Adaptation of pluripotent stem cells to StemFlex Medium

Introduction

The use of pluripotent stem cells (PSCs) in research has expanded vastly with the advent of induced pluripotent stem cells (iPSCs). Methods for maintaining PSCs in culture have also developed from feeder-dependent to feeder-free systems. The benefits of this evolution of culture systems are vast, including a simplified workflow for routine culture of PSCs, as well as simplified downstream protocols for gene editing and differentiation. One such feeder-free system includes Gibco™ StemFlex™ Medium, which provides a flexible selection of passaging reagent, extracellular matrix, as well as feeding schedule. In addition, StemFlex Medium has been shown to support PSCs through stressful events, including single-cell passaging, recovery from genome editing, and subsequent clonal expansion following flow sorting. Researchers are sometimes apprehensive about changing culture systems due to the sensitive nature of PSCs. In this application note, we discuss the relative ease of adaptation of pluripotent stem cells into StemFlex Medium from a feeder-dependent system and another feeder-free system (mTeSR™1 medium).

Suggested workflow

The recommended workflow differs only in the initial passaging method, which is dependent upon if transitioning from a different feeder-free culture system, such as mTeSR1 medium on a Matrigel™ or Gibco™ Geltrex™ matrix, or a feeder-dependent culture system. For detailed instructions on transitioning to StemFlex Medium, refer to the instructions in the StemFlex Medium Kit User Guide (Pub. No. MAN0016431, pp 5 and 6), which follows the outline shown in Figure 1.

Technical tips

- Allow at least two passages in StemFlex Medium for full adaptation
- For frozen vials, thaw into original medium and substrate
  - Alternatively, cryopreserved PSC stocks that easily recover from cryopreservation can be thawed directly into StemFlex Medium; however, some cell lines may benefit from one passage in the original culture system prior to transition

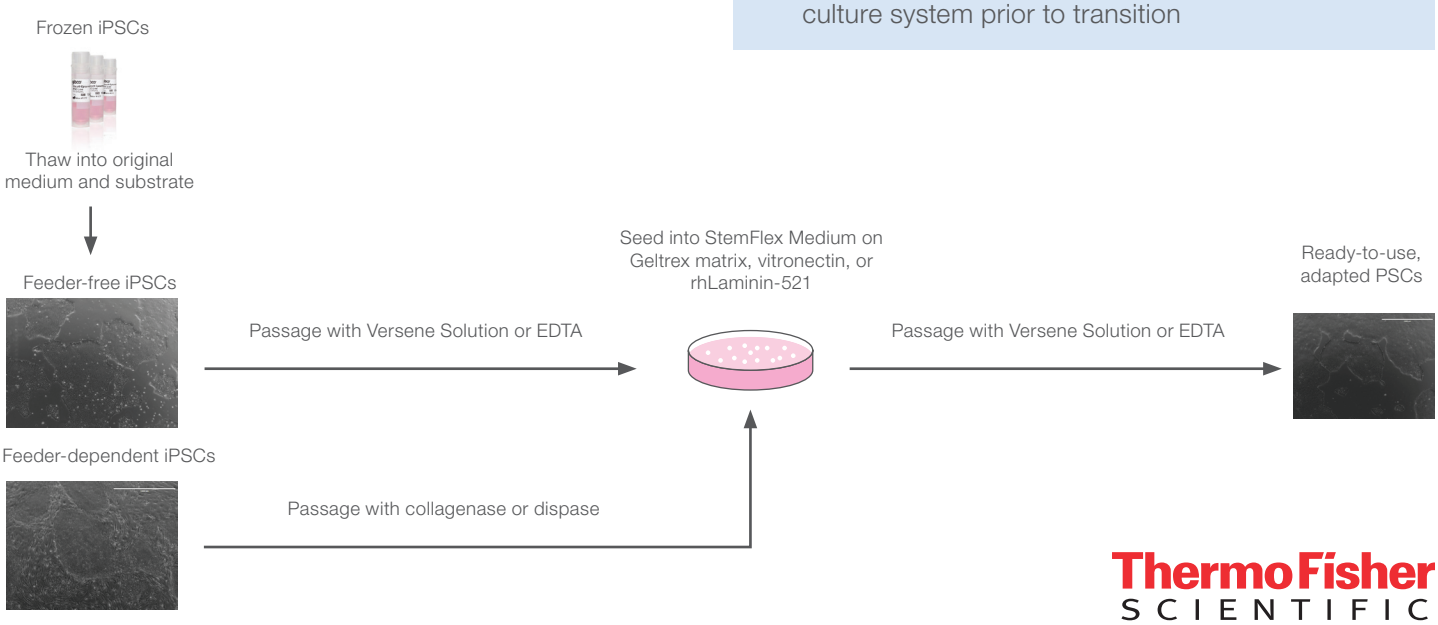

Figure 1. Suggested workflow for adaptation into StemFlex Medium.

Briefly, for feeder-free adaptation, we recommend culturing in the current system to a passaging confluency of 60–85%, then passaging into StemFlex Medium using the "clump cell passaging" protocol onto Geltrex matrix. This passaging method makes use of Gibco™ Versene™ Solution or 500 µM EDTA as the passaging reagent, resulting in small clumps of cells. For feeder-dependent stem cell cultures, the initial step is to grow the feeder-dependent cultures to a passaging confluency of 60–85% with round colonies that are not overcrowded. Then, use collagenase or dispase to help dislodge the colonies. It is recommended to feed transitioned feeder-dependent cultures daily on this first passage in StemFlex Medium. Difficult-to-transition lines may benefit from plating on Gibco™ rhLaminin-521 as an alternative matrix. After this initial passage into StemFlex Medium, one additional passage using the clump cell passaging protocol is recommended to complete the adaptation process.

Results

Adaptation from mTeSR1 medium and Matrigel matrix

In this example, iPSC lines from mTeSR1 medium on Matrigel matrix were adapted to StemFlex Medium over 2 passages. The data presented demonstrates the effectiveness of the simple adaptation protocol. We allowed established cultures grown in mTeSR1 medium on Matrigel matrix to recover from cryopreservation for one passage in this system and subsequently performed clump cell passaging using Versene Solution. Cells were seeded in StemFlex Medium on Geltrex matrix or vitronectin (rhVTN-N) substrate. Figure 2 shows the growth of the iPSCs from clump cell passaging in the second passage. Cells show robust growth as expected in StemFlex Medium. Furthermore, cells maintain pluripotency as shown by high expression levels of the intracellular marker OCT4.

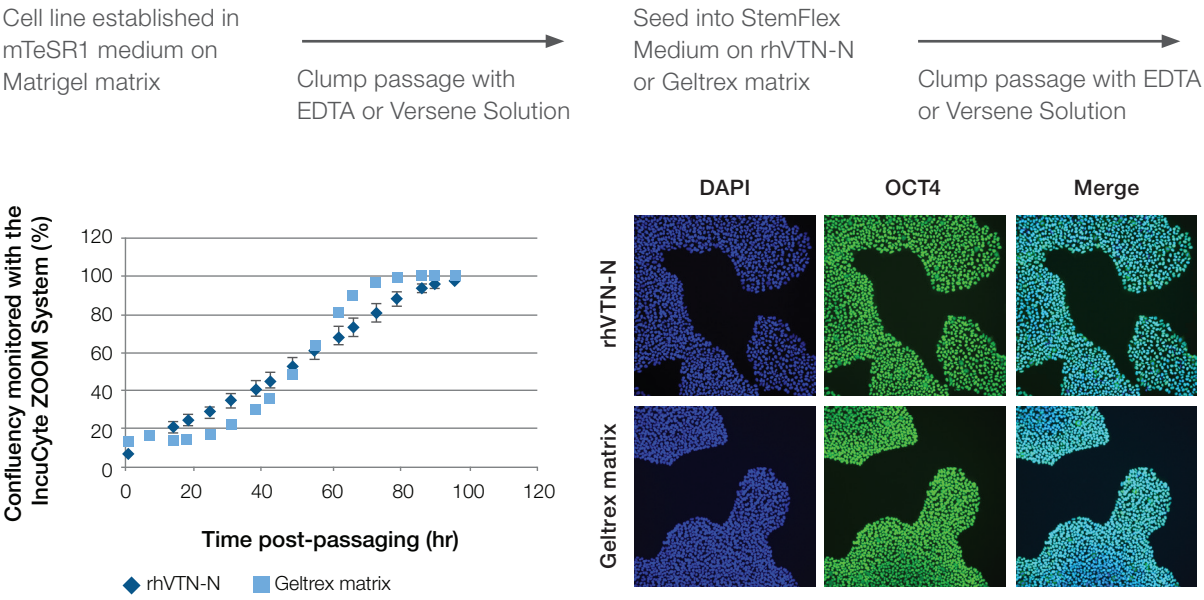

Figure 2. Adaptation of PSCs from mTeSR1 medium on a Matrigel matrix to StemFlex Medium on a Geltrex matrix or rhVTN-N substrate. Existing PSC lines in other feeder-free systems can be easily transitioned to StemFlex Medium following a minimum of two passages for full adaptation.

Adaptation from feeder-dependent systems

When adapting cells from feeder-dependent cultures, the initial transfer into StemFlex Medium involves a few more steps, but it is still straightforward. After dissociation of the colonies via collagenase or dispase, the residual dissociation solution is removed through a series of media exchanges. The second passage is performed using the clump cell passaging protocol with Versene Solution to complete the adaptation process. Figure 3 shows the growth of the iPSCs from clump cell passaging in the second passage. Cells show robust growth as expected in StemFlex Medium on both matrices. Furthermore, cells maintain pluripotency as shown by high expression levels of the intracellular marker OCT4.

Conclusions

Here we present workflows for transition of existing PSC lines to feeder-free StemFlex Medium, whether the cells were grown using a feeder-dependent or feeder-free system. This transition will allow you to take advantage of all of the benefits that StemFlex Medium offers, including a flexible selection of feeding schedule, passaging reagent, and matrix, as well as optimal support in applications such as gene editing and somatic cell reprogramming.

Refer to the StemFlex Medium Kit User Guide (Pub. No. MAN0016431) for detailed passaging instructions for feeder-free and feeder-dependent culture systems.

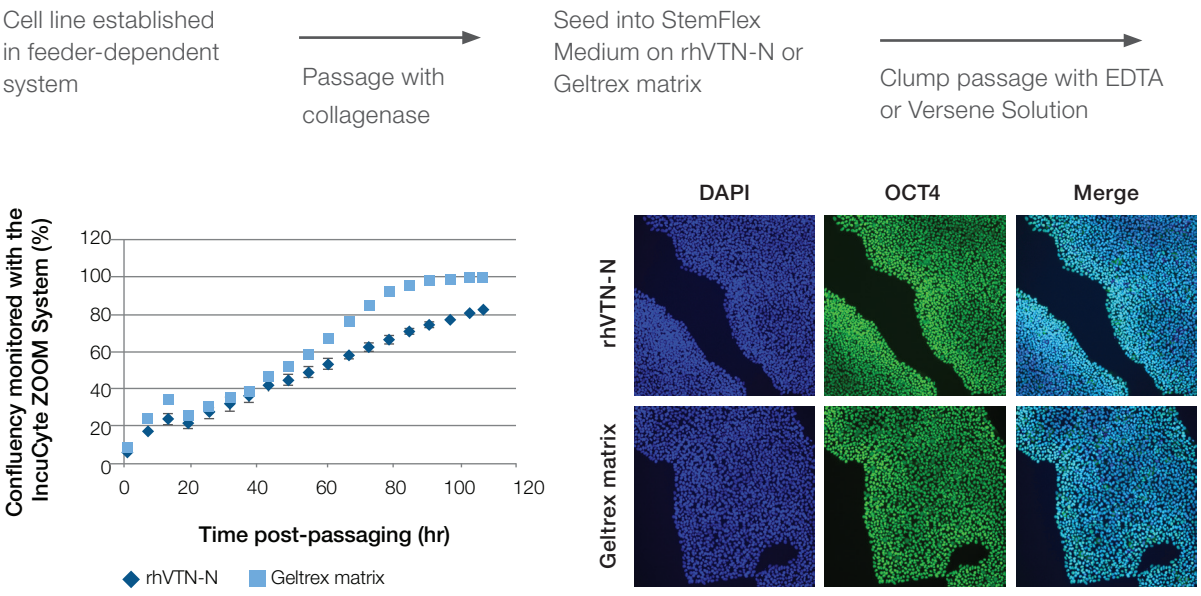

Figure 3. Adaptation of PSCs from a feeder-dependent system to StemFlex Medium on a Geltrex matrix or rhVTN-N substrate. Existing PSC lines in feeder-dependent systems can be easily transitioned to StemFlex Medium following a minimum of two passages for full adaptation.

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