Enabling Successful Reprogramming of Peripheral Blood Mononuclear cells to Induced Pluripotent Stem Cells with the CytoTune® –iPS 2.0 Sendai Reprogramming Kit

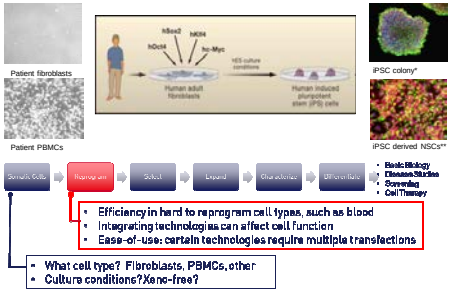
Jennifer Crean, Connie S. Lebakken, Laurie J. Reichling, Chad MacArthur, Uma Lakshmipathy and Bonnie Hammer
Life Technologies, 501 Charmany Drive, Madison, WI USA, 53719 and 1500 Faraday Avenue, Carlsbad, CA 92008

life technologies
A Thermo Fisher Scientific Brand

ABSTRACT

Human induced pluripotent stem cells (iPSCs) derived from adult somatic cells hold great promise for disease modeling and may provide new cell sources for clinical therapies. Currently the majority of iPSCs are derived from donor fibroblast cells which are obtained by a skin biopsy and expanded prior to reprogramming. Utilizing a donor blood sample as a source for reprogramming is attractive as blood can be easily obtained from most patients, and large banks of frozen peripheral blood mononuclear cell (PBMC) samples from patients are available to researchers. Reprogramming from peripheral blood sources has been challenging due to cytotoxicity and low reprogramming efficiencies. Our goal was to enable the successful and consistent generation of iPSCs from frozen PBMCs utilizing our CytoTune® iPS Sendai reprogramming tools. We optimized conditions throughout the workflow including culture conditions, timing of transduction and transduction methodology which led to increased reprogramming efficiencies from donor PBMCs. When these optimizations were used in conjunction with the CytoTune® –iPS 2.0 Sendai Reprogramming Kit, we observed reprogramming efficiencies of greater than 2% on MEF feeder layers in KSR-based iPSC medium and greater than 1% on Geltrex™ or Vitronectin substrates in Essential 8™ Medium. Together these improvements support the efficient and reproducible reprogramming of PBMCs and should provide a reliable tool to generate iPSCs from existing and future PBMC sources.

Working to Improve the iPSC workflow



MATERIALS AND METHODS

PBMCs were obtained from AllCells and Sanguine BioSciences
StemPro® CD34+ cord blood cells, StemPro ® 34 basal medium , Knockout DMEM-F12, Essential 8™ Medium, KSR, bFGF, SCF, GM-CSF, IL-3, FLT-3. IL-6, TPO, MEM Non-essential Amino Acids, 2-mercaptoethanol, GlutaMax™, ICC kits, Gibco® PSC Neural Induction Medium, Vitronectin, Geltrex®, CytoTune® EmGFP, CytoTune ® iPSC reprogramming kits were obtained from Life Technologies
Vector Red was obtained from Vector Laboratories.

For reprogramming efficiency calculations, cells were fixed between days 17 and 21 of reprogramming with 4% paraformaldehyde in PBS for 15 min. They were then rinsed with PBS and stained with Vector Red alkaline phosphatase detection reagent following manufacturers protocols and imaged with a Fuji FLA-5100 imager. Images were analyzed utilizing ImageJ software. Efficiency calculations were determined by dividing the number of alkaline phosphatase positive colonies by the number of live cells plated onto the substrate following transductions.

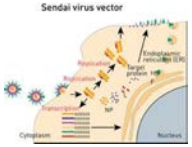
*iPSC colony shown was generated using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit under feeder-free conditions using Essential 8™ Medium and vitronectin. These cells were stained for pluripotency markers OCT4 and SSEA4 using the PSC (OCT4, SSEA4) ICC Kit (A25526). Images were acquired on an EVOS® FL Imaging System. ** Neural stem cells (NSCs) were generated from a patient-derived iPSC line using Gibco® PSC Neural Induction Medium (A1647801). Cells were cultured on a Geltrex® coated chamber slide and then stained for NSC markers using the Human Neural Stem Cell ICC Kit (Cat. no. A24354).

Figure 1. CytoTune®-iPS 2.0 Sendai Reprogramming Kit addresses challenges in reprogramming

Non-Integrating technology based on an RNA virus that replicates exclusively in the cytoplasm and cannot produce infectious particles.

Efficiency in hard to reprogram cell types, or with limited samples. The Sendai virus has broad tropism and can efficiently transduce a wide range of cell types.

A single transduction is required and reprogramming can be done under feeder-free conditions.



iPSC colonies derived from fibroblasts, CD34+ cord blood or Peripheral Blood Mononuclear Cells (PBMCs)

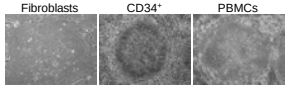
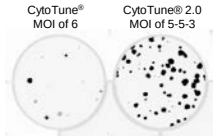


Figure 2. CytoTune®-iPS 2.0 Sendai Reprogramming Kit reprograms PBMCs with higher efficiency than CytoTune®

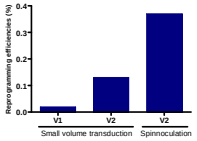


Frozen Peripheral Blood Mononuclear Cells (PBMCs) were thawed and cultured in StemPro® 34 medium containing 100 ng/mL SCF, 100 ng/mL FLT3, 20 ng/mL TPO and 10 ng/mL IL-6 for 4 days prior to transduction of 250,000 cells with 4 µg/mL polybrene in a total volume of 300 µL with CytoTune® reprogramming reagents (MOI of 6 for each viral vector; hOct3/4, hSox2, hKlf4 and hc-Myc) or CytoTune® 2.0 reprogramming reagents (MOI of 5 for KOS and hc-Myc vectors and MOI of 3 for hKlf4 vector). After 24 hours, the medium was exchanged and the cells were cultured an additional two days in the above medium. Three days following transduction, the cells were plated onto a feeder layer of irradiated mouse embryonic fibroblasts (MEFs) in StemPro® 34 medium in the absence of cytokines. On day 7 following transduction, the cells were transitioned to DMEM-F12 based iPSC medium. Emerging iPSC colonies were maintained on the MEFs and medium was exchanged daily. Results shown are from the same lot of PBMCs handled as a single culture prior to transduction.

Figure 3. Reprogramming efficiencies can be greatly affected by cytokine supplements

A single lot of PBMCs were thawed and cultured in StemPro®34 medium containing either 100 ng/mL SCF, 100 ng/mL FLT3, 20 ng/mL TPO and 10 ng/mL IL-6) (Cytokine mix A) or 100 ng/mL SCF, 100 ng/mL FLT3, 20 ng/mL IL-3 and 20 ng/mL IL-6) (Cytokine mix B) for 4 days prior to transduction as above. In the small volume transduction format we observed decreased reprogramming efficiencies for some lots of PBMCs in the presence of TPO.

Figure 4. Spinoculation transduction protocol improves reprogramming efficiencies



PBMCs were cultured and transduced as above with the exception that one sample was transduced utilizing a spinoculation protocol in which cells and virus were mixed in 1 ml total medium and then centrifuged in a 12 well plate at 2250 rpm for 90 min (plate-based spinoculation). One day after transduction, the virus was removed and the cultures were maintained as described in Figure 2 on MEFs.

Figure 5. Recommended reprogramming workflow with CytoTune® -iPSC 2.0 reprogramming kit utilizing MEF feeders

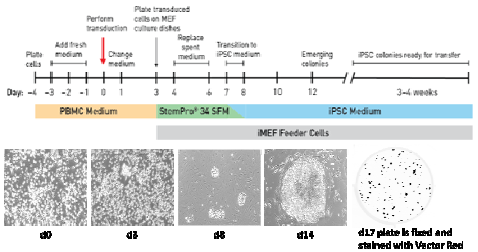
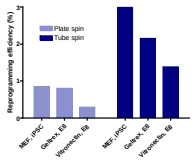
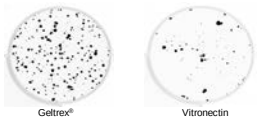


Figure 6. Tube-based spinoculation increases reprogramming efficiencies and enables reprogramming with feeder-free conditions on vitronectin

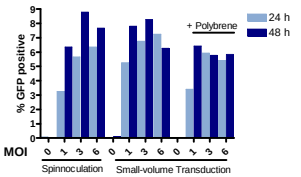


Alkaline-phosphatase positive colonies at d17 post-transduction



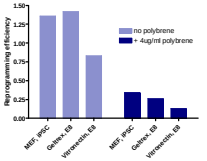
PBMCs were cultured four days and then transduced by spinoculation in a 12-well plate or in a sterile, round-bottom tube for 30 min at 2250 rpm at room temperature in a volume of 1 ml total with a MOI of 5-5-3. Cells transduced in the tube were transferred with the virus and media to a 12-well plate for overnight incubation and cultured as descried above. On day three post transduction, the cells were plated onto MEFs, Geltrex® or Vitronectin, and transitioned at day 7 into either iPSC medium or Essential 8™ Medium. On day 17 the plates were fixed, stained with Vector Red and reprogramming efficiencies were calculated as described above. The data shown here are from two separate PBMC lots (the graph on the left and the plate images on the right). For the plates on the right, 18,750 cells were plated onto each substrate on day 3 post transduction.

Figure 7. Transduction efficiencies using CytoTune® EmGFP Sendai Fluorescence Reporter do not reflect differences observed in reprogramming efficiencies



To begin to understand the differences that were observed between the transduction methodologies, we utilized the CytoTune® EmGFP Sendai Fluorescence Reporter in a series of transduction experiments. We carried out the spinoculation protocol and the small-volume transduction protocol in the presence or absence of 4 µg/mL polybrene hexadimethrine bromide (polybrene). We observed a MOI-dependent increase in the percentage of positive cells that was more apparent at 24 hours post transduction compared to 48 hours post transduction. The percentage of GFP positive cells were comparable across methods suggesting transduction efficiency is not driving the increase observed in reprogramming efficiencies and polybrene does not increase transduction efficiencies.

Figure 8. Polybrene negatively affects PBMC reprogramming efficiencies



The optimized tube-based spinoculation transduction protocol was tested in the presence or absence of 4 µg/mL polybrene. A decrease in reprogramming efficiency was observed in the presence of polybrene.

CONCLUSIONS

CytoTune® –iPS 2.0 Sendai Reprogramming Kit enables successful reprogramming of PBMCs

Reprogramming efficiencies vary between lot of PBMCs

Protocol modifications including spinoculation, removal of polybrene and a change in recommended cytokine additions in Stem Pro® 34 medium increase observed reprogramming efficiencies and enable a feeder-free reprogramming workflow

ACKNOWLEDGEMENTS

We would like to thank our colleagues who helped support this work, especially Aaron Trow, Kevin Monroe, Michael Hancock, Marian Piekarczyk and Chetana Revankar. Thanks to Sandy Kuligowski, Shayne Boucher, Mohan Vemuri, Emily Gu and David Kuninger for helpful discussion and exchange of reagents.

For Research Use Only. Not for use in diagnostic procedures.