invitrogen

Efficient iPSC Generation from Various Blood-derived Cell Types

Chad C. MacArthur, Uma Lakshmipathy
Cell Biology, Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008

ABSTRACT

A major use of induced pluripotent cells (iPSCs) is the ability to create patient-derived cells that may be used in a number of different applications including disease modeling, drug screening, and personalized medicine. Fibroblasts collected by skin punch biopsy are a common source of cells used to create iPSC, but this collection process is more inconvenient to the patient than a standard blood draw. Peripheral blood contains a variety of different cells types which have been shown to be able to be reprogrammed, including CD34+ hematopoietic stem cells, erythroblasts, and T-cells. We used varying methodologies; including upfront isolation and culture of the cells, reprogramming methods, media, and culture conditions, to develop optimized protocols that achieved high efficiency reprogramming of a number of different cell types isolated from blood. The relative convenience of collection and the high number of reprogrammable cells make blood a viable source of cells to generate patient-derived iPSC.

INTRODUCTION

In the past decade since Yamanaka's landmark discovery of iPSC generation, the iPSC field has made many advances. Numerous technologies have been developed to allow for high efficiency and footprint-free generation of iPSC from a wide variety of cell types. Fibroblasts were the first cells to be reprogrammed, and remain a commonly used source for iPSC generation. As the field has grown and researchers seek to create iPSC from an ever growing pool of patients, blood has become an increasingly popular source of cells for iPSC generation. Blood is more convenient to collect from a patient than a skin-punch, and blood cells can be processed and reprogramming initiated in less than a week, where as establishment of fibroblast cultures prior to reprogramming typically takes 2-3 weeks. Peripheral blood also contains a multitude of different cell types, allowing the researcher more freedom to choose a specific cell type to fit their needs.
Here, we looked at different methods to enable high efficiency reprogramming of different cell types; bulk PBMCs, and T-cells. We looked at the effect of cryopreservation and different cryopreservation media on reprogramming of PBMCs. We also looked at the effect on reprogramming of culturing either PBMCs or purified CD3+ cells in different T-cell media and on different substrates.

MATERIALS AND METHODS

PBMCs were isolated from whole blood using Ficoll-Paque density gradient centrifugation.

PBMC Cryopreservation
After isolation, PBMCs were cryopreserved in one of three freezing media; 40% Fetal Bovine Serum (FBS) + 10% DMSO, PSC Cryopreservation Medium, or Synth-a-Freeze™. Cells were stored in liquid nitrogen, then thawed and asses for viability using trypan blue exclusion.

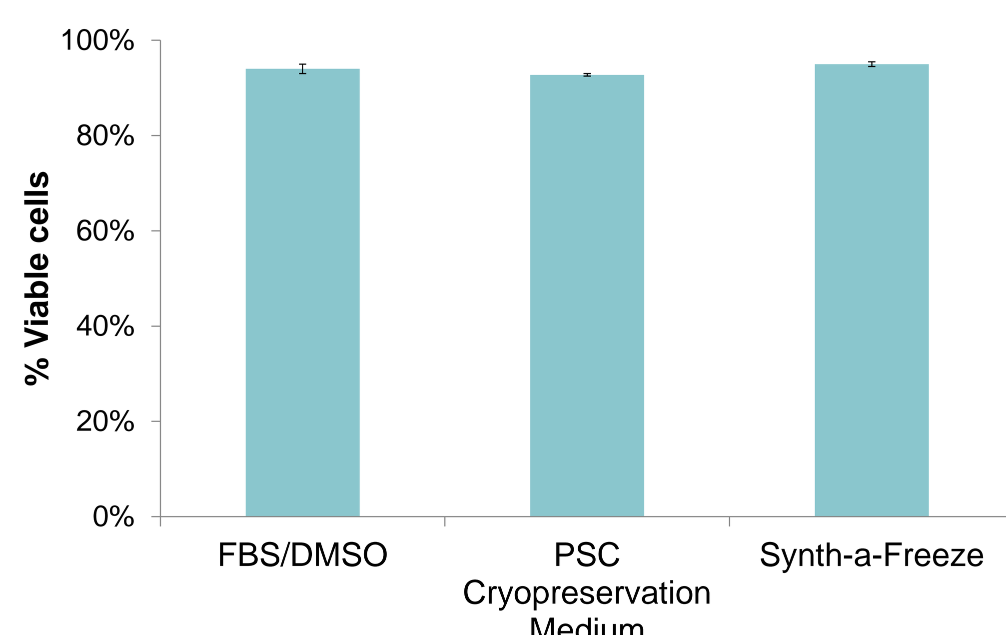
PBMC Reprogramming
PBMCs were cultured in StemPro™34 SFM, plus SCF (100ng/ml), FLT3 (100ng/ml), IL-3 (20 ng/ml), IL-6 (20ng/ml) for 4 days after isolation or thaw. Cells were then transduced with CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Virus was removed after 24 hours, and cells were plated onto rh-Vitronectin (VTN) at 3 days after transduction. At 7 days after transduction, medium was changed to Essential 8™ Medium. At 21 days after transduction cells were analyzed for alkaline phosphatase activity using the Vector® Red Alkaline Phosphatase stain.

T-Cell Reprogramming (PBMC)
Isolated PBMCs were cultured with Dynabeads™ Human T-Expander CD3/CD28 and 100U/mL recombinant human IL-2 in either AIM V™ Serum free medium containing 5% Immune Cell Serum Replacement, or CTS™ OpTMizer™ T Cell Expansion SFM for seven days. Cells were then removed from beads, and either stained for CD3,CD8, and CD4 and analyzed by flow cytometry, or reprogrammed with a protocol modified from Seki et. al.[1]. Cells were plated onto CD3 antibody coated plates, then transduced with CytoTune™ 2.0. After 48 hours, cells were plated onto either VTN, or rhLaminin-521 in Essential 8™ Medium. At 18 days after transduction, cells were analyzed for AP activity.

T-Cell Reprogramming (CD3+ Cells)
CD3+ cells were purified from whole blood using the the Dynabeads™ FlowComp™ Human CD3 Kit, according to manufacturer instructions. Purified CD3+ cells were cultured and reprogrammed as described above for PBMCs.

RESULTS

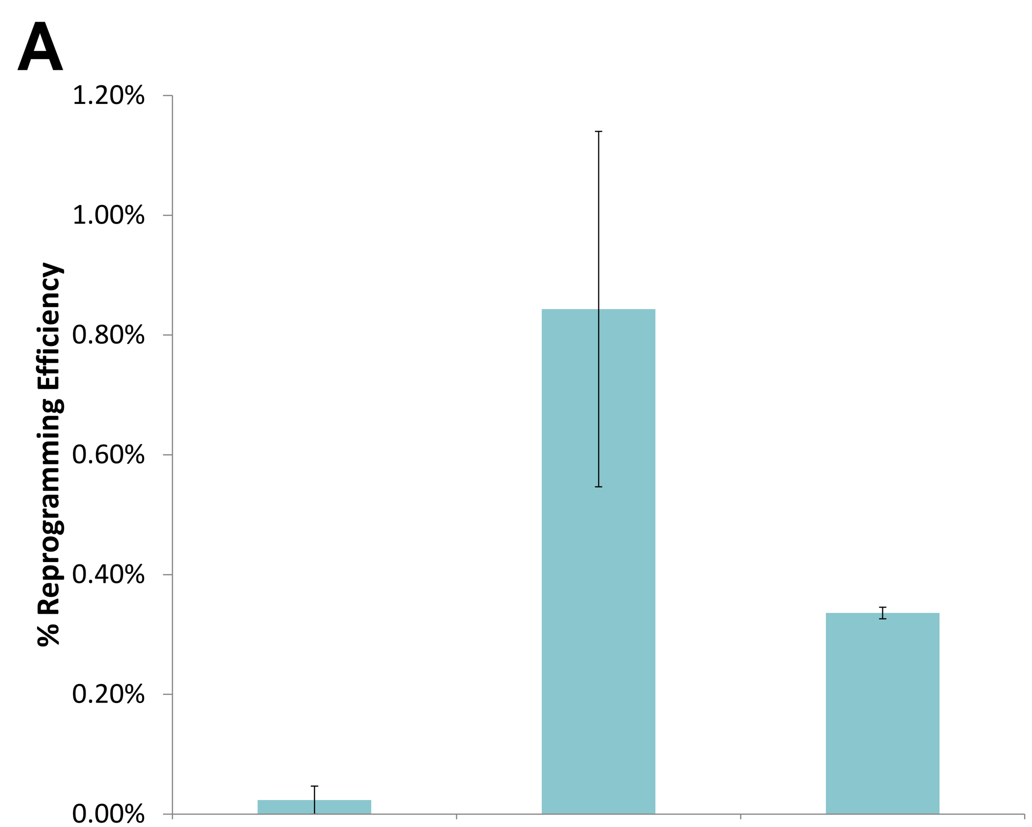
Figure 1. Defined, xeno-free freezing media perform similarly to traditional FBS-containing media.



PBMCs were isolated from whole blood from two different donors, and cryopreserved in one of three different media; traditional freezing medium containing 40% FBS and 10% DMSO, PSC Cryopreservation Medium, and Synth-a-freeze™. After cryopreservation, cells were thawed and evaluated for viability via Trypan blue and counted on a Countess™ II automated cell counter. Data shown is mean across two donors (n=2).

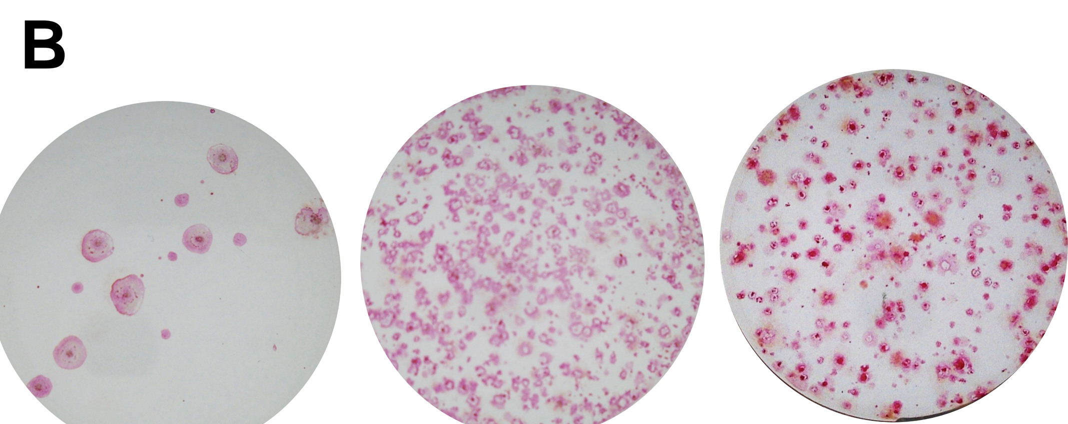
Figure 2. Cryopreserved PBMCs show decreased reprogramming efficiency compared to fresh PBMCs

A



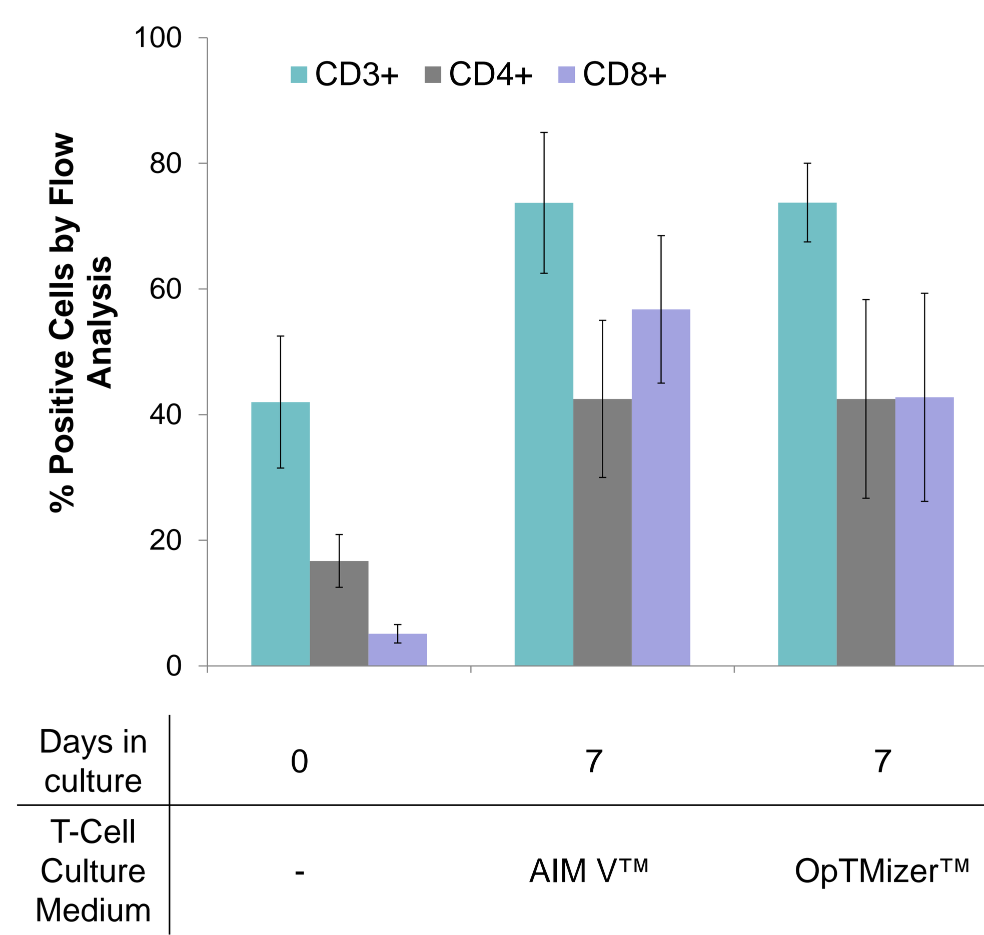
Days in Culture	0	4	4
Fresh	+	+	-
Cryopreserved / Recovered	-	-	+

B



PBMCs were isolated from whole blood from two different donors, and reprogrammed with CytoTune™ 2.0 either immediately; after four days of culture in PBMC medium; or after cryopreservation, recovery, and four days of culture in PBMC medium. At the end of reprogramming, cells were stained for alkaline phosphatase (AP), and AP positive colonies were counted. Reprogramming efficiency is expressed relative to the number of cells plated down (A). Data shown is mean across two donors (n=2). Representative images of AP stained colonies are also shown (B).

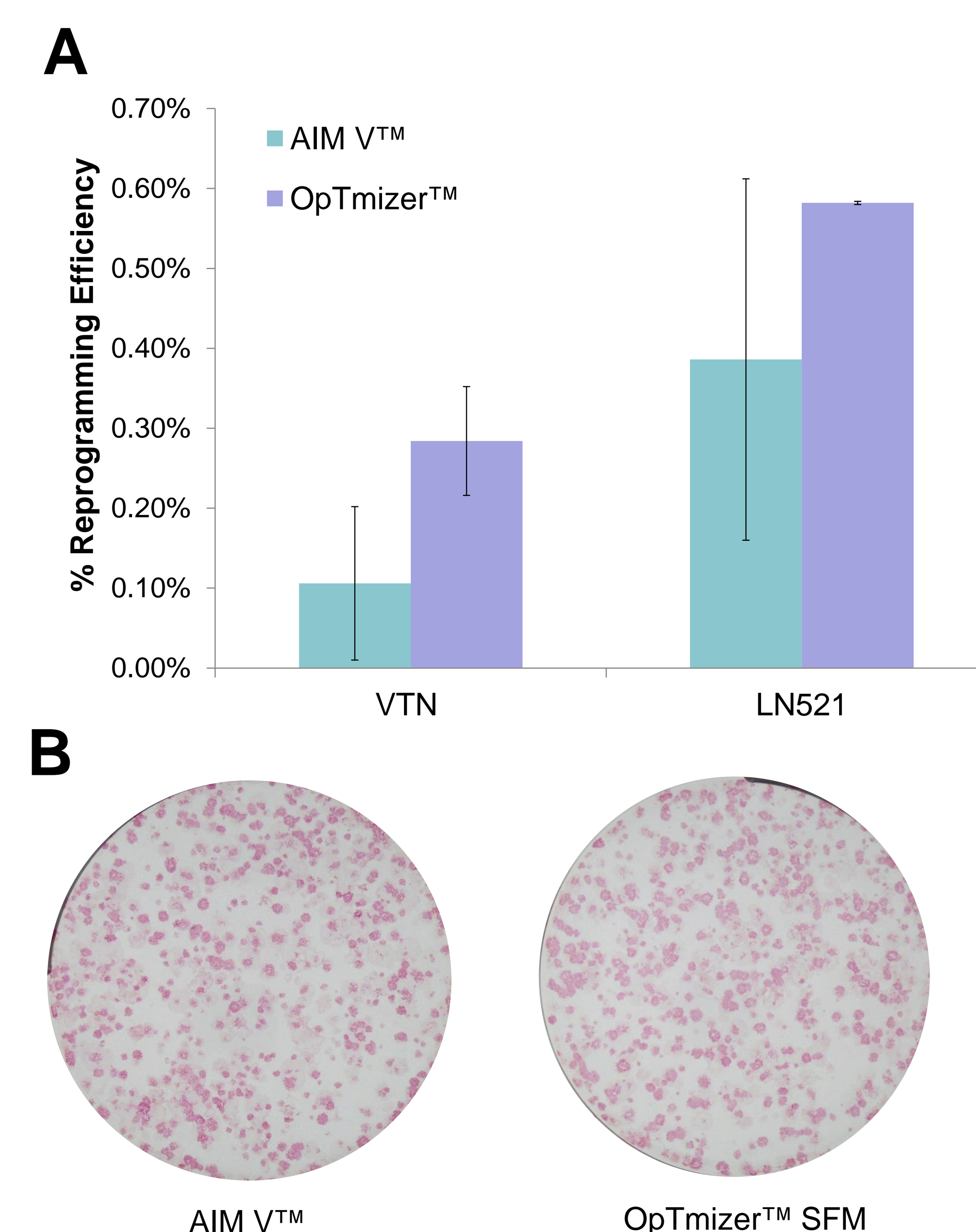
Figure 3. Activation and culture of PBMC in T-cell media enriches the CD3+ population.



Days in culture	0	7	7
T-Cell Culture Medium	-	AIM V™	OpTMizer™

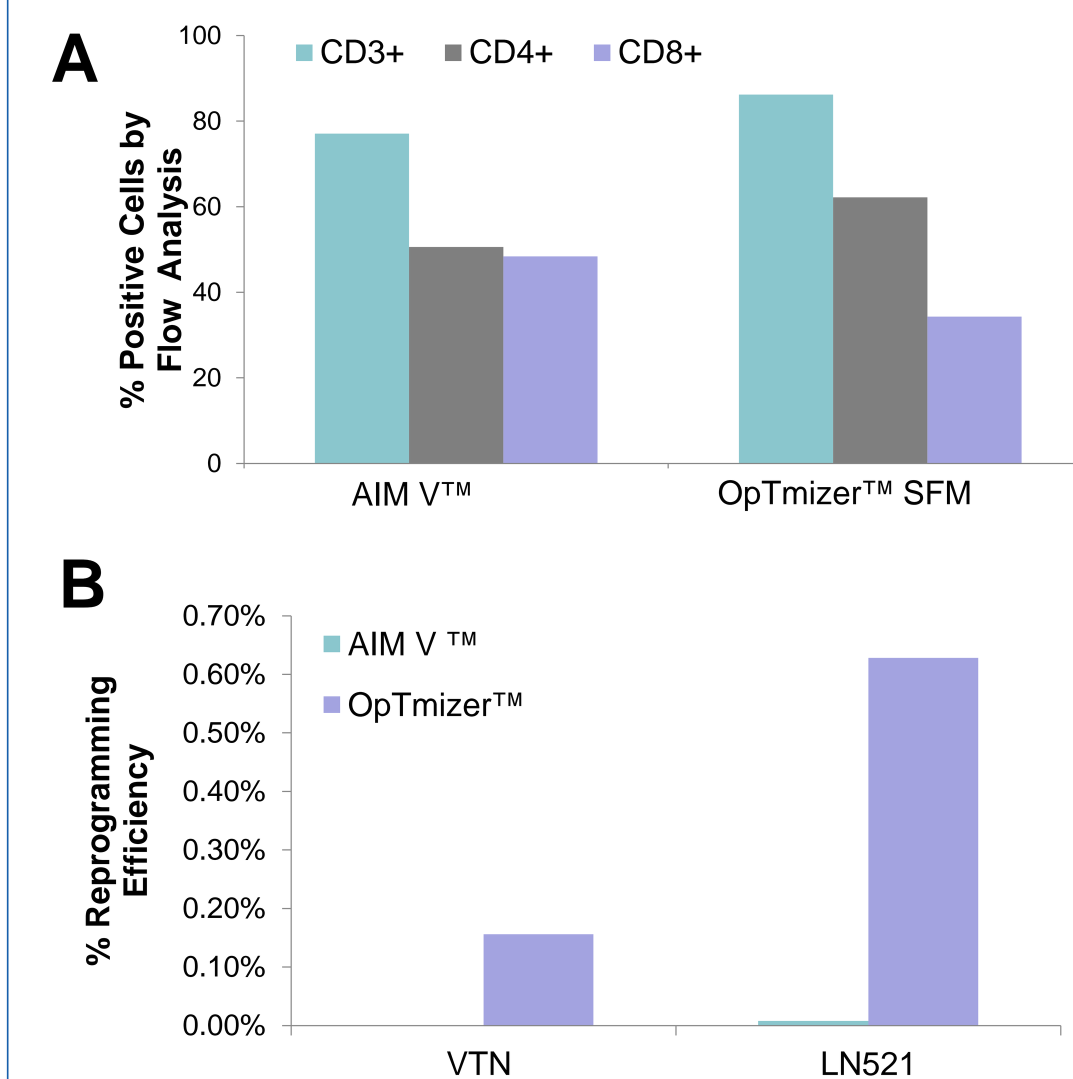
PBMCs were isolated from whole blood from two different donors, activated with CD3/CD28 Dynabeads™, and cultured for 7 days in one of two T-cell expansion medium containing 100 U/mL rhIL-2, either AIM V™ + 5% Immune Cell Serum Replacement, or OpTmizer™ SFM. After 7 days in culture, in order to analyze the T-cell population, cells were stained with CD3, CD4, and CD8 antibodies and analyzed by flow cytometry (A), and compared to similar analysis of cells performed immediately after PBMC isolation. Data shown is mean across two donors (n=2)

Figure 4. Culture and expansion of PBMC-derived T-cells in OpTmizer™ SFM, & plating on LN521 allow for high efficiency reprogramming



PBMCs were isolated and cultured as described in Figure 3. After 7 days in culture, cells were plated onto CD3 antibody coated plates and transduced with CytoTune™ 2.0. After 48 hours, cells were plated onto VTN or Laminin 521, and cultured in Essential 8™ medium. 18 days after transduction, cells were analyzed for reprogramming efficiency by AP stain. Reprogramming efficiency is expressed relative to the number of cells plated down (A). Data shown is the mean across two donors (n=2). Representative images of AP stained colonies on LN521 are also shown (B).

Figure 5. Culture and expansion of purified CD3+ cells in OpTmizer™ SFM, and plating on LN521 allow for high efficiency reprogramming



CD3+ cells were isolated from whole blood from two different donors using the Dynabeads™ FlowComp™ Human CD3 Kit, according to manufacturer instructions. After isolation, cells were activated with CD3/CD28 Dynabeads™, and cultured for 7 days in one of two T-cell expansion medium containing 100 U/mL rhIL-2, either AIM V™ + 5% Immune Cell Serum Replacement, or OpTmizer™ SFM. After 7 days in culture, cells from one of the donors had failed to expand, so only cells from the other donor were used in subsequent experiments. Cells were stained with CD3, CD4, and CD8 antibodies and analyzed by flow cytometry (A). Cells were also plated onto CD3 antibody coated plates and reprogrammed with CytoTune™ 2.0, as described in Figure 4. 18 days after transduction, cells were analyzed for reprogramming efficiency by AP stain. Reprogramming efficiency is expressed relative to the number of cells plated down (B).

CONCLUSIONS

Defined, serum-free medium can be used for the cryopreservation of PBMCs, while maintaining a high (>90%) viability after thaw.

Cryopreserved and thawed PBMCs reprogram at a lower efficiency than PBMCs that have not undergone cryopreservation.

PBMCs can be successfully reprogrammed immediately after isolation from whole blood, but at significantly lower efficiency.

Reprogramming of T-cells is enhanced by culture and expansion in OpTmizer™ SFM, and plating of cells onto LN521 substrate.

PBMCs cultured in the same conditions as purified CD3+ cells yield a population with similar T-cell marker expression and reprogramming efficiency. CD3 purification does not seem to be necessary for T-cell reprogramming.

Cells derived from different donors was a major source of variability, and this variability was consistent across experiments; cells from one donor consistently yielded a higher reprogramming efficiency.

REFERENCES

1. Seki T, Yuasa S, Fukuda K (2012) Generation of induced pluripotent stem cells from a small amount of human peripheral blood using a combination of activated T cells and Sendai virus. Nat Protoc;7(4):718-28.

TRADEMARKS/LICENSING

© 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. CytoTune™ is a registered trademark of ID Pharma Co., Ltd. Essential 8™ is a trademark of Cellular Dynamics International, Inc. Vector® Red is a trademark of Vector Laboratories. For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC