# Optimized Media and Workflow for the Expansion of Human Pluripotent Stem Cells as Aggregates in Suspension Cultures

Eric Jervis[1], Karen Markwick[1], Kyle Hukezalie[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Steven Woodside[1]

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

## INTRODUCTION

3D suspension culture enables scale-up of human pluripotent stem cell (hPSC) manufacturing. However, media and methods optimized for 2D adherent cultures can lead to low volumetric productivity and laborious workflow in suspension cultures. To overcome these limitations, we have developed fed-batch media based on either mTeSR™1 (BSA-containing) or TeSR™-E8™ (animal component-free) for hPSC expansion as aggregates in suspension cultures. Fed-batch feeding protocols are more efficient and cost-effective than batch medium changes because only exhausted components are replenished. Suspension cultures were fed daily using either half-medium changes of standard 2D medium, or fed-batch optimized medium and feeding protocols. With observed growth rates, aggregates required passaging every 3 or 4 days as clumps of 5 - 10 cells obtained by treatment with Gentle Cell Dissociation Reagent (GCDR). Optimization was performed by iteratively modifying the feed solution to maintain consistent nutrient levels and maximal growth rate while maintaining cell quality. The concentration of feeding solutions was optimized to minimize volume changes during feeding cycles, which can negatively impact mixing during cell culture. Robust and repeatable cell expansion during scale-up can be achieved by selecting the appropriate feeding schedule. Control and optimized fed-batch formulations demonstrated between 1.4- and 1.8-fold expansion per day, > 90% viability, > 90% expression of OCT-4 and TRA-1-60, in vitro trilineage differentiation, and normal karyotype (n = 8 independent cultures). Suspension culture-optimized mTeSR™3D and TeSR™-E8™3D fed-batch media enable cost-effective production of hPSCs as aggregates with an efficient workflow and high cell quality.

## METHODS

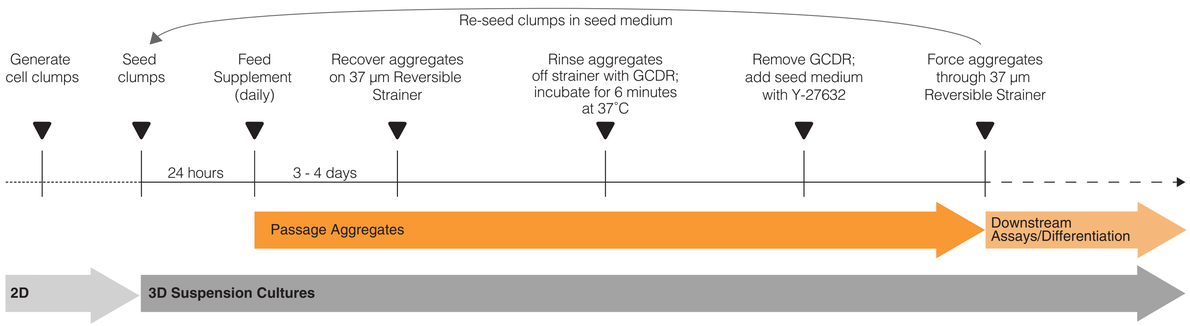


**FIGURE 1. Workflow for the Expansion of hPSCs as Aggregates in Suspension Culture**

High-quality adherent hPSC cultures are dissociated to clumps using Gentle Cell Dissociation Reagent (GCDR; Catalog #07174). Cell clumps are resuspended (1 - 4 x $10^5$ viable cells/mL) in complete seed medium with 10 µM Y-27632 (Catalog #72302). Fed-batch feed supplement is added daily starting 24 hours after inoculating cell clumps. After 3 or 4 days, aggregates are recovered using a 37 µm Reversible Strainer (Catalog #27250 or 27215), and then incubated in GCDR at 37°C for 6 minutes. GCDR is removed and aggregates are resuspended in complete seed medium with 10 µM Y-27632. Immediately after resuspension, aggregates are forced through a 37 µm Reversible Strainer to generate cell clumps. Clumps are re-seeded into a fresh culture vessel at 1 - 4 x $10^5$ viable cells/mL. No aggregate handling or medium exchanges are required between passages. Cells in clumps were counted by lysing a small volume of the clump suspension and staining nuclei with DAPI. Nuclei were counted using an image-based cell counter.

## RESULTS

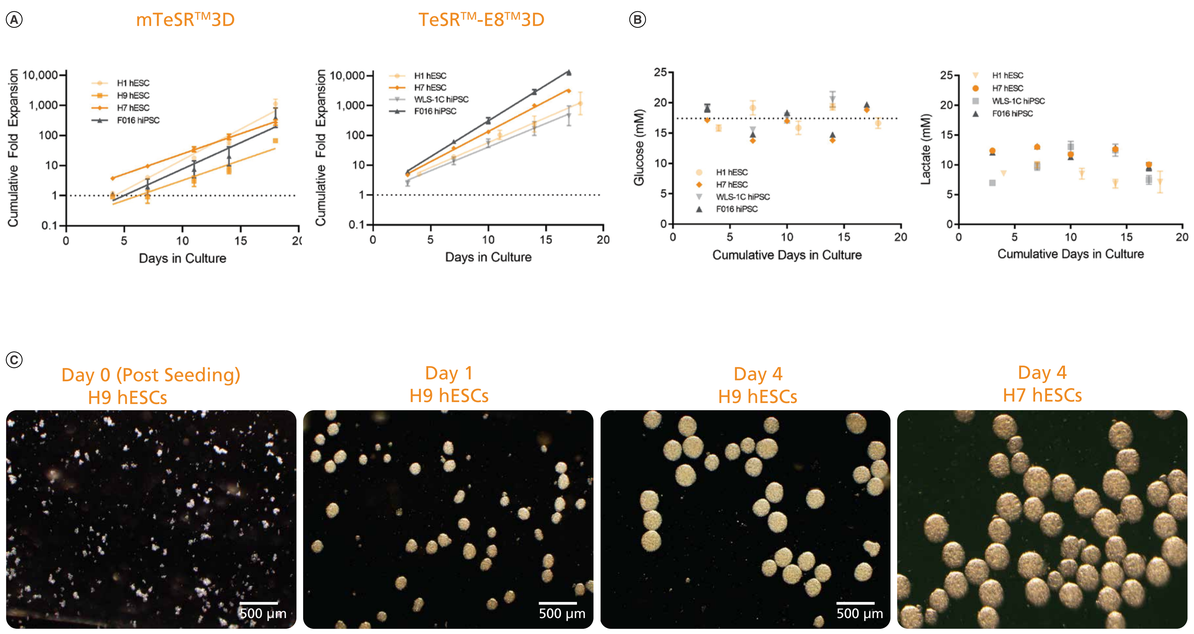


**FIGURE 2. Expansion and Metabolite Measurements from hPSCs Cultured as Aggregates in Suspension Using mTeSR™3D or TeSR™-E8™3D**

**(A)** Growth curves for hPSCs expanded over 5 passages in mTeSR™3D or TeSR™-E8™3D. **(B)** Glucose and lactate measurements of two cell lines expanded in TeSR™-E8™3D. Glucose is maintained at an optimal concentration during expansion, and lactate does not reach concentrations greater than 12 mM. The dotted line represents the glucose concentration in the complete seed medium. Error bars in (A) and (B) are ± SD (n = 3 in A, n = 4 in B). **(C)** Representative images of hPSC aggregates in suspension culture. H9 human embryonic stem cells (hESCs) over a single passage (Days 0, 1, and 4) are shown. H7 hESCs at the end of a passage are shown at higher magnification. The dimpled morphology is a key characteristic of undifferentiated hPSC aggregates.

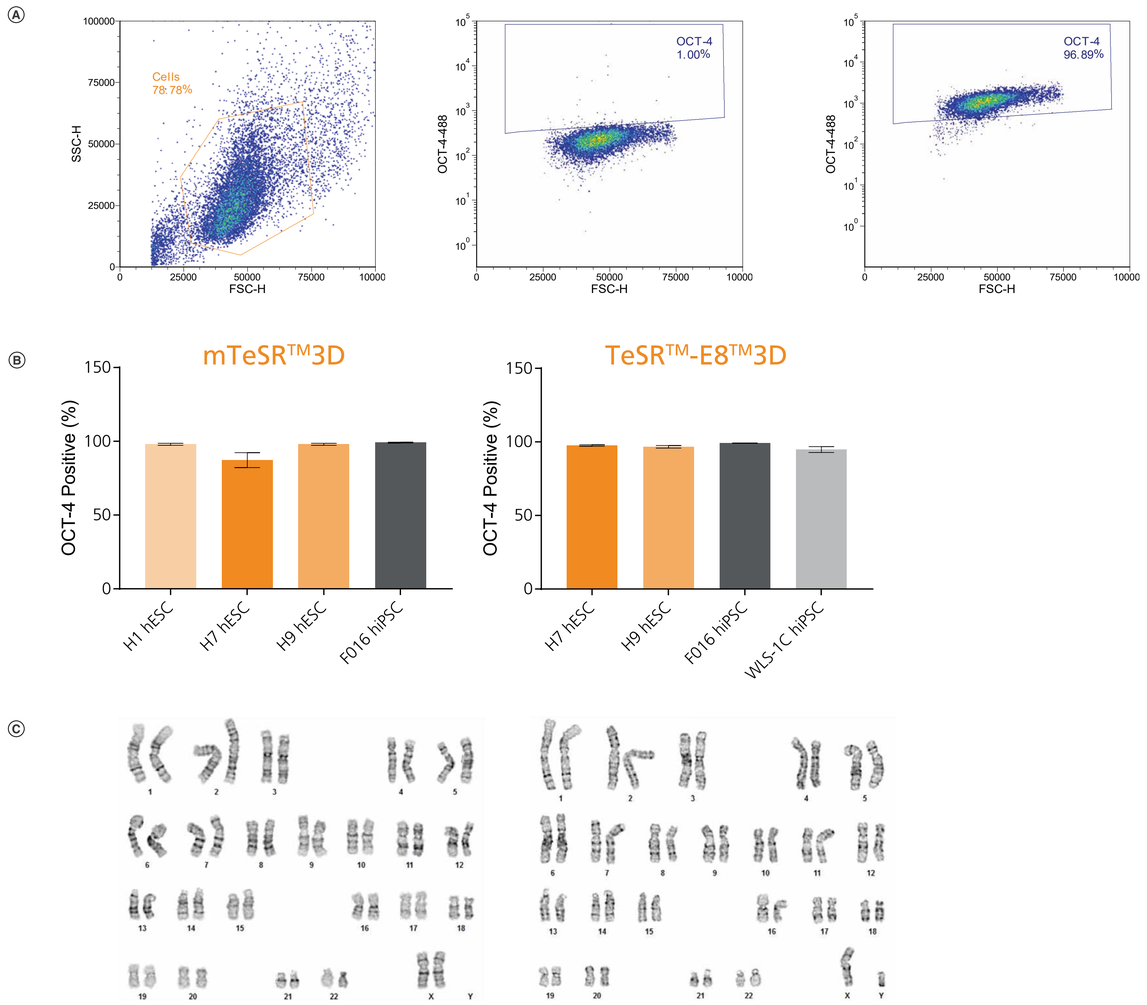


**FIGURE 3. hPSCs Cultured as Aggregates Using mTeSR™3D or TeSR™-E8™3D Express Markers of Undifferentiated hPSCs and Have Normal Karyotypes**

**(A)** A representative example of the flow cytometry plots for OCT-4 expression. H9 hESCs expanded in TeSR™-E8™3D are shown. **(B)** Summary OCT-4 data for multiple hPSC lines expanded in mTeSR™3D (left panel) and TeSR™-E8™3D (right panel). Flow cytometry was performed after 5 passages of expansion in suspension. Error bars are ± SD (n = 3). **(C)** G-banding karyotype results for H9 hESCs and WLS-1C human induced pluripotent stem cells (hiPSCs) expanded over 5 passages in TeSR™-E8™3D. hPSCs expanded in mTeSR™3D also maintain normal karyotype (data not shown).

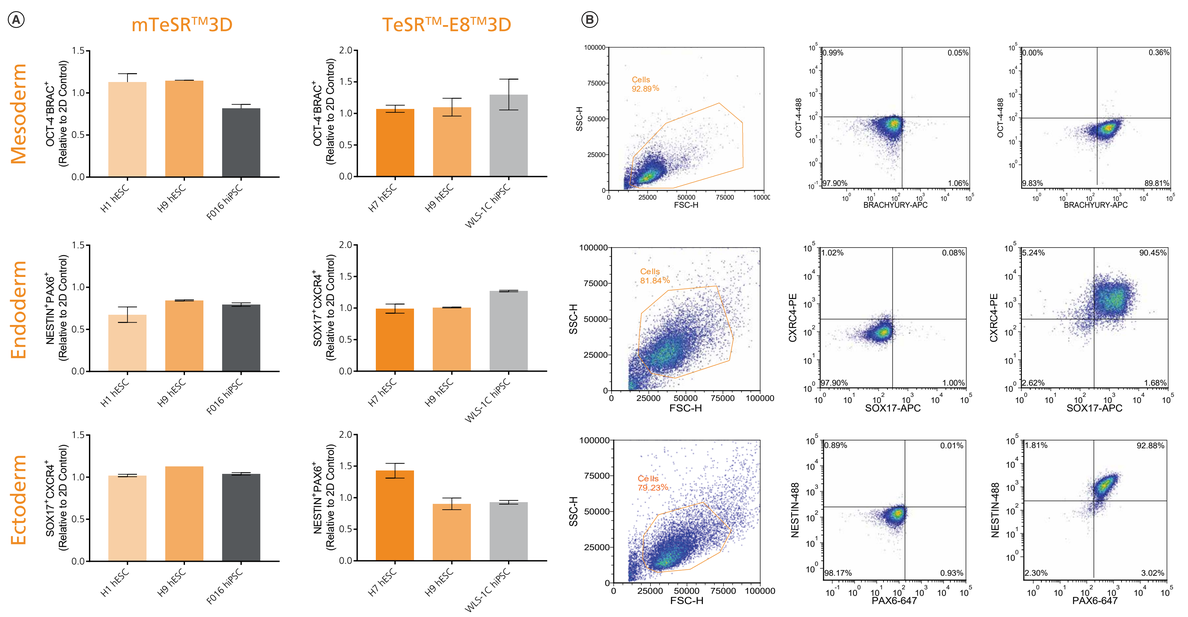


**FIGURE 4. hPSC Aggregates Expanded in mTeSR™3D or TeSR™-E8™ can be Differentiated into the Three Germ Layers**

After 5 passages the differentiation capacity of expanded hPSCs was assessed using the STEMdiff™ Trilineage Differentiation Kit. **(A)** Summary data for differentiation of multiple hESC and hiPSC lines to the three germ layers using STEMdiff™ Trilineage Differentiation Kit. Data are presented as marker expression relative to 2D control. Error bars are ± SD (n = 3). **(B)** Representative flow cytometry plots demonstrating successful differentiation to mesoderm, endoderm, and ectoderm after 5 passages of hPSCs as aggregates in suspension culture.

## Summary

- hPSCs expanded in mTeSR™3D or TeSR™-E8™3D highly express markers of undifferentiated hPSCs, maintain a normal karyotype, and maintain the capacity to differentiate to the three germ layers
- With our suspension culture workflow, scaling up hPSCs is efficient, convenient, cost-effective, and does not require passaging on weekends