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Development of a Robust Next Generation Feeder-Free Pluripotent Stem Cell Medium

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ABSTRACT

Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While a number of PSC feeder-free medium systems exist, there are many challenges encountered by stem cell scientists across the PSC workflow. Here we sought to improve the robustness and versatility of traditional PSC culture medium systems by utilizing several rounds of Definitive Screening and Custom Design of Experiments (DOEs) to identify and optimize critical medium components. Through assessment of over 100 different formulations, an optimum medium composition was identified which provides compatibility across the PSC workflow from somatic cell reprogramming, PSC expansion, downstream differentiation, as well as providing support in gene editing applications. This system additionally provides versatility, allowing for every-other-day or weekend-free feed schedules and compatibility with a broad range of passaging reagents and matrices. We demonstrate that this system maintains normal PSC properties, including (1) expression of canonical pluripotency markers, including Tra1-60 and OCT4, (2) maintains trilineage differentiation potential, and (3) exhibits normal karyotype over long-term passaging. Together this system provides a robust next-generation stem cell medium system for today's PSC workflow needs.

INTRODUCTION

The goals of this research were to (1) provide a robust feeder-free culture medium system for routine culture of human PSCs, (2) ensure that changes made to the medium system did not negatively impact maintenance of key PSC properties including pluripotency, karyotype, and trilineage differentiation potential, (3) confirm compatibility across the workflow from somatic cell reprogramming through to downstream differentiation, and (4) determine system applicability in gene editing workflows.

Six Sigma Design Excellence principles were used to guide StemFlex™ Medium* development. An iterative approach was taken to system optimization with an example of one such DOE optimization experiment being shown in Fig. 1.

Figure 1. Example DOE for Optimization of PSC Culture Medium Performance

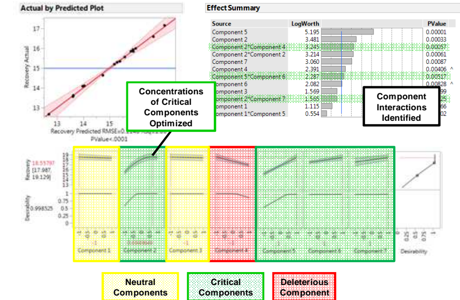
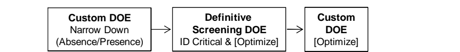


Figure 2. Example Iterative Approach Taken for Further System Optimization



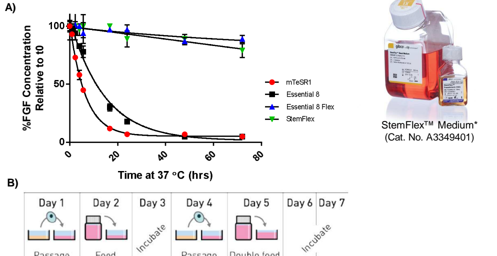
Through assessment of multiple experimental outputs and implementing a series of iterative DOEs such as that outlined in Fig. 2, the formulation was streamlined and optimized. Final formulations were subsequently tested through internal and external alpha testing to select the final formulation of StemFlex™ Medium.* This formulation offers versatility in selection of extracellular matrix and passaging reagent (Fig. 3).

Figure 3. Versatility of StemFlex™ Medium System

Flexible Matrix Selection	Flexibility in Level of Dissociation
• Geltrex™ Matrix (Cat. No. A14133)* • Vitronectin Recombinant Human Protein, Truncated (VTN-N; Cat. No. A14700)* • Recombinant Human Laminin-521 (rhLaminin-521; Cat. No. A29248)*	• *Clump Cell Passaging:* Versene Solution (Cat. No. 15040) or 500 µM EDTA* • *2-3 Cell Clusters:* StemPro™ Accutase™ (Cat. No. A11105)** • *Single Cell Passaging:* TrypLE™ Select Enzyme (Cat. No. 12563) +/- RevitaCell™ Supplement (Cat. No. A2644501)*

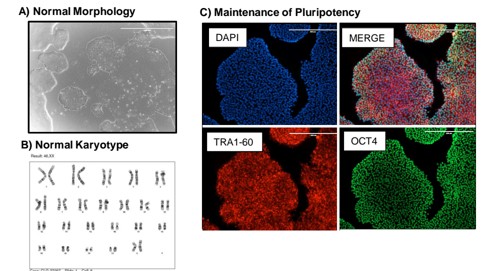
RESULTS

Figure 4. Maintains Pluripotency More Consistently While Enabling Weekend-Free Feed Schedule Option



Through stabilization of wild-type FGF2 in the formulation, FGF2 levels are able to persist in the medium formulation upon incubation at 37 °C, 5% CO_2 (Fig. 4A), allowing StemFlex™ Medium* to support alternative feed schedules. A typical weekend-free feed schedule is presented in Fig. 4B in which up to a 29% reduction in medium and decreased labor can be achieved. For additional feed schedules refer to www.thermofisher.com/stemflex.

Figure 5. StemFlex™* Supports Routine Maintenance of PSCs



H9 ESCs cultured in StemFlex™* Medium on Geltrex™* were propagated for >50 passages using Versene Solution* for passaging while implementing the weekend-free feeding schedule outlined in Fig. 4B. As presented in Fig. 5, PSCs were shown to maintain normal (A) morphology as assessed by phase contrast microscopy using the EVOS® FL Cell Imaging Station, (B) karyotype as assessed by G-band Karyotyping, and (C) pluripotency as assessed using the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Cat. No. A24881).

In development of StemFlex™ Medium* it was important to ensure that compatibility with existing reagents across the PSC workflow was maintained, from somatic cell reprogramming through to downstream differentiation. Therefore, assessment of feeder-free reprogramming using CytoTune™-iPS Sendai Reprogramming Kit (Cat. No. A16517)* for somatic cell reprogramming was conducted to explore the utility of StemFlex™ Medium.*

Figure 6. CytoTune™-iPS Sendai Reprogramming Workflow

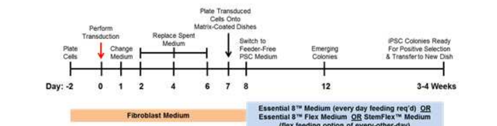
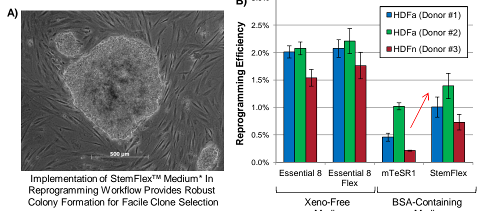


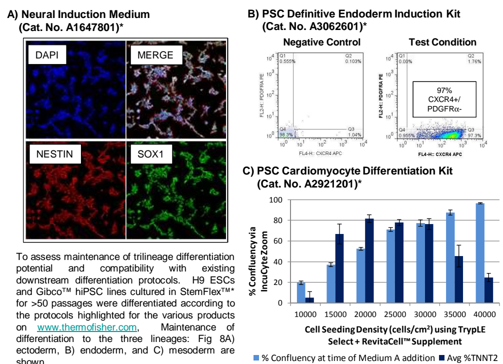
Figure 7. Compatibilty with CytoTune-iPS™ Sendai Reprogramming Kit*, Offering Alternative Every-Other-Day Feed Schedule



Implementation of StemFlex™ Medium* In Reprogramming Workflow Provides Robust Colony Formation for Facile Clone Selection

21 Days post-reprogramming with transfer to Geltrex™ Matrix* on Day 7 followed by implementation of feeder-free medium on Day 8 using the feed schedules outlined in Fig. 6, robust colony size is observed for the StemFlex™ Medium* system with low outgrowth of untransduced fibroblasts (Fig. 7A). On Day 21 alkaline phosphatase staining was completed and colony counting was performed using the Essen IncuCyte® ZOOM to determine the reprogramming efficiency (Fig. 7B). Regardless of the every-other-day feeding schedule for the Essential 8™ Flex Medium* system, this xeno-free medium system was shown to be comparable to the daily fed Essential 8™ Medium* system across the donors. For the BSA-containing media systems, the efficiency of somatic cell reprogramming was shown to be decreased relative to the xeno-free media systems. However, StemFlex™ Medium* was shown to have higher reprogramming efficiency relative to mTeSR™1.

Figure 8. Maintenance of Trilineage Differentiation Potential and Compatibility with Existing Downstream Differentiation Kits from Thermo Fisher Scientific



To assess maintenance of trilineage differentiation potential and compatibility with existing downstream differentiation protocols. H9 ESCs and Gibco™ hiPSC lines cultured in StemFlex™* for >50 passages were differentiated according to the protocols highlighted for the various products on www.thermofisher.com. Maintenance of differentiation to the three lineages: Fig 8A) ectoderm, B) endoderm, and C) mesoderm are shown.

While shown to be a robust feeder-free medium offering modern conveniences and with compatibility across the workflow, a key area of focus for StemFlex™ Medium* development is its role in helping cells through stressful workflows, including Cas9 gene editing. Supporting PSCs in recovery post-electroporation and following clonal expansion.

Figure 9. Neon™ Electroporation-Based Gene Editing Workflow for Assessment

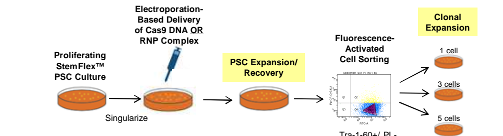
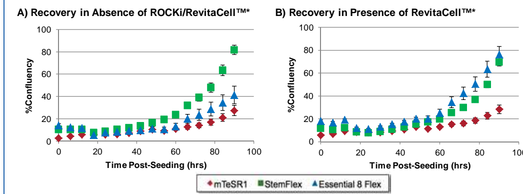
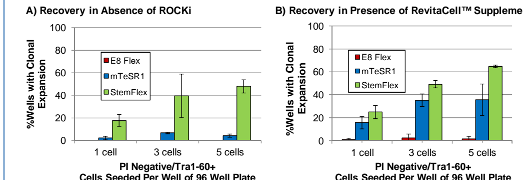


Figure 10. StemFlex™ Medium* Provides Improved Cell Survival of PSCs Following Electroporation-Based Delivery of CRISPR-Cas9 RNP Complex



Following electroporation of Cas9 RNP complex according to the protocol outlined at www.thermofisher.com/stemflexelectro, Gibco™ Episomal hiPS cells (Cat. No. A18945)* demonstrated optimal recovery in the StemFlex™ Medium* condition. At this cell seeding density (50,000 viable cells/cm²) no advantage was gained for the StemFlex™ Medium* system by incorporation of RevitaCell™ Supplement (Cat. No. A2644501)*, a ROCKi containing supplement, for the first 24 hours post-electroporation.

Figure 11. StemFlex™ Medium* Provides Up to 5-Fold Improvement in Clonal Expansion of PSCs Following FACS-Sorting



To determine the utility of the StemFlex™ Medium* system in supporting PSCs in another critical juncture in the editing workflow (Fig 9), Gibco™ Episomal hiPS cells (Cat. No. A18945)* were adapted into the various media systems on rhLaminin-521*, subsequently singularized using TrypLE™ Select*, underwent flow sorting for live (i.e., propidium iodide negative), pluripotent (i.e., Tra1-60) cells, and were seeded at 1, 3, or 5 viable cells/well of a 96-well plate in the A) absence of ROCK inhibitor or B) presence of RevitaCell Supplement (Cat. No. A2644501).* Refer to procedural guidelines provided at www.thermofisher.com/stemflexelectro. PSCs were subsequently fed following 3 days, followed by every 2-3 days thereafter. On Day 14, whole well imaging was performed using the IncuCyte® ZOOM. These data demonstrate the robustness of the StemFlex™ Medium* system in providing 18% clonal survival when seeding at 1 cell per well in the absence of ROCKi (Fig. 11A). Inclusion of RevitaCell™ Supplement* for the first 72 hours as shown in Fig. 11B, resulted in an increase in cell survival providing ~25% of the wells showing clonal expansion upon seeding of 1 viable cell/well.

CONCLUSIONS

The data presented here highlights the development of the StemFlex™ Medium* and its utility in the following: (1) routine culture of PSCs, providing modern convinces including weekend-free flexible feeding schedule option, (2) compatibility with existing reagents across the PSC workflow, and (3) provides optimal cell survival to support critical steps in the gene editing workflow. For additional information on StemFlex™ Medium* visit www.thermofisher.com/stemflex. For additional information on the applicability of the StemFlex™ Medium* system in gene editing workflows please refer to Dr. Lise Munsie's presentation at www.thermofisher.com/24hoursondemand.

For a free sample of StemFlex™ Medium*, please visit Thermo Fisher Scientific Booth #507.

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TRADEMARKS/LICENSING

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