Culture of High-Quality Human Pluripotent Stem Cells with Versatile Workflows Using mTeSR™ Plus, a New Stabilized TeSR™ Maintenance Medium

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INTRODUCTION

Human pluripotent stem cells (hPSCs) require specialized culture media to promote expansion while maintaining self-renewal and pluripotency. To date, the majority of culture systems require daily medium changes in order to replenish levels of critical components and eliminate accumulated metabolic waste. This is time-consuming when maintaining multiple cell lines, especially in hPSC core facilities, and typically requires operators to change medium over the weekend. mTeSR™ Plus, based on the mTeSR™1 formulation, was specifically developed to ensure truly versatile feeding schedules while maintaining high-quality hPSC cultures. The stabilization of FGF2 levels over 72 hours at 37°C, combined with an enhanced buffering capacity, supports flexibility for every other day or weekend-free schedules. We investigated key cell quality parameters of hPSCs cultured for ≥ 10 passages in mTeSR™ Plus compared with cells in mTeSR™1 and found that hPSC morphology, marker and gene expression, differentiation potential, cloning efficiency, and karyotype were all comparable. In summary, mTeSR™ Plus is an improved medium that promotes a more consistent cell culture environment, enabling versatile workflows while maintaining high-quality hPSCs that are fully compatible with established genome editing and differentiation protocols.

METHODS

hPSCs were cultured for up to 30 passages in mTeSR™ Plus with a reduced feeding schedule or in mTeSR™1 with daily feeding. Expression of OCT4 and TRA-1-60 was assessed by flow cytometry every 5 passages and transcriptome analysis was performed by RNA sequencing after 10 passages. Differentiation potential was evaluated for cells cultured in each medium after ≥ 5 passages using STEMdiff™ Trilineage Differentiation Kit. Cultures were monitored for recurrent genetic abnormalities every 5 passages using the hPSC Genetic Analysis Kit and characterized by G-banding after 30 passages. Gene knockout was performed using the ArciTect™ CRISPR-Cas9 system, and cloning efficiency in the different media was measured with the addition of CloneR™.

RESULTS

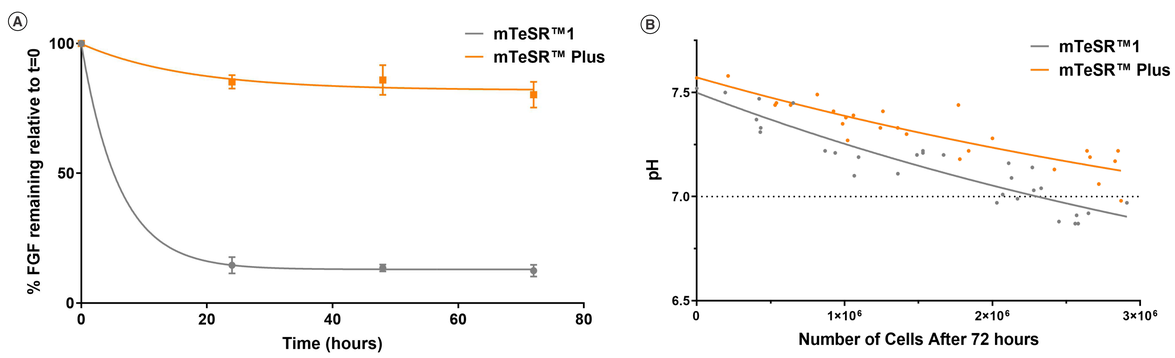

FIGURE 1. mTeSR™ Plus Maintains Consistent Levels of FGF2 and Optimal pH Levels Throughout a Weekend-Free Protocol

(A) FGF2 levels in mTeSR™ Plus remain at 80.3 ± 2.9% of T = 0 levels at 72 hours when kept at 37°C (measured by ELISA). **(B)** The pH of spent medium from hPSCs cultured in mTeSR™ Plus is higher than that for hPSCs cultured in mTeSR™1 at similar cell densities. Cultures were fed double the standard medium volume, and pH and cell numbers from one well of a 6-well plate were measured after a 72-hour period without feeding. The range of cell numbers shown represents different densities that would be observed throughout a typical passage, demonstrating that feeds can be skipped for two days at any time during routine maintenance using mTeSR™ Plus while maintaining a pH above 7.0.

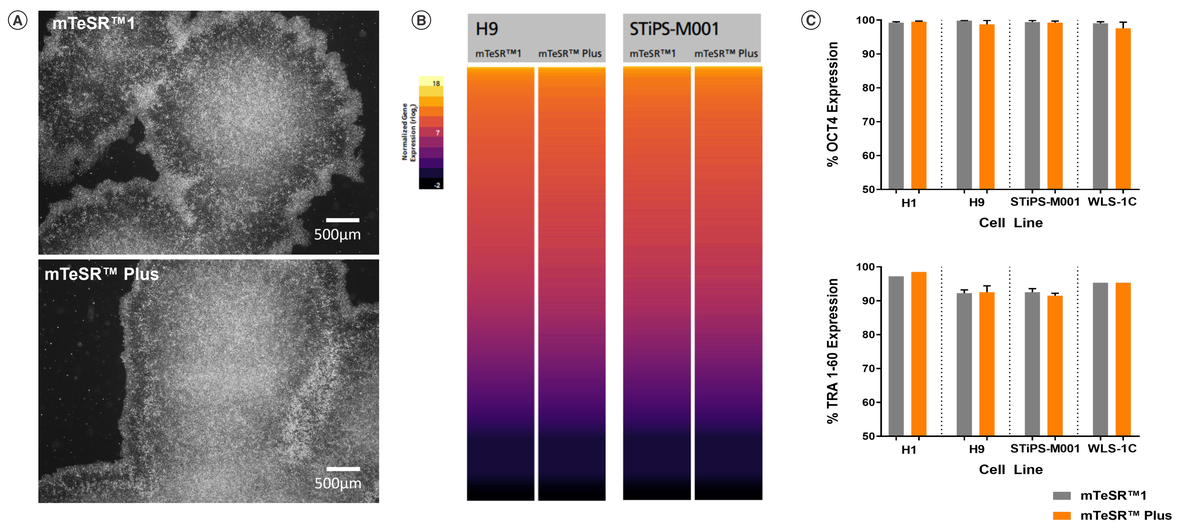

FIGURE 2. hPSCs Cultured in mTeSR™ Plus Medium with Restricted Feeding Display Improved Morphology and Retain Comparable Gene and Marker Expression

(A) Human ES cells (H9) cultured in mTeSR™1 or mTeSR™ Plus on Corning® Matrigel® with restricted feeding. Images were taken on day 7 after seeding. hPSC colonies grown in mTeSR™ Plus are larger in size and exhibit tighter cellular packing at the edges, resulting in greater border definition compared to mTeSR™1 when feeding is restricted. **(B,C)** hPSCs were cultured for ≥ 10 passages with either mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds). **(B)** Transcriptome analysis of H9 and STiPS-M001 maintained in mTeSR™ Plus shows a gene expression profile indistinguishable from cultures maintained in mTeSR™1 by RNAseq. Heat map displays all 19,665 genes measured for each condition. **(C)** Human ES (H1, H9) and iPS (WLS-1C, STiPS-M001) cells were characterized using flow cytometry for undifferentiated cell markers OCT4 and TRA-1-60. Graphs show average expression (± SEM) results from analyses of duplicate wells measured at passages 5 and 10.

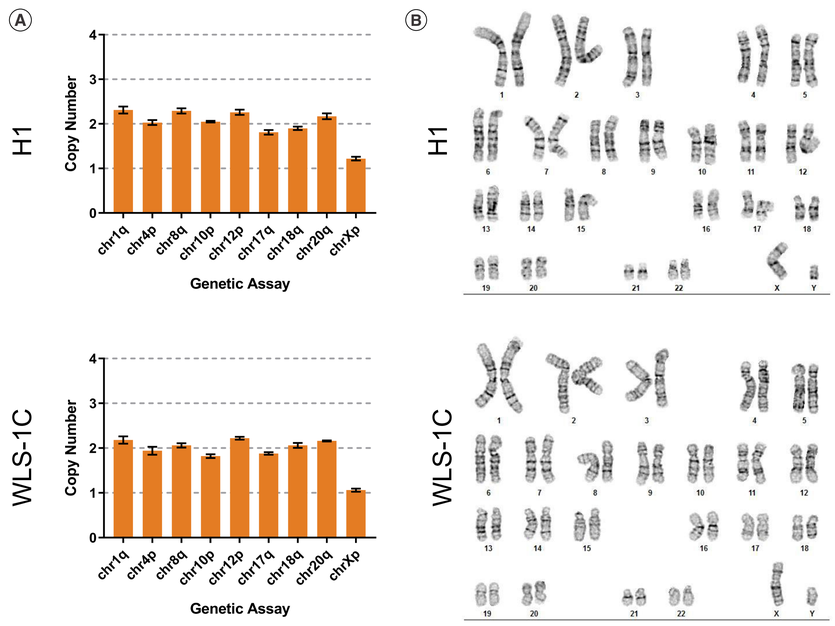

FIGURE 3. hPSCs Cultured in mTeSR™ Plus with Restricted Feeding Maintain a Normal Karyotype

Human ES (H1) and iPS (WLS-1C) cells were cultured in mTeSR™ Plus for 30 passages. **(A)** Cultures were screened using the hPSC Genetic Analysis Kit every 5 passages and no abnormalities were detected (passage 30 shown). **(B)** At passage 30, cultures displayed a normal karyotype by G-banding.

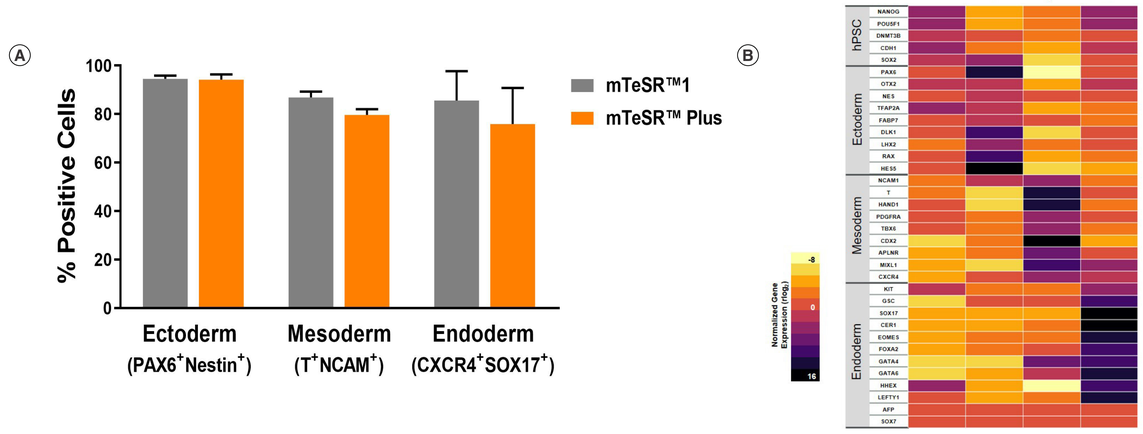

FIGURE 4. hPSCs Maintained in mTeSR™ Plus With Restricted Feeding Have Comparable Differentiation Efficiencies to hPSCs Maintained in mTeSR™1

(A) Human ES (H1, H9) and iPS (WLS-1C, STiPS-M001) cells were maintained in mTeSR™1 (daily feeds) or mTeSR™ Plus (restricted feeds). hPSCs were differentiated using directed differentiation protocols and subjected to flow cytometry analysis using the markers indicated. Graphs show average expression results (± SEM) from the four cell lines. **(B)** Additional analysis of WLS-1C cells differentiated from mTeSR™ Plus cultures showed clear upregulation of appropriate germ layer-specific markers when assessed using the hPSC Trilineage Differentiation qPCR Array.

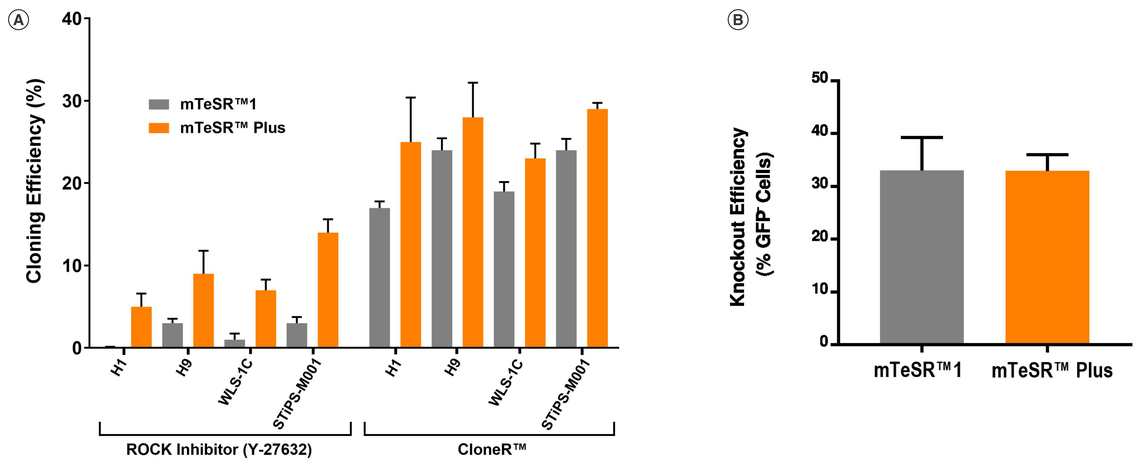

FIGURE 5. High Cloning and Gene Editing Efficiency of hPSCs in mTeSR™ Plus

(A) hPSCs (H1, H9, WLS-1C, STiPS-M001; n = 3 per cell line) plated in mTeSR™ Plus with 10 µM Y-27632 or CloneR™ demonstrate cloning efficiencies equal to or greater than hPSCs in mTeSR™1 with the same supplement. hPSCs were seeded at clonal density (25 cells/cm^2) on Vitronectin XF™-coated plates. **(B)** H1-eGFP ES cells were electroporated with RNP complexes targeting the eGFP transgene (15:30 pmol Cas9:gRNA) and knockout efficiency was measured at 72 hours following electroporation. Knockout efficiency (± SEM) was measured as % GFP negative (GFP-) cells in test condition subtracted by % GFP- cells in non-electroporated controls (n = 3).

Summary

- mTeSR™ Plus is an improved medium formulation that promotes a more consistent cell culture environment, enabling versatile maintenance workflows
- High cell quality is maintained in mTeSR™ Plus, even when using reduced feeding schedules; gene and marker expression, genetic stability, and differentiation potential are unaltered when compared with mTeSR™1 cultures fed daily
- mTeSR™ Plus is fully compatible with workflows using established gene editing and cloning protocols