CYTOTUNE®-IPS 2.0 SENDAI REPROGRAMMING KIT FOR HIGH EFFICIENCY REPROGRAMMING OF FIBROBLASTS AND BLOOD-DERIVED CELLS

life technologies
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ABSTRACT

Induced pluripotent stem cells (iPSCs) are valuable research tools for many different applications, including drug discovery, disease modeling, and regenerative medicine. Given the importance and potential of iPSC, it is vital that researchers have access to reprogramming methods that can safely and efficiently generate iPSCs from a wide variety of patient-derived cell types. The CytoTune®-iPS Sendai Reprogramming kit has emerged as an efficient method for generation of footprint-free iPSC from a wide variety of cell types, including blood cells. However, issues that were observed in some donor cells included toxicity, low reprogramming efficiency, and persistence of the virus beyond passage 10.
To address the above challenges, novel configurations of the virus were identified and used for the development of the CytoTune®-iPS 2.0 Sendai Reprogramming Kit. The resulting new configuration consists of the Oct4, Sox2, and Klf4 genes in a single polycistronic vector in a new backbone, with cMyc remaining on a separate vector, and an additional separate Klf4 vector used to enhance efficiency. The polycistronic vector was designed for greater efficiency of reprogramming in a new backbone that allows for lower cytotoxicity and faster viral clearance. The additional Klf4 vector further aids in maximum efficiency with minimal viral load. Consistent with this, CytoTune®-iPS 2.0 was found to have 2-5 fold greater efficiency of reprogramming in fibroblast and blood reprogramming. In addition, the level of toxicity in primary cells was significantly reduced with CytoTune®-iPS 2.0, relative to the previous version. Clones derived with CytoTune®-iPS 2.0 had positive pluripotent marker expression, tri-lineage differentiation potential, and normal karyotype. In addition, the viral backbone was diluted from the majority of clones as early as passage 3. Thus, the CytoTune®-iPS 2.0 Sendai Reprogramming kit provides a robust system for higher efficiency, low toxicity, and faster viral clearance from resulting iPSC clones.

INTRODUCTION

Since the discovery of iPSC in 2006 by Shinya Yamanaka(1), many different methods have emerged for generating iPSCs. Aspects of a useful reprogramming method include ease-of-use, high efficiency of reprogramming, ability to reprogram different cell types, and footprint–free iPSCs. CytoTune®-iPS is a modified, non-transmissable form of Sendai virus, which is an RNA-based mouse parainfluenza virus (2,3). This virus is used to deliver the transgenes necessary to generate iPSCs, and is advantageous because it can infect a wide range of different cells types, and never integrates into the host cell genome, resulting in footprint-free iPSCs (4).
The initial version of CytoTune®-iPS is an efficient method for generation of footprint-free iPSC from a wide variety of cell types with a single application. However, issues were observed in some cell types, including toxicity, low reprogramming efficiency, and persistence of the virus beyond passage 10. To address these issues, a new configuration of CytoTune®-iPS was developed, using a polycistronic vector containing Klf4, Oct4, and Sox2 on one vector, with a new backbone to allow for greater efficiency of reprogramming, lower cytotoxicity, and faster viral clearance.

MATERIALS AND METHODS

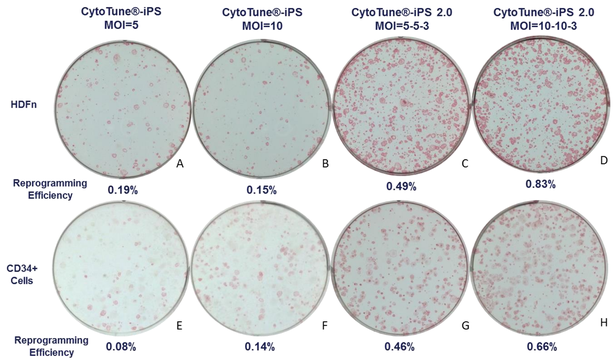
Vector® Red Alkaline Phosphatase kit was obtained from Vector Laboratories. Incucyte® FLR Instrument was obtained from Essen Biosciences. BJ HDFn were obtained from ATCC. All other reagents were obtained from Life Technologies.

BJ HDFn fibroblasts were cultured in fibroblast medium (DMEM containing 10% FBS and 1% NEAA). On the day of transduction, CytoTune® viral vectors were added at the indicated MOI (for CytoTune®-iPS, an MOI of 5 indicates that MOI for each vector; Oct4, Sox2, Klf4, cMyc; for CytoTune®-iPS 2.0, an MOI of 5-5-3 indicates that respective MOI for the KOS, cMyc, and Klf4 vectors). Virus was removed the following day. Cells were harvested seven days later and plated onto either mouse embryonic fibroblasts (MEF) for feeder-dependent conditions; or Geltrex® matrix or vitronectin for feeder-independent conditions. The next day medium was changed to human iPSC medium (DMEM/F-12 with 20% KSR, 1% NEAA, 55 mM 2-mercaptoethanol, 4 ng/mL bFGF) for feeder-dependent conditions, or Essential 8™ Medium for feeder-free conditions. Cells were cultured until Day 21, then picked for expansion, or stained with Vector® Red Alkaline Phosphatase stain for efficiency determination.

CD34+ cells were cultured in complete StemPro®34 medium containing cytokines (SCF 100ng/mL, IL-3 50 ng/mL, GM-CSF 25 ng/mL). On the day of transduction, CytoTune® viral vectors were added at the indicated MOI. Virus was removed the following day. On day 3 after transduction, cells were plated onto either mouse embryonic fibroblasts (feeder-dependent conditions), or Geltrex® matrix, or vitronectin (feeder-free conditions). Four days later, StemPro®34 medium was transitioned to human iPSC medium for feeder-dependent conditions, or Essential 8™ Medium for feeder-free conditions. Cells were cultured until Day 19, then picked for expansion, or stained with Vector® Red Alkaline Phosphatase stain for efficiency determination.

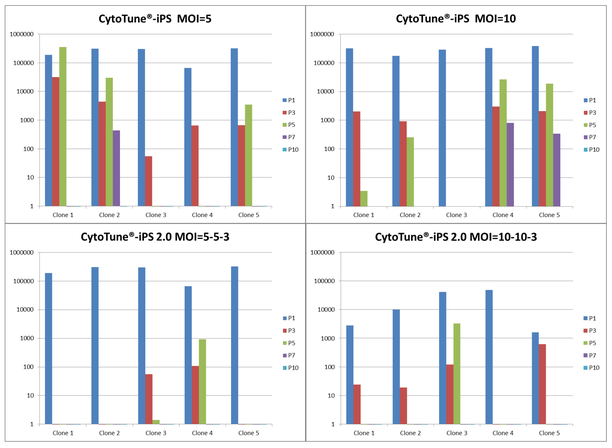
RESULTS

Figure 1. Reprogramming with the CytoTune®-iPS 2.0 kit results in higher efficiencies than the CytoTune®-iPS kit



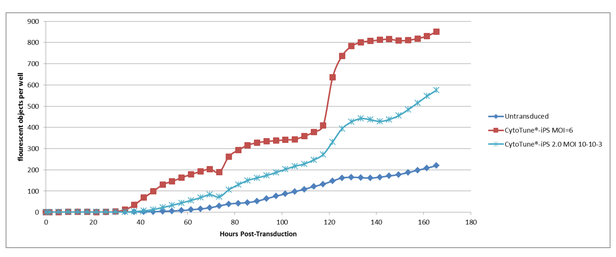
HDFn were transduced according to standard protocol with either CytoTune®-iPS MOI 5 (A), CytoTune®-iPS MOI 10 (B), CytoTune®-iPS 2.0 MOI 5-5-3 (C), or CytoTune®-iPS 2.0 MOI 10-10-3 (D). CD34+ cells were transduced according to standard protocol with either CytoTune®-iPS MOI 5 (E), CytoTune®-iPS MOI 10 (F), CytoTune®-iPS 2.0 MOI 5-5-3 (G), or CytoTune®-iPS 2.0 MOI 10-10-3 (H). Standard feeder-dependent reprogramming protocols were followed, and cells were stained for Alkaline Phosphatase activity at 19 (CD34+) or 21 (BJ HDFn) days following transduction. Percent efficiency is calculated as the percent of AP positive colonies relative to the number of cells plated down on Day 7 after transduction (50,000).

Figure 2. CytoTune®-iPS 2.0 vectors clear from iPSC clones faster than CytoTune®-iPS vectors



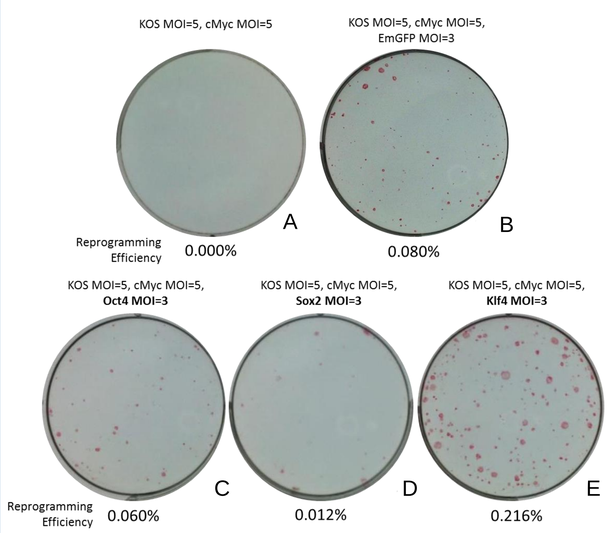
iPSC clones were generated from BJ HDFn using the indicated MOI and version of CytoTune®-iPS or CytoTune®-iPS 2.0. iPSC clones were cultured on feeder-dependent conditions for 10 passages, and RNA was isolated and analyzed at every other passage. qPCR was used to detect the presence of the Sendai viral vector backbone, and values were normalized against expression levels in untransduced BJ HDFn.

Figure 3. CytoTune®-iPS 2.0 vectors are less cytotoxic than CytoTune®-iPS vectors



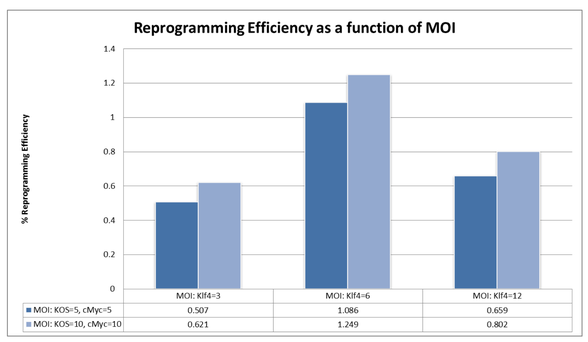
BJ HDFn were transduced with the indicated MOI of virus. To measure cytotoxicity, SYTOX® Green dye was added to the fibroblast medium at a concentration of 150 nM. Cells were imaged every fours hours using the Incucyte® FLR instrument. Cells were cultured for a total of 166 hours, and fibroblast medium containing SYTOX® Green was changed every other day. The number of green fluorescent objects per well (dead cells) were determined using the Incucyte® FLR analysis software.

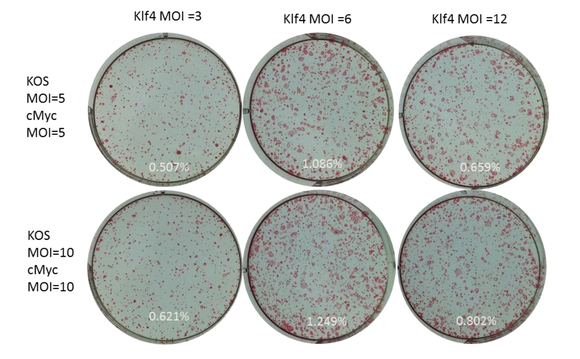
Figure 4. An additional Klf4 vector significantly increases CytoTune®-iPS 2.0 mediated reprogramming efficiencies



HDFn were transduced according to standard protocol with either CytoTune®-iPS 2.0 KOS MOI 5, CytoTune®-iPS 2.0 cMyc MOI 5 (A); CytoTune®-iPS 2.0 KOS MOI 5, CytoTune®-iPS 2.0 cMyc MOI 5, CytoTune® EmGFP MOI 3 (B); CytoTune®-iPS 2.0 KOS MOI 5, CytoTune®-iPS 2.0 cMyc MOI 5, CytoTune®-iPS Oct4 MOI 3 (C); CytoTune®-iPS 2.0 KOS MOI 5, CytoTune®-iPS 2.0 cMyc MOI 5, CytoTune®-iPS Sox2 MOI 3 (D); or CytoTune®-iPS 2.0 KOS MOI 5, CytoTune®-iPS 2.0 cMyc MOI 5, CytoTune®-iPS Klf4 MOI 3 (E). Standard feeder-dependent reprogramming protocols were followed, and cells were stained for Alkaline Phosphatase activity at 21 days following transduction. Percent efficiency is calculated as the percent of AP positive colonies relative to the number of cells plated down on Day 7 after transduction (50,000).

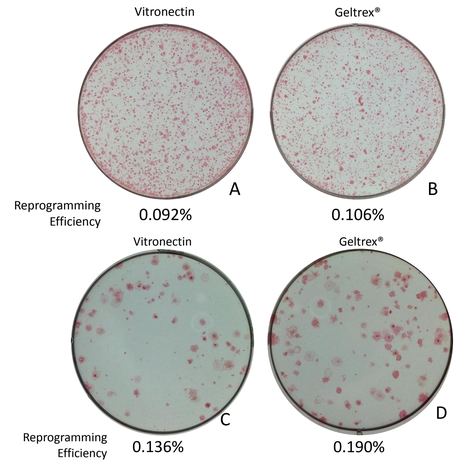
Figure 5. An increase in the MOI of the Klf4 vector increases reprogramming efficiencies





BJ HDFn were transduced with the indicated MOI of CytoTune®-iPS 2.0 vectors. Standard feeder-dependent reprogramming protocols were followed. After 21 days, cells were stained for Alkaline phosphatase activity. AP positive colonies were counted, and percent efficiency is expressed as the percent of AP positive colonies relative to the number of cells plated down on Day 7 after transduction. Graphical representation of data (A), and images of wells containing AP-positive colonies (B) are shown.

Figure 6. HDFn and CD34+ cells can be reprogrammed with the CytoTune®-iPS 2.0 kit on feeder-free conditions



HDFn were transduced according to standard protocol with CytoTune®-iPS 2.0, MOI 5-5-3. Standard feeder-free reprogramming protocols were followed, and cells were plated down onto either vitronectin (A), or Geltrex® matrix (B) at a density of 100,000 cells per well of a 6-well plate. CD34+ cells were transduced according to standard protocol with CytoTune®-iPS 2.0, MOI 5-5-3. Standard feeder-free reprogramming protocols were followed, and cells were plated down onto either vitronectin (C), or Geltrex® matrix (D) at a density of 50,000 cells per well of a 6-well plate. Cells were stained for Alkaline Phosphatase activity at 19 (CD34+) or 21 days (HDFn) following transduction. Percent efficiency is calculated as the percent of AP positive colonies relative to the number of cells plated down on Day 3 (CD34+) or Day 7 (HDFn) after transduction.

CONCLUSIONS

- The CytoTune®-iPS 2.0 kit yields a greater efficiency of reprogramming than the CytoTune®-iPS kit by two-fold in fibroblasts, and five-fold in blood-derived (e.g. CD34+) cells .
- iPSCs generated using CytoTune®-iPS 2.0 vectors display viral clearance faster that clones generated using CytoTune®-iPS vectors.
- CytoTune®-iPS 2.0 vectors show greater than two-fold lower toxicity in BJ HDFn than CytoTune®-iPS vectors.
- CytoTune®-iPS 2.0 vectors KOS and cMyc alone are not sufficient for reprogramming; a third vector is required. Addition of an “empty vector” such as EmGFP results in successful reprogramming.
- An additional Klf4 vector results in the greatest increase in reprogramming efficiency, indicating an enhancing effect resulting from high Klf4 expression.
- An increase in the MOI of the Klf4 vector can cause a concomitant increase in reprogramming efficiency.
- The CytoTune®-iPS 2.0 kit can be used to reprogram HDFn and blood-derived cells (CD34+) on feeder-free conditions (Essential 8™ Medium).

REFERENCES

1. Takahashi, K., and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.
2. Fusaki, N., Ban, H., Nishiyama, A., Saeki, K., and Hasegawa, M. (2009) Efficient induction of transgene free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci *85*, 348-362.
3. Seki, T., Yuasa, S., Oda, M., Egashira, T., Yae, K., Kusumoto, D., Nakata, H., Tohyama, S., Hashimoto, H., Kodaira, M., Okada, Y., Seimiya, H., Fusaki, N., Hasegawa, M., and Fukuda, K. (2010) Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell *7*, 11-14
4. Macarthur, C.C., Fontes, A., Ravinder, N., Kuninger, D., Kaur, J., Bailey, M., Taliana, A., Vemuri, M.C., Lieu, P.T. (2012) Generation of human-induced pluripotent stem cells by a nonintegrating RNA Sendai virus vector in feeder-free or xeno-free conditions. Stem Cells Int. 564-612.

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