Reprogramming of somatic cells to iPSCs using the CytoTune-iPS 2.0 kit with feeder-free media systems

Introduction

The ability to reprogram somatic cells to generate induced pluripotent stem cells (iPSCs) has created exciting opportunities for both basic research and future clinical applications. These iPSCs circumvent many of the ethical concerns associated with the use of human embryonic stem cells, while providing a continuous pool of cells that retain the basic genetic makeup of the somatic cells from which they were derived. Therefore, iPSCs are great tools for many research applications, including disease modeling, drug discovery, toxicological assessment, as well as assessment of regenerative therapy.

The Invitrogen™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit is our most efficient, non-integrating reprogramming product that utilizes Sendai virus to reprogram fibroblasts or any number of blood-derived cells to iPSCs. Initial transduction protocols vary depending on the somatic cell source. Following transduction, the cells are transferred to either (1) a layer of irradiated or mitomycin C–treated fibroblasts for feeder-dependent PSC expansion of clones or (2) an extracellular matrix for feeder-free PSC expansion of clones. The day following transfer onward to clonal selection, cells are fed with either feeder-dependent (e.g., based on Gibco™ KnockOut™ Serum Replacement – Multi-Species) or feeder-free (e.g., Gibco™ Essential 8™, Essential 8™ Flex, or StemFlex™) culture medium.

While feeder-dependent PSC expansion of clones has been shown to be more efficient, many researchers prefer to initiate their cultures with a feeder-free PSC system to avoid subsequent challenges in transitioning their clones from feeder-dependent to feeder-free systems. An additional benefit of using feeder-free culture systems from the beginning is that they are simpler systems, requiring fewer reagents and less time. In this application note, we discuss the use of Essential 8, Essential 8 Flex, and StemFlex feeder-free media in the CytoTune-iPS 2.0 Sendai Reprogramming Kit workflow, and outline the minor protocol updates that allow for every-other-day feeding with Essential 8 Flex or StemFlex Medium.

Suggested workflow

The recommended workflow updates for feeder-free systems occur at day 7 in the reprogramming protocols, whether reprogramming fibroblasts or CD34+ cells (Figures 1 and 2). Refer to the CytoTune-iPS 2.0 Sendai Reprogramming Kit User Guide (Pub. No. MAN0009378) for specific workflow instructions.

Fibroblast reprogramming workflow (Figure 1):
At day 7, transduced human dermal fibroblasts are plated in fibroblast medium onto vessels coated with one of three extracellular matrices (Gibco™ Vitronectin Recombinant Human Protein, Truncated (VTN-N), Geltrex™ matrix, or rhLaminin-521). At day 8, the medium is then switched to one of the three options for feeder-free growth medium (Essential 8, Essential 8 Flex, or StemFlex Medium). Subsequently, cells are fed either every day for Essential 8 Medium or every other day for Essential 8 Flex Medium or StemFlex Medium until colonies are ready for picking and transfer (3–4 weeks posttransduction).

CD34+ blood cell reprogramming workflow (Figure 2):
At day 3, transduced CD34+ blood cells are transferred to plates containing Gibco™ StemPro™-34 serum-free medium (SFM) and either vitronectin or Geltrex matrix. At day 7, cells begin transitioning to one of the three options for feeder-free growth medium (Essential 8, Essential 8 Flex, or StemFlex Medium) by removing half of the StemPro-34 SFM and replacing that volume with feeder-free PSC medium. On day 8, spent medium is removed completely and replaced with feeder-free PSC medium. Subsequently, cells are fed either every day for Essential 8 Medium or every other day for Essential 8 Flex Medium or StemFlex Medium until colonies emerge and are ready to be picked and replated (generally 3–4 weeks posttransduction).

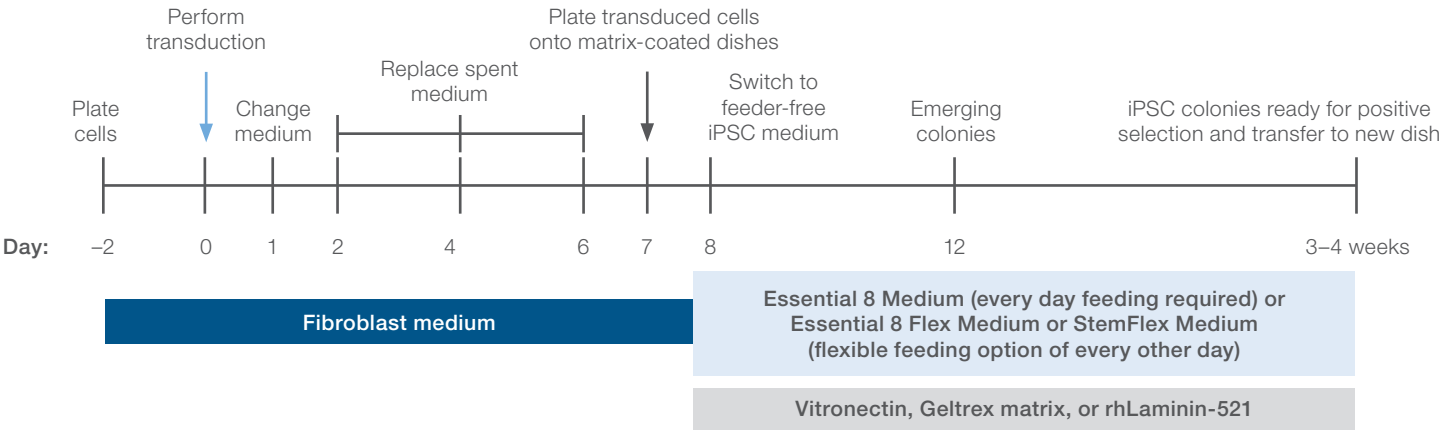
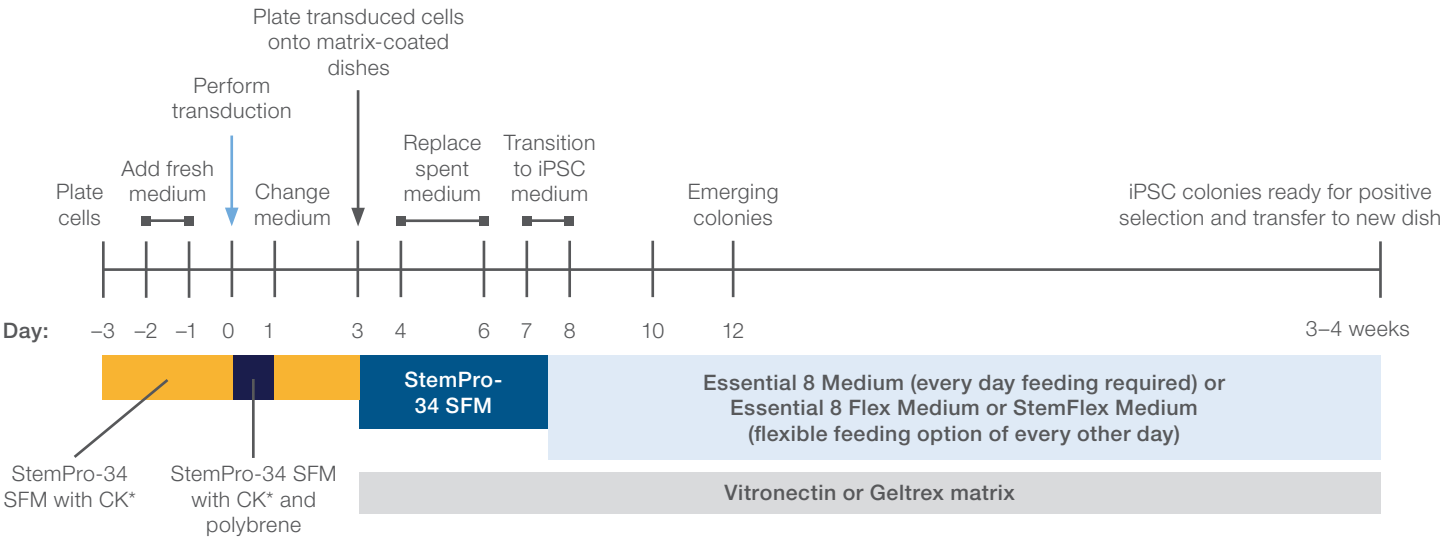


Figure 1. Schematic workflow for somatic cell reprogramming of human dermal fibroblasts to iPSCs using feeder-free media systems.



* CK = cytokines (SCF, IL-3, and GM-CSF).

Figure 2. Schematic workflow for somatic cell reprogramming of CD34+ blood cells to iPSCs using feeder-free media systems.

Results

Live-cell staining of newly reprogrammed colonies

Live-cell stains can be helpful in identifying PSC colonies for subsequent clonal selection, as shown in Figure 3. Pluripotent clones can subsequently be harvested via manual colony picking and transferred to a freshly coated tissue culture vessel.

Somatic cell reprogramming of human dermal fibroblasts using the CytoTune-iPS 2.0 kit

A variety of medium and matrix combinations are possible when completing somatic cell reprogramming under feeder-free conditions. Here we compare the relative reprogramming efficiencies of human dermal fibroblasts from adult (HDFa) or neonatal (HDFn) tissue in our feeder-free growth media to mTeSR™1 Medium from STEMCELL Technologies (Figure 4).

Regardless of the every-other-day feeding schedule for Essential 8 Flex Medium, this xeno-free PSC medium was comparable to the daily feeding schedule of Essential 8 Medium across the donors and on both the vitronectin (rhVTN-N) and Geltrex matrices. For the BSA-containing PSC media, the efficiency of somatic cell reprogramming was decreased relative to the xeno-free media. However, StemFlex Medium had higher reprogramming efficiency relative to mTeSR1 Medium. Again, comparable results were observed between the rhVTN-N and Geltrex matrices.

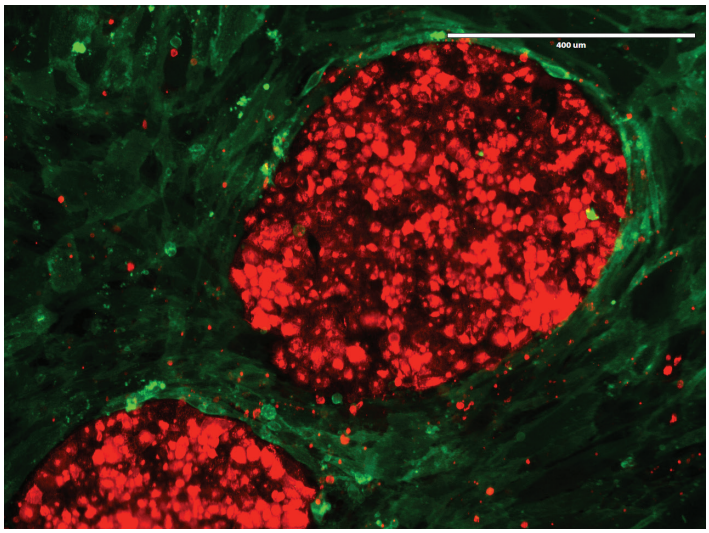


Figure 3. Live-cell staining of day 21 iPSCs. Gibco™ Human Dermal Fibroblasts, Neonatal (Cat. No. C0045C) were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. On day 7, cells were transferred to rhLaminin-521, and on day 8 onward, cells were fed every other day with StemFlex Medium. The image demonstrates staining of day 21 iPSC colonies with the Invitrogen™ TRA-1-60 Alexa Fluor™ 594 Conjugate Kit for Live-Cell Imaging (shown in red; Cat. No. A24882) and counterstaining of untransduced fibroblasts with the Invitrogen™ CD44 Alexa Fluor™ 488 Conjugate Kit for Live-Cell Imaging (shown in green; Cat. No. A25528).

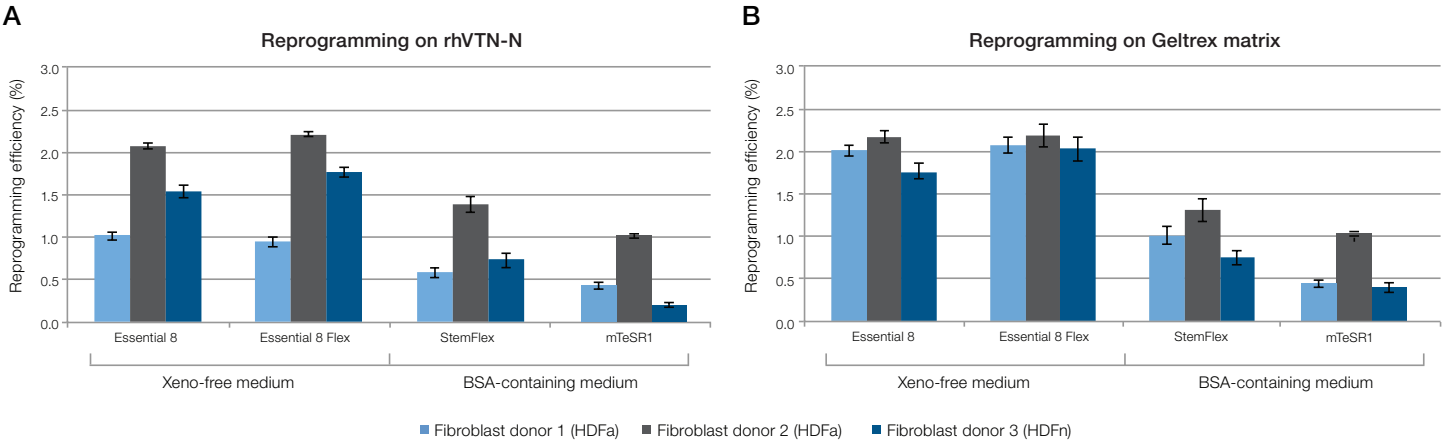


Figure 4. Reprogramming efficiency of human dermal fibroblasts using feeder-free medium conditions on Geltrex and rhVTN-N substrates. Fibroblasts from three donors, two adult and one neonatal, were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. On day 7, 50,000 viable cells were transferred per well of a 6-well plate onto either **(A)** rhVTN-N or **(B)** Geltrex matrices, and on day 8 onward, were either fed daily with Essential 8 Medium or mTeSR1 Medium, or every other day with Essential 8 Flex Medium or StemFlex Medium. On day 21, alkaline phosphatase staining was completed and colony counting was performed using the IncuCyte™ ZOOM System to determine the reprogramming efficiency (percentage reprogramming efficiency = colonies counted/50,000 viable cells seeded x 100; n = 3 per condition).

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Implementation of rhLaminin-521 to improve reprogramming efficiency

For some donors, survival of newly reprogrammed cells during the transition process, where cells are harvested and transferred to an extracellular matrix, can be improved by using rhLaminin-521 matrix. This increased cell survival during transition provides improved reprogramming efficiency (Figure 5).

Somatic cell reprogramming of CD34⁺ blood cells using the CytoTune-iPS 2.0 kit

Here we demonstrate data comparing the relative reprogramming efficiency of CD34⁺ blood cells in our feeder-free growth media relative to mTeSR1 Medium (Figure 6).

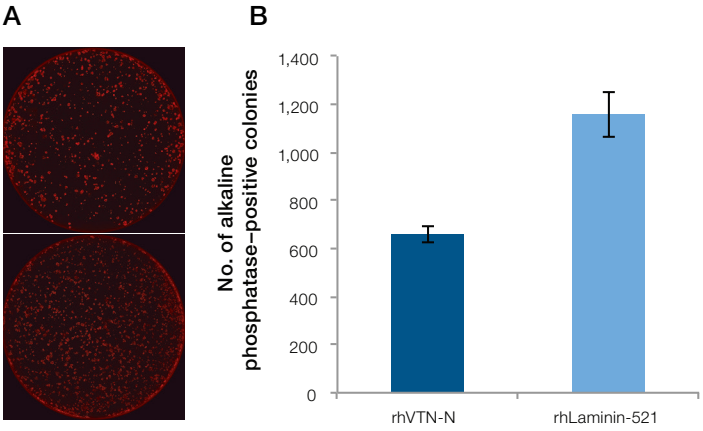

Figure 5. rhLaminin-521 provides optimum somatic cell reprogramming efficiency. Human Dermal Fibroblasts, Neonatal were expanded in Gibco™ Medium 106 (Cat. No. M106500) with Low Serum Growth Supplement (LSGS, Cat. No. S00310). Cells were then reprogrammed in KnockOut Serum Replacement–based medium using the CytoTune-iPS 2.0 Sendai Reprogramming Kit at a multiplicity of infection (MOI) of 5:5:3. On day 7 posttransduction, newly reprogrammed fibroblasts were passaged onto rhLaminin-521 (Cat. No. A29248) or rhVTN-N (Cat. No. A14700) matrices and were fed daily with Essential 8 Medium (Cat. No. A15170). On day 21 posttransduction, the number of alkaline phosphatase–positive colonies was determined per condition (n = 3 per condition). **(A)** Representative alkaline phosphatase imaging. **(B)** Bar graph depicting the number of alkaline phosphatase–positive colonies achieved for the rhVTN-N and rhLaminin-521 conditions.

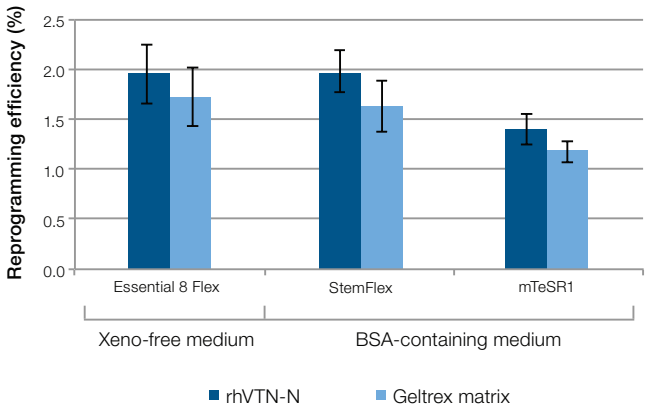

Figure 6. Reprogramming efficiency of CD34⁺ blood cells assessed under feeder-free conditions on Geltrex and rhVTN-N substrates. Gibco™ StemPro™ CD34⁺ cells (Cat. No. A14059) were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. On day 3, 50,000 viable cells were transferred per well of a 6-well plate onto either rhVTN-N or Geltrex matrices. On day 7 onward, cells were either fed daily with mTeSR1 Medium or every other day with Essential 8 Flex Medium or StemFlex Medium. On day 14, large iPSC colonies had emerged, alkaline phosphatase staining was completed, and colony counting was performed using the IncuCyte ZOOM System to determine the reprogramming efficiency (percentage reprogramming efficiency = colonies counted/50,000 viable cells seeded x 100; n = 3 per condition).

Conclusions

Together, these data demonstrate the relative efficiencies of feeder-free, pluripotent growth media systems in somatic cell reprogramming and show that all of the systems are compatible with the CytoTune-iPS 2.0 Sendai Reprogramming Kit. Additionally, these data demonstrate the flexibility of Essential 8 Flex Medium and StemFlex Medium in affording every-other-day feeding schedules to simplify the workflow.

For a detailed protocol outlining use of the CytoTune-iPS 2.0 Sendai Reprogramming Kit, refer to Pub. No. MAN0009378 for feeder-free reprogramming of human dermal fibroblasts (pp 16–20) and CD34⁺ blood cells (pp 39–44).

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