A Simple Functional Assay Demonstrating Pluripotency Using Directed Differentiation to All Three Germ Layers in Monolayer Culture

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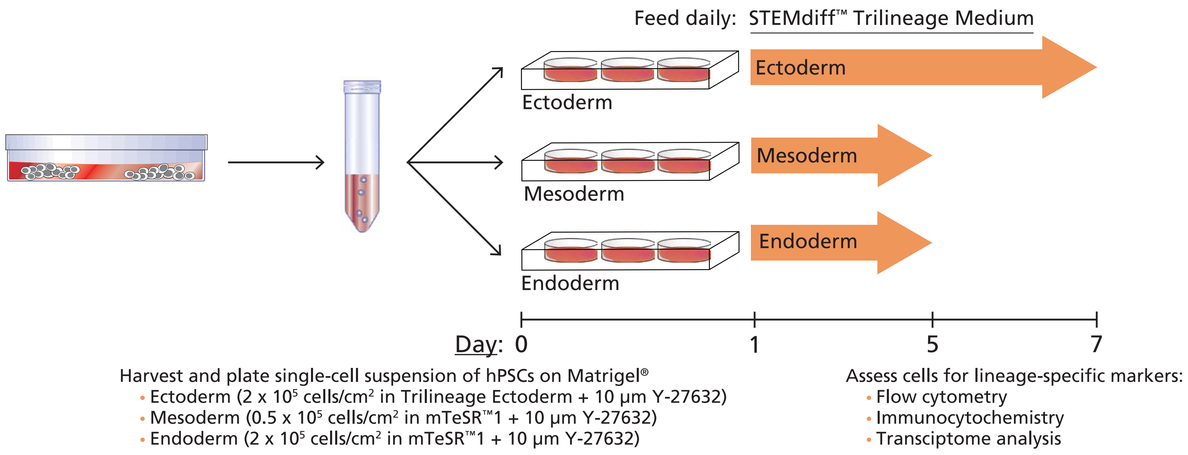
Introduction

Assessing the pluripotency of human pluripotent stem cell (hPSC) lines requires a functional demonstration of differentiation into ectoderm, mesoderm and endoderm lineages representing the three germ layers. The current gold standard *in vivo* teratoma assay is time consuming, costly and requires access to animals and specialized resources. Alternately, spontaneous *in vitro* differentiation of embryoid bodies (EBs) in serum-containing medium is inefficient and unpredictable in terms of differentiation kinetics and lineages produced, and thus not used as a replacement for the teratoma assay. We have developed a monolayer-based hPSC culture assay using STEMdiff™ Trilineage Ectoderm, Mesoderm and Endoderm Media and protocols specific for each germ layer, which clearly establishes trilineage differentiation potential within one week.

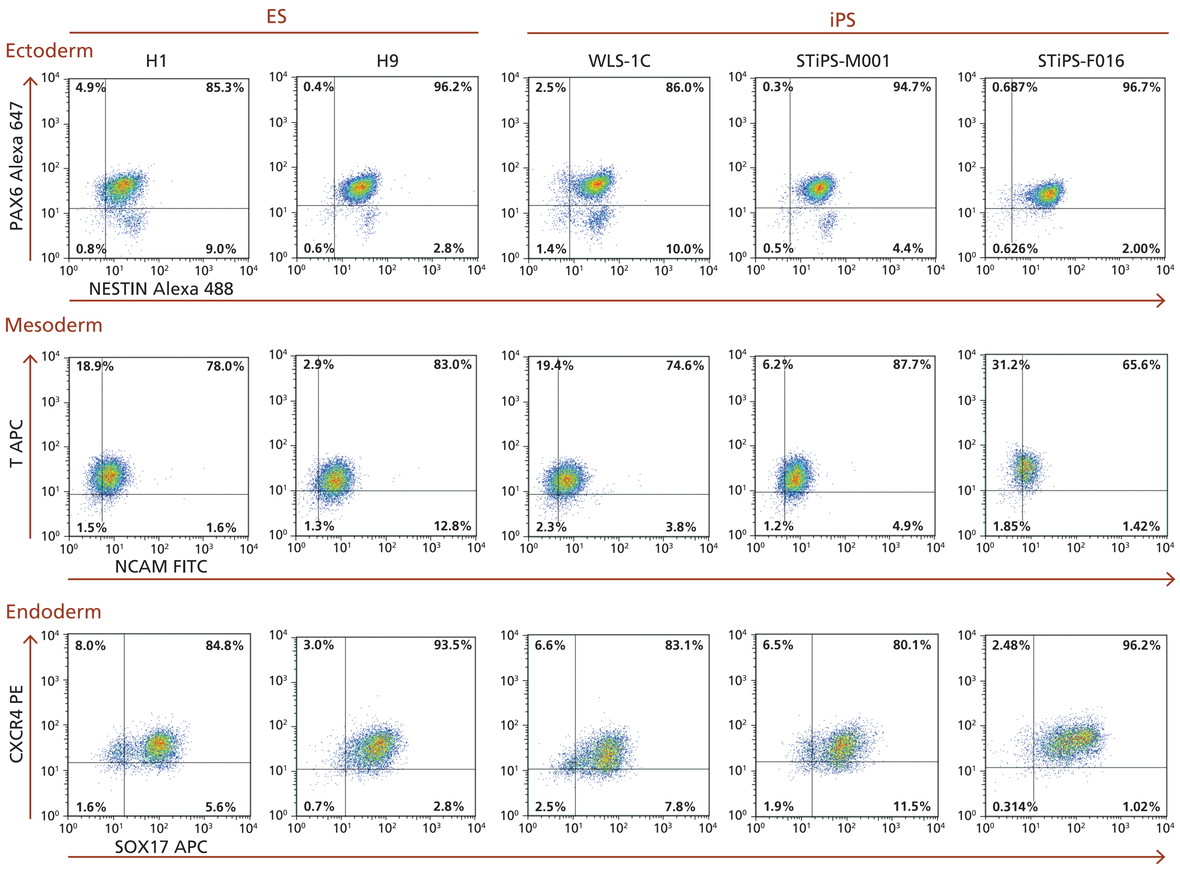
Methods

FIGURE 1: Protocol Schematic for STEMdiff™ Trilineage Differentiation Kit



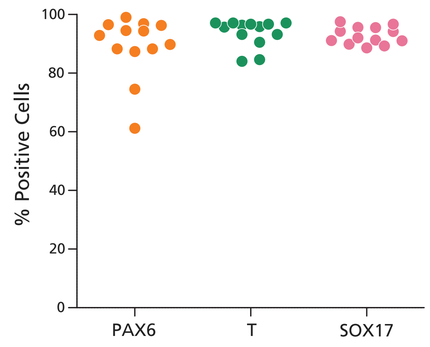
Results

FIGURE 2: Efficient Differentiation to All Three Germ Layers Assessed by Flow Cytometry



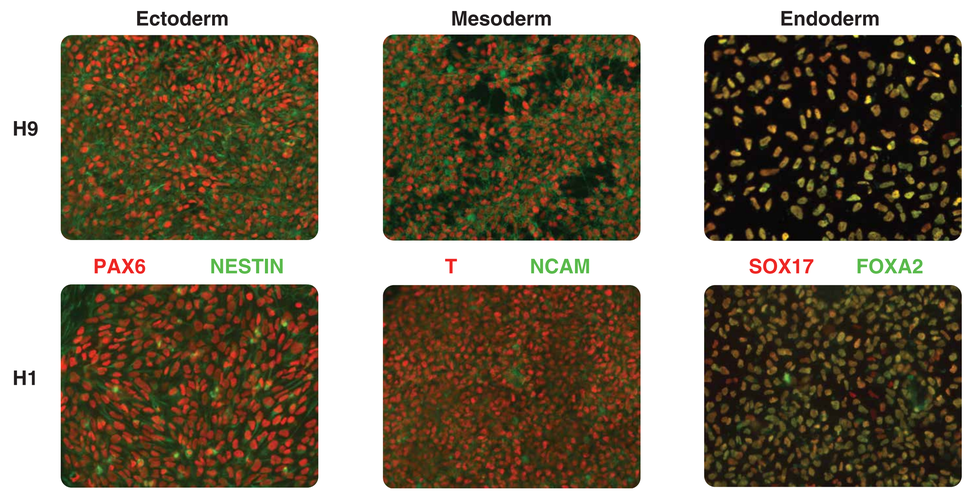
A panel of human ES and iPS cell lines maintained in mTeSR™1 were assayed for pluripotency using STEMdiff™ Trilineage Differentiation Kit and analyzed for marker expression using flow cytometry. Ectoderm differentiation gave rise to PAX6⁺NESTIN⁺ cells, mesoderm differentiation gave rise to Brachyury (T)⁺NCAM⁺ cells, and endoderm differentiation gave rise to SOX17⁺CXCR4⁺ cells. Gates were set based on isotype controls.

FIGURE 3: Reproducible Differentiation Using STEMdiff™ Trilineage Differentiation Kit



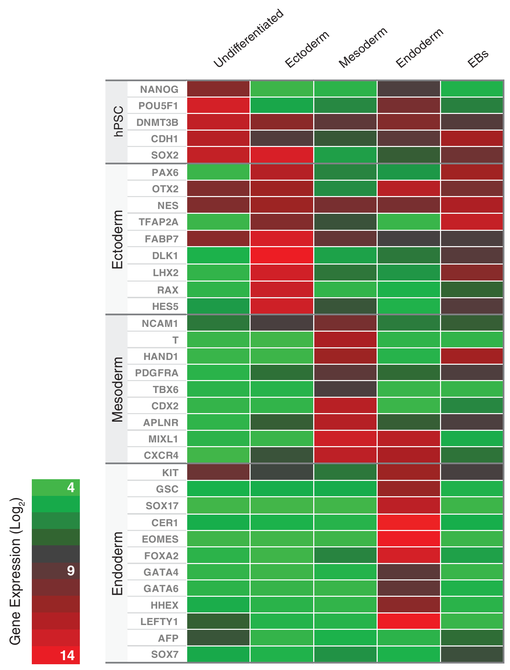
Summary of percentage of positive cells representing each germ layer generated using STEMdiff™ Trilineage Differentiation Kit quantitatively assessed by flow cytometry (n = 13 biological replicates, including 2 ES and 3 iPS cell lines). The markers evaluated for each germ layer are shown on the X-axis.

Figure 4. Efficient Differentiation to All Three Germ Layers Assessed by Immunocytochemistry



Human PSCs maintained in mTeSR™1 were assayed for pluripotency using STEMdiff™ Trilineage Differentiation Kit and analyzed for marker expression using immunocytochemistry. Ectoderm differentiation gave rise to PAX6⁺NESTIN⁺ cells, mesoderm differentiation gave rise to T⁺NCAM⁺ cells, and endoderm differentiation gave rise to SOX17⁺FOXA2⁺ cells.

FIGURE 5: Transcriptome Analysis Confirms Lineage-Specific Marker Expression of Cells Generated Using STEMdiff™ Trilineage Differentiation Kit



H9 cells were maintained in mTeSR™1 and differentiated *in vitro* using either the STEMdiff™ Trilineage Differentiation Kit or in a 10-day embryoid body (EB) protocol using serum-containing medium. Microarray analysis was performed to assess gene expression of the undifferentiated cells, differentiated ectoderm, mesoderm and endoderm cells from the kit, and cells from the EBs. Cells differentiated using the STEMdiff™ Trilineage Differentiation Kit showed clear upregulation of appropriate germ layer-specific markers, whereas cells from the EBs predominantly showed upregulation of ectoderm markers.

Summary

STEMdiff™ Trilineage Differentiation Kit is a simple culture assay that provides:

- Reproducible directed differentiation to each of the three germ layers
- High efficiency differentiation to each germ layer gives clear, easy-to-interpret assay results
- Demonstration of the pluripotency of hPSC lines within one week

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