Rapid Assessment of Pluripotency Using Directed Differentiation to all Three Germ Layers

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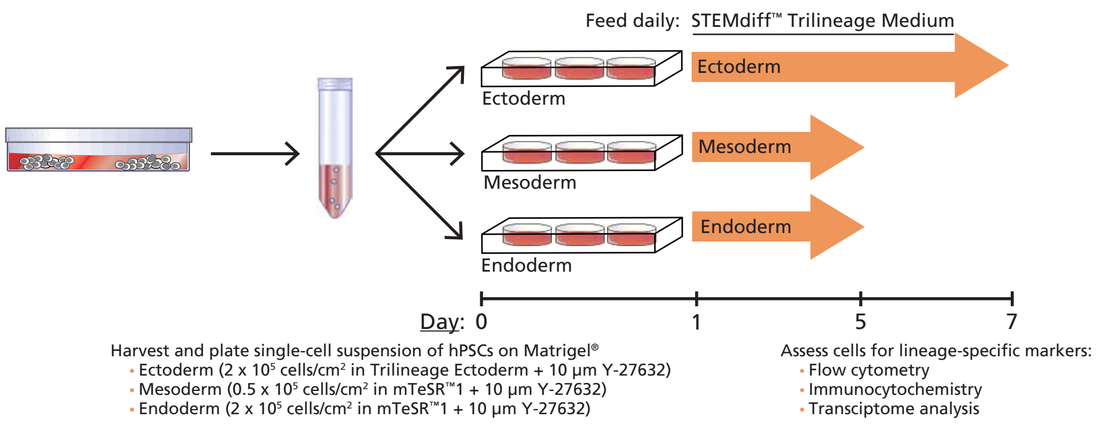
Introduction

Human pluripotent stem cells (hPSCs) possess the ability to differentiate into ectoderm, mesoderm and endoderm lineages representing the three germ layers. The current gold standard to demonstrate this pluripotent potential is to generate teratomas in immunodeficient mice. This in vivo assay is time consuming, costly, difficult to quantify and requires access to animals and other specialized resources. Spontaneous differentiation through the formation of embryoid bodies (EBs) in serum-containing medium is an alternative, but can be highly variable and unpredictable, often showing a differentiation bias towards a specific lineage. To address this problem we have developed the STEMdiff™ Trilineage Differentiation Kit, a simple and reproducible monolayer-based culture assay to demonstrate pluripotency using media and protocols specific for each of the three germ layers

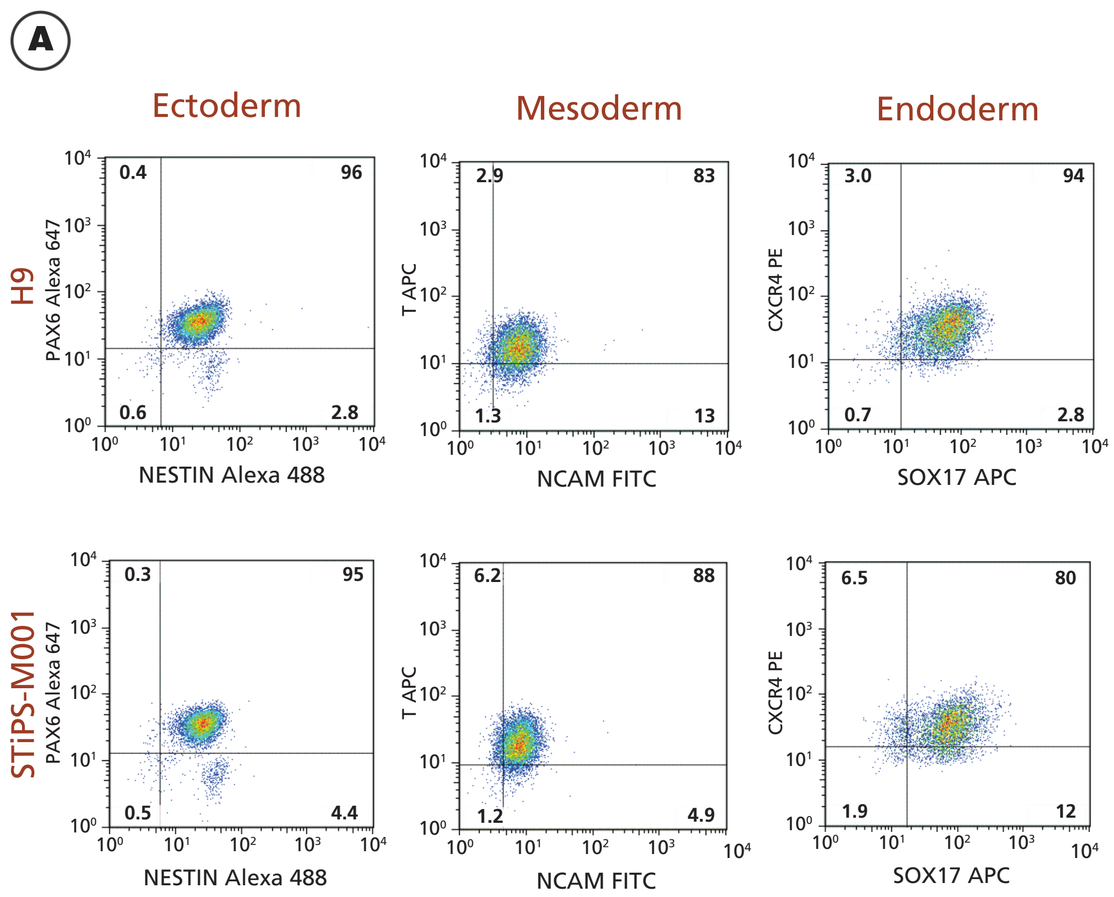
Methods

FIGURE 1: Protocol Schematic for STEMdiff™ Trilineage Differentiation Kit



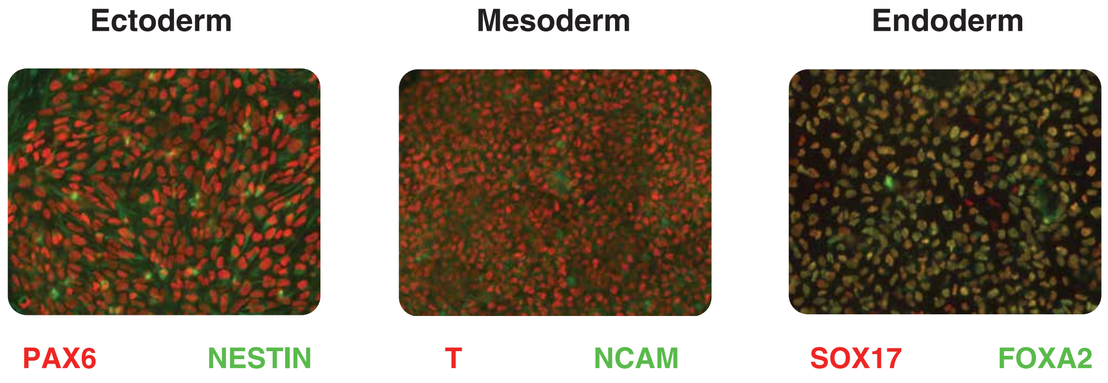
Results

FIGURE 2: Efficient and Reproducible Differentiation to all Three Germ Layers Assessed by Flow Cytometry



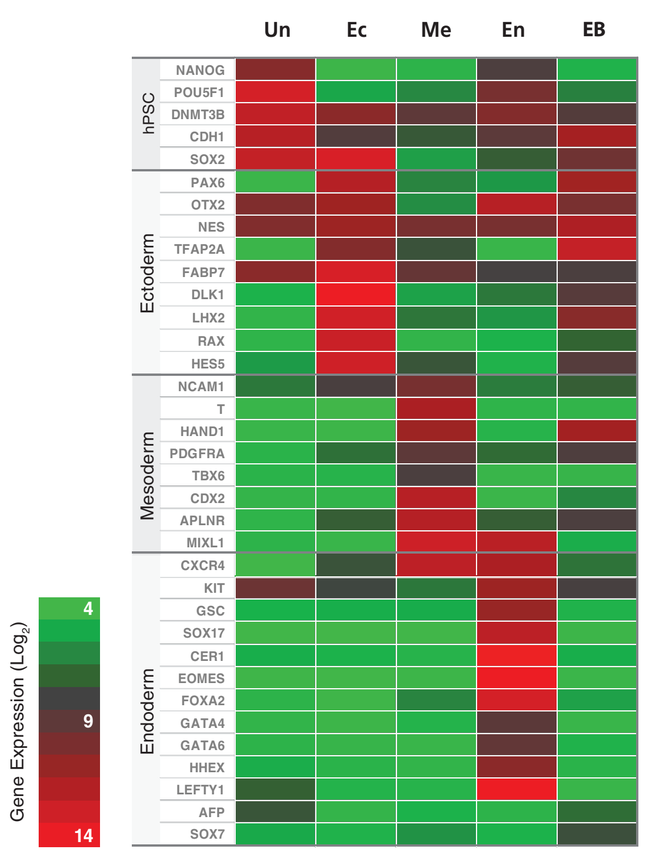
Human ES and iPS cell lines maintained in mTeSR™1 were assayed for pluripotency using the STEMdiff™ Trilineage Differentiation Kit and analyzed for marker expression using flow cytometry. **(A)** Representative flow cytometry plots of H9 ES cells and STiPS-M001 iPS cells showing differentiation to PAX6⁺NESTIN⁺ ectoderm cells, T⁺NCAM⁺ mesoderm cells, and SOX17⁺CXCR4⁺ endoderm cells. **(B)** Summary of differentiation to all three germ layers (n = 13 biological replicates, including 2 ES and 3 iPS cell lines) with the markers evaluated for each germ layer shown on the X-axis.

FIGURE 3: Efficient Differentiation to all Three Germ Layers Assessed by Immunocytochemistry



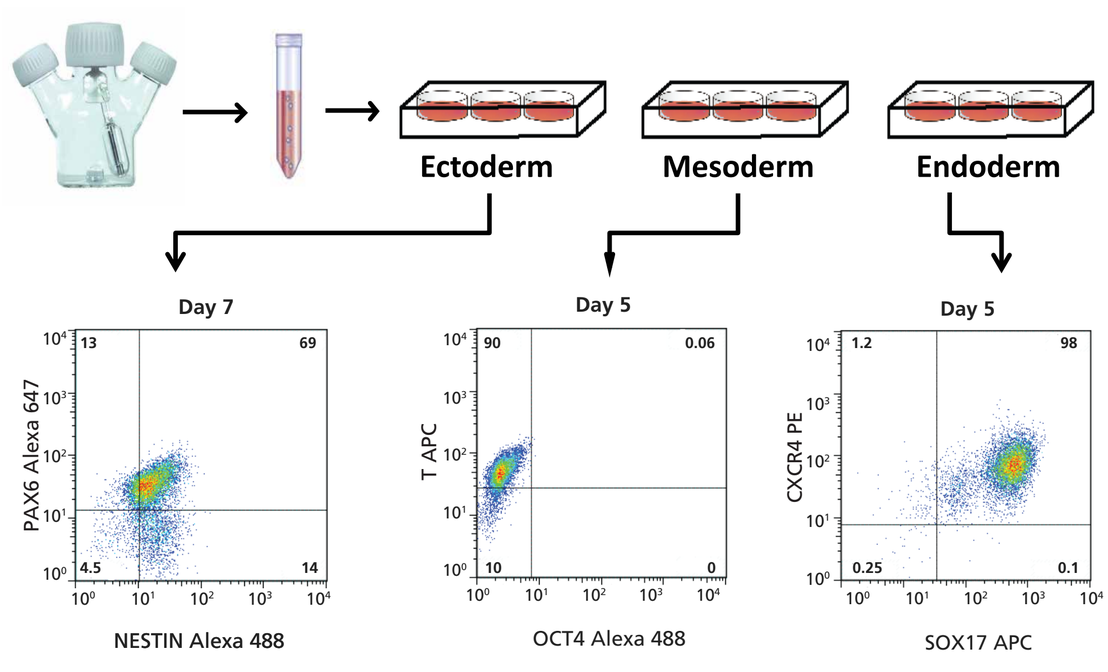
Human PSCs maintained in mTeSR™1 were assayed for pluripotency using the STEMdiff™ Trilineage Differentiation Kit and analyzed for marker expression using immunocytochemistry. Representative images of H1 ES cells show differentiation to PAX6⁺NESTIN⁺ ectoderm cells, T⁺NCAM⁺ mesoderm cells, and SOX17⁺FOXA2⁺ endoderm cells.

FIGURE 4: Transcriptome Analysis Confirms Lineage-Specific Marker Expression of Cells Generated Using STEMdiff™ Trilineage Differentiation Kit



Microarray analysis was performed to assess gene expression of H9 cells before and after differentiation. Samples compared were: undifferentiated cells **(Un)** maintained in mTeSR™1, ectoderm **(Ec)**, mesoderm **(Me)** and endoderm **(En)** differentiated using the STEMdiff™ Trilineage Differentiation Kit, and cells differentiated using a 10-day EB protocol in serum -containing medium **(EB)**. Cells differentiated using the STEMdiff™ Trilineage Differentiation Kit showed clear upregulation of appropriate germ layer -specific markers, whereas EBs primarily showed upregulation of ectoderm markers.

FIGURE 5: hPSC from Suspension Cultures in mTeSR™3D can be Differentiated to all 3 Germ Layers



Spinner flasks or 6-well plates on an orbital shaker were used for hPSC scale-up as aggregates in suspension culture using mTeSR™3D. After 5 passages, cultures were assessed for differentiation capacity using STEMdiff™ Trilineage Differentiation Kit. Aggregates were dissociated to single cells and seeded into the monolayer protocols for ectoderm, mesoderm, and endoderm differentiation, yielding high percentages of differentiated cells as assessed by flow cytometry (representative plots show differentiation of H1 human ES cells).

Summary

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

- Reproducible directed differentiation to each of the three germ layers
- Clear, easy-to-interpret assay results
- Demonstration of the pluripotency of hPSC lines within one week