 2: Assessing Spontaneous Differentiation



Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
H9-Undiff	0.71	0.15	-0.49	-0.56
H9-EB-Day2	-0.26	0.97	-0.08	-0.18
H9-EB-Day4	-1.92	2.18	1.22	0.04
H9-EB-Day7	-3.25	2.35	2.09	0.63
H9-EB-Day9	-3.23	2.36	3.48	1.30
H9-EB-Day11	-3.74	2.40	4.16	1.37
H9-EB-Day14	-4.67	2.36	4.79	1.61

Spontaneous trilineage differentiation of H9 ESCs was detected by Day 7 using the TaqMan® hPSC Scorecard™ Panel.

Figure 3: Supported Sample Preparation Methods

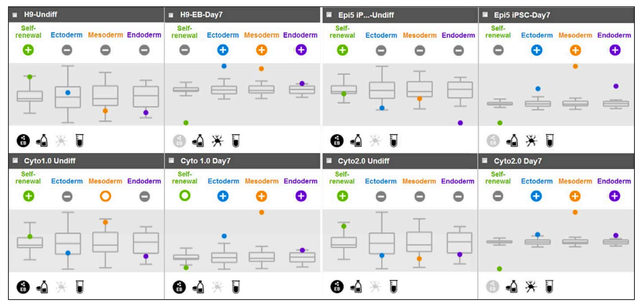


Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
H9 TRIzol	0.25	-0.69	-0.52	-0.86
H9 CellstoCt	0.04	-0.45	-0.34	-0.62
H9 CellsDirect	0.52	-0.91	-0.45	-0.94

H9 ESCs were harvested with various RNA isolation and cDNA synthesis methods. These methods have minimal impact on the results generated from the TaqMan® hPSC Scorecard™ Panel and as a result, the most applicable method can be chosen by the user.

Results

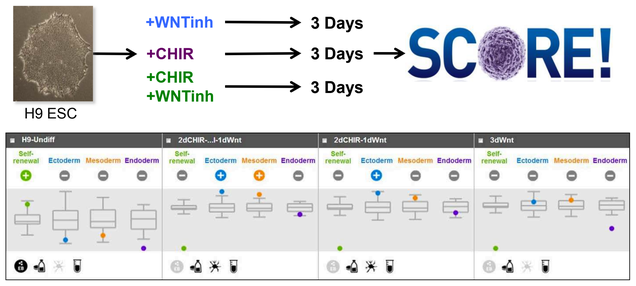
Figure 4: Confirmation of Functional Pluripotency of iPS Clones Derived Using Three Different Reprogramming Methods



Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
H9-Undiff	0.71	0.15	-0.49	-0.56
H9-EB-Day7	-3.25	2.35	2.09	0.63
Epi5 iPSC-Undiff	-0.17	-0.82	-0.39	-1.51
Epi5 iPSC-Day7	-2.96	2.33	5.80	2.73
Cyto1.0 Undiff	0.24	-0.34	0.75	-0.46
Cyto 1.0 Day7	-0.80	1.68	3.55	0.57
Cyto2.0 Undiff	0.43	-0.46	-0.54	-0.40
Cyto2.0 Day7	-4.88	1.36	5.42	1.19

Undifferentiated iPSCs and iPSCs spontaneously differentiated for 7 days by EB formation were analyzed using the TaqMan® hPSC Scorecard™ Panel and functional pluripotency was confirmed. The iPSCs were derived from human fibroblasts using three different reprogramming methods: the Epi5™ Episomal iPSC Reprogramming Kit, CytoTune®-iPS Sendai Reprogramming Kit, and CytoTune™-iPS 2.0 Sendai Reprogramming Kit.

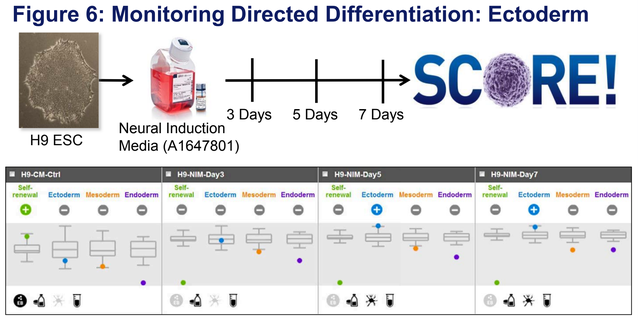
Figure 5: Monitoring Directed Differentiation: Mesoderm



Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
H9-Undiff	0.60	-0.77	-0.60	-1.10
2dCHIR-1dBasal-1dWnt	-3.76	1.50	1.23	-0.62
2dCHIR-1dWnt	-2.90	0.99	0.65	-0.39
3dWnt	-3.39	0.28	0.42	-1.82

The progression of pluripotent stem cells undergoing directed mesoderm differentiation under different combinations of cytokines was monitored using the TaqMan® hPSC Scorecard™ Panel. A clear change in expression was apparent by day 3 of differentiation.

Figure 6: Monitoring Directed Differentiation: Ectoderm

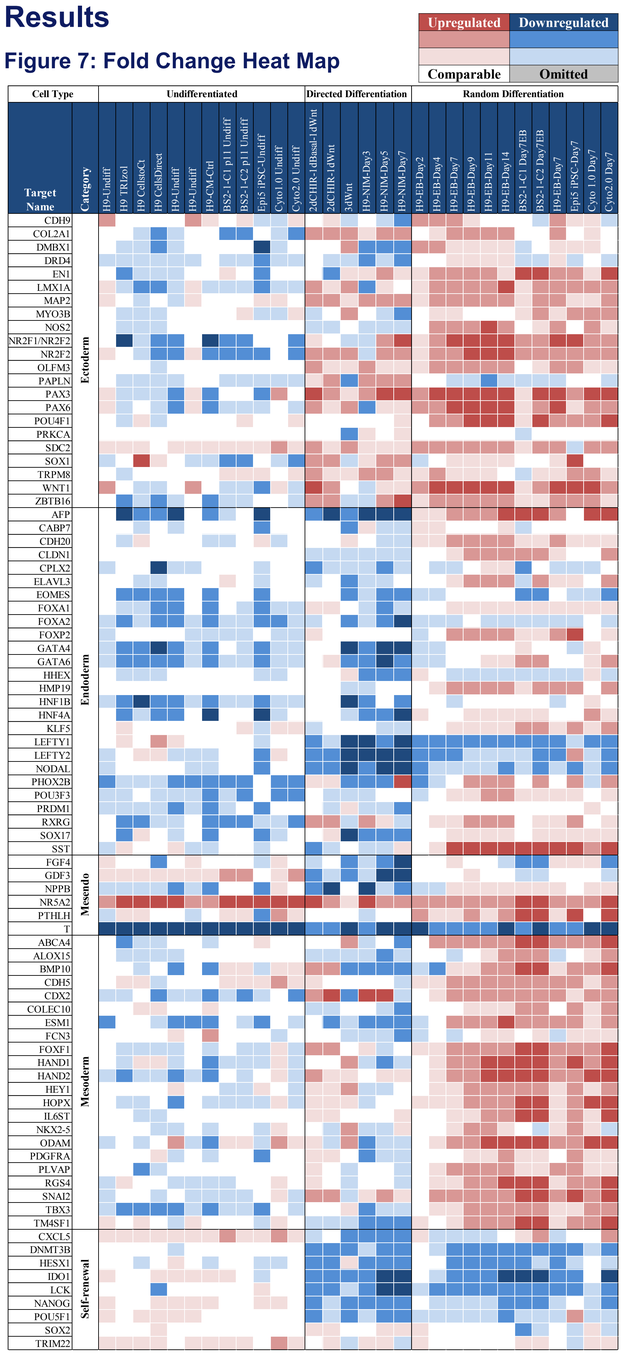


Sample Name	Self-renewal	Ectoderm	Mesoderm	Endoderm
H9-CM-Ctrl	0.61	-0.53	-0.80	-1.57
H9-NIM-Day3	-3.62	-0.11	-1.06	-1.79
H9-NIM-Day5	-4.56	1.16	-1.12	-1.99
H9-NIM-Day7	-5.62	0.96	-1.75	-1.73

The progression of pluripotent stem cells undergoing directed ectoderm differentiation was monitored using the TaqMan® hPSC Scorecard™ Panel over a 5 day period. A clear change in expression was apparent by day 5 of differentiation.

Results

Figure 7: Fold Change Heat Map



This heat map displays the fold changes relative to the TaqMan® hPSC Scorecard™ Panel's validated reference standard across each gene for all samples presented in this poster.

Conclusions

- The 94-gene TaqMan® hPSC Scorecard™ Panel offers a fast, high-throughput alternative to traditional characterization methods for qualifying human pluripotent stem cells. The hPSC Scorecard™ identified unique signatures of undifferentiated pluripotent stem cells and their differentiated progeny.
- The TaqMan® hPSC Scorecard™ Panel accurately characterized ESCs, iPSCs derived using different reprogramming methodologies, and effectively monitored directed differentiation into the Mesoderm and Endoderm lineages.

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