Weekend-Free Protocol to Culture Human Pluripotent Stem Cells using mTeSR™1 or TeSR™-E8™

Melanie D. Kardel[1], Raymond Kwok[1], Jessica Norberg[1], Melanie Olson[1], Erik B. Hadley[1], Jennifer Antonchuk[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Sharon A. Louis[1]

[1] STEMCELL Technologies Inc., Vancouver, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, B.C., Canada

Introduction

Human pluripotent stem cells (hPSCs) have enormous potential for use in basic research, disease modeling, drug screening, and regenerative medicine; however, culturing hPSCs is labor-intensive. Feeder-free culture using mTeSR™1 or TeSR™-E8™ media removes the labor requirement of maintaining and preparing feeder cells, but still requires vigilant monitoring of colony morphology to determine the ideal passaging day and daily medium changes, with the accepted exception to allow one double-volume medium change followed by a single day without feeding each passage. These protocols routinely require researchers to work on the weekend, which is problematic for many labs with regards to scheduling experiments or staff. A "weekend-free" (WF) protocol would require a 7-day passaging interval and permit undifferentiated cells to be maintained when left for two days without a medium change. By controlling the aggregate size and density such that cells were routinely passaged at 7-day intervals, we were able to eliminate the need to feed cultures for two days after seeding, and developed a WF protocol for use with mTeSR™1 and TeSR™-E8™ media.

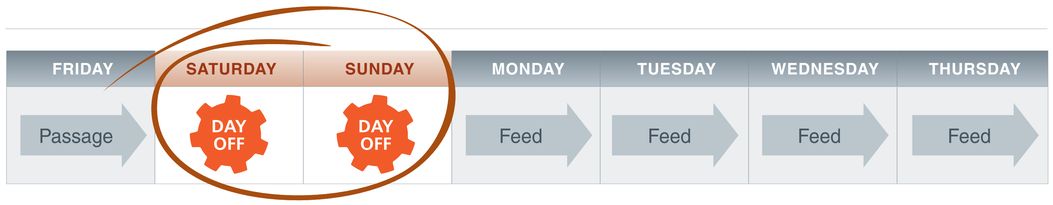

Figure 1. Overview of the weekend-free protocol.

Materials & Methods

Experiments were performed using H1 or H9 human embryonic stem (ES) cells, or WLS-1C or WLS-4D1 human induced pluripotent stem (iPS) cells maintained in either mTeSR™1 (Catalog #05850) or TeSR™-E8™ (Catalog #05940) on Matrigel® (Corning®) unless otherwise indicated. For each experiment, duplicate wells were independently maintained in parallel for at least 10 passages.

Passaging Cells

Cells were passaged every 7 days using Dispase (Catalog #07923) or Gentle Cell Dissociation Reagent (Catalog #07174) according to the recommended protocols. Briefly, cells were incubated with either passaging reagent, which was then removed and replaced with fresh mTeSR™1 or TeSR™-E8™ medium. Cells were scraped into culture medium using a cell scraper, and cell aggregates were transferred to a tube and pipetted up and down until the aggregates in suspension were approximately 80 - 150 µm in diameter. Cell aggregates were counted and then plated at a density of 35 - 50 aggregates/cm^2 onto fresh matrix-coated dishes containing the appropriate TeSR™ medium.

Feeding Schedules

We compared the WF protocol to a standard 7-day passaging protocol. In the standard protocol, cells were passaged on Friday, medium was exchanged on Saturday with a double-volume feed, no feed on Sunday, followed by daily medium changes Monday - Thursday. In the WF protocol (Figure 1), cells were passaged on Friday, left undisturbed Saturday and Sunday with no medium change, followed by daily medium changes Monday - Thursday.

Culture Assessment

At each passage, cells were characterized by observing cell morphology, percent undifferentiated cells [(# undifferentiated colonies/total # colonies) x 100%], and fold expansion (# aggregates harvested/# aggregates seeded). Expression of hPSC markers was assessed by flow cytometry at the end of each experiment. Differentiation experiments and karyotype analysis were performed after cells had been maintained for at least 10 passages using the WF protocol.

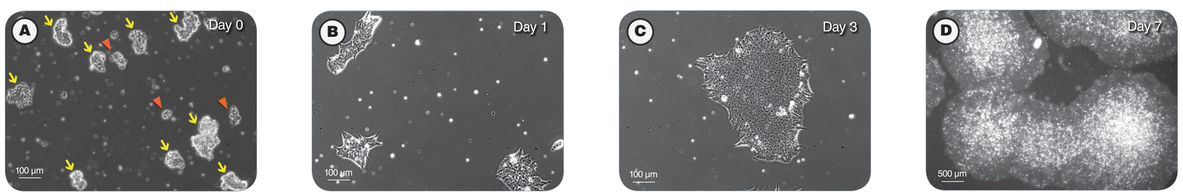

Figure 2. Colony growth during 7-day culture. **(A)** Aggregate suspension containing optimally-sized aggregates (80 - 150 µm in diameter, yellow arrows). Single cells and aggregates of <60 µm in diameter (red arrowheads) should be minimized. **(B)** Representative day 1 colonies after a medium change. **(C)** Representative day 3 colony. **(D)** Representative day 7 colonies ready for passaging.

Results

hPSC cultures maintained using the WF protocol were equivalent to those maintained using the standard protocol: cultures exhibited excellent undifferentiated morphology, high rates of expansion, expression of pluripotent stem cell markers, and a normal karyotype. We assessed functional pluripotency of cells maintained for at least 10 passages with the standard or WF 7-day passaging protocols by using *in vitro* directed differentiation to cell types representing all 3 germ layers. Comparable efficiency of differentiation was observed for ectoderm using STEMdiff™ Neural Induction Medium (Catalog #05831), mesoderm using a published cardiomyocyte differentiation protocol, and endoderm using the STEMdiff™ Definitive Endoderm Kit (Catalog #05110).

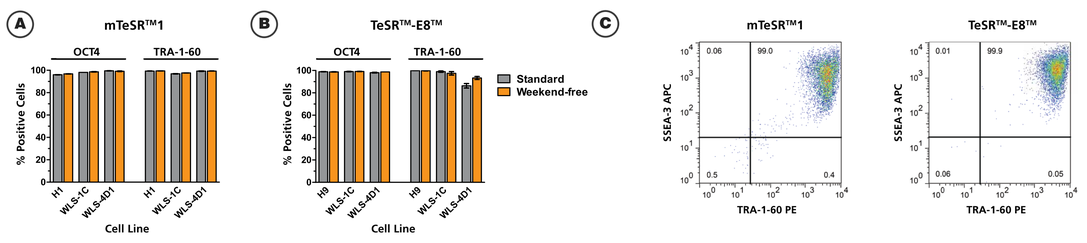

Figure 3. High expression of hPSC markers in cells cultured with the WF protocol. **(A, B)** Expression of pluripotent stem cell markers OCT4 and TRA-1-60 was high after 10 - 11 passages in cells maintained in mTeSR™1 **(A)** or TeSR™-E8™ **(B)** using standard (grey) or WF (orange) protocols (average of duplicate wells shown). **(C)** Representative examples of WF cultured cells show co-staining of TRA-1-60 and SSEA-3 on nearly all cells (left: H1, mTeSR™1; right: H9, TeSR™-E8™).

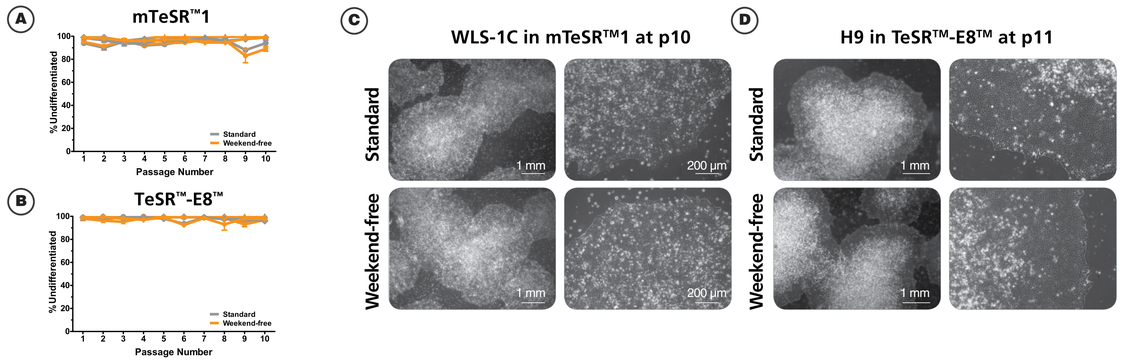

Figure 4. Normal undifferentiated colony morphology is maintained in WF cultures. **(A, B)** The percentage of colonies with undifferentiated morphology at each passage was determined by visual assessment of cultures of H1 or H9 (circles), WLS-1C (triangles) or WLS-4D1 (diamonds) cells maintained in mTeSR™1 **(A)** or TeSR™-E8™ **(B)** using standard (grey) or weekend-free (orange) protocols. All conditions showed consistently high percentages of undifferentiated colonies over 10 passages. **(C, D)** Representative images indicate comparable undifferentiated morphology in standard and WF conditions. Colonies are large and round with well-defined edges, phase-bright centers, and minimal differentiation. Cells within the colonies are homogeneous and tightly packed with prominent nucleoli and a high nuclear to cytoplasmic ratio.

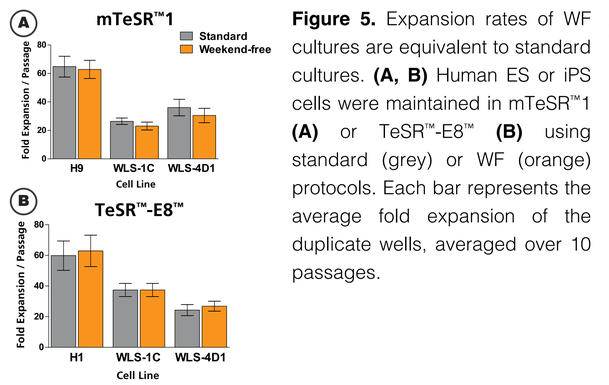

Figure 5. Expansion rates of WF cultures are equivalent to standard cultures. **(A, B)** Human ES or iPS cells were maintained in mTeSR™1 **(A)** or TeSR™-E8™ **(B)** using standard (grey) or WF (orange) protocols. Each bar represents the average fold expansion of the duplicate wells, averaged over 10 passages.

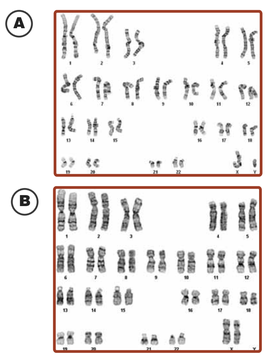

Figure 6. Normal karyotype of cells cultured with the WF protocol. **(A, B)** A normal karyotype was retained after >10 passages using the WF protocol for WLS-4D1 iPS cells cultured in mTeSR™1 **(A)** and for H9 ES cells cultured in TeSR™-E8™ **(B)**.

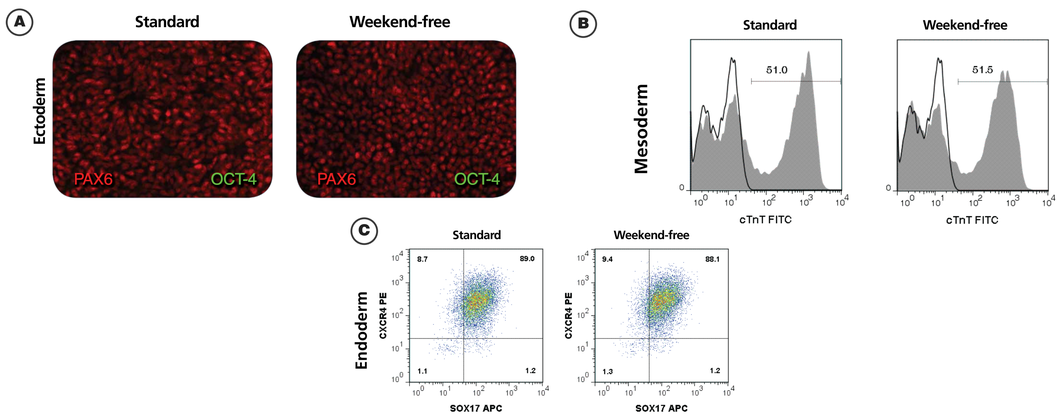

Figure 7. Ability to differentiate to three germ layers is retained in cells cultured with the WF protocol. **(A-C)** Differentiation efficiencies of WLS-4D1 cells maintained in mTeSR™1 for >10 passages with standard WF protocols were comparable for each of the following cell types: **(A)** PAX6$^+$ neural progenitor cells (red) with virtually no OCT4$^+$ undifferentiated cells (green) remaining (200X). **(B)** cTnT$^+$ (cardiac troponin T) cardiomyocytes. **(C)** SOX17$^+$CXCR4$^+$ definitive endoderm cells.

Weekend-free culture in a completely defined culture system was achieved using mTeSR™1 and TeSR™-E8™ in combination with the defined recombinant matrix protein Vitronectin XF™ (developed and manufactured by Primorigen Biosciences). H1 human ES cells were equivalently maintained in mTeSR™1 and TeSR™-E8™ using the standard and WF protocols, for ≥5 passages on Vitronectin XF™.

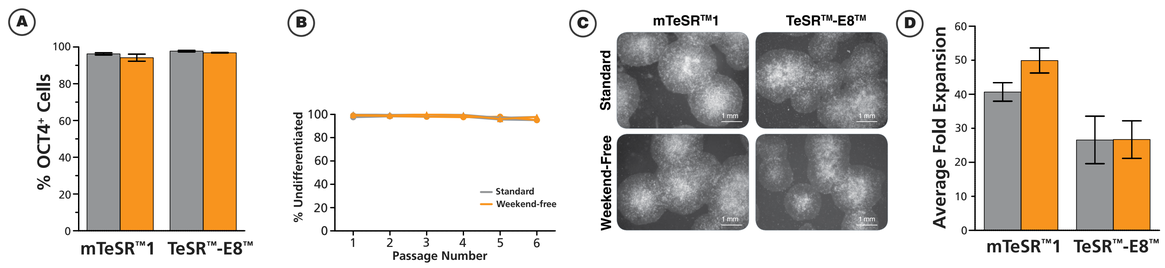

Figure 8. Vitronectin XF™ supports hPSC maintenance in a weekend-free protocol. **(A)** After 6 passages on Vitronectin XF™, virtually all cells expressed OCT4 (average of duplicate wells). **(B)** Visual assessment at each passage showed equally high percentages of undifferentiated colonies maintained in either mTeSR™1 (circles) or TeSR™-E8™ (triangles) using standard (grey) or WF (orange) protocols. **(C)** Representative images of H1 colonies in mTeSR™1 and TeSR™-E8™ show high quality morphology. **(D)** Expansion rates were equivalent for standard (grey) and WF (orange) protocols (average of duplicate wells, averaged over 5 passages).

Summary

By controlling hPSC aggregate size and seeding density from mTeSR™1 or TeSR™-E8™ cultures, we established a 7-day passaging interval, and showed that no medium change was required for the first 2 days after cell seeding. Cultures maintained using this "weekend-free" protocol were equivalent to those maintained using a standard (control) protocol. In practical terms, hPSCs cultured in TeSR™ media could be passaged on a Friday, and then left unmanipulated for Saturday and Sunday, thus providing a convenient weekend-free protocol for maintaining hPSCs.