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Tools that support consistent generation of high quality iPSC using Sendai virus reprogramming for translational research

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ABSTRACT

Induced pluripotent stem cell (iPSC) research is rapidly moving towards translational and clinical applications. A prerequisite for these applications is consistent production of high quality iPSC lines from different donor cells. In addition, the initial stages of iPSC generation need to be further streamlined to minimize extra effort and costs associated with clones that fail to expand, or do not meet quality standards for downstream use. Methods that enhance consistency between different donors and tools that facilitate identification, elimination and quantification of residual reprogramming factors enable faster generation of high quality iPSC clones, suitable for use in downstream applications.

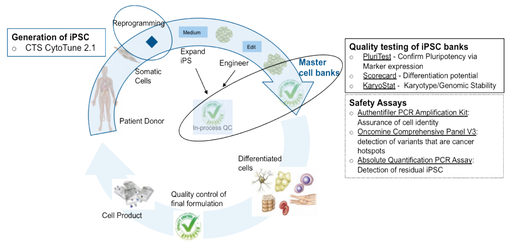
Previously, we reported the first off-the-shelf reprogramming kit designed for clinical and translational research. Here, we describe xeno-free workflows and associated tools that can be used in conjunction with this kit to further streamline iPSC generation and help ensure the consistent creation of high quality iPSCs. A combination of hypoxia, matrix and seeding density was shown to offer consistent iPSC generation from difficult to reprogram donor cells. In addition, a panel of antibodies specific for unreprogrammed & partially reprogrammed cells along with an assay that detects residual Sendai virus enabled visualization and elimination of unwanted cells, thereby enriching for high quality, footprint-free iPSC. Lastly, a qPCR-based absolute quantification assay was developed to determine the copy number of Sendai virus present in iPSC clones for further confirmation of foot-print free iPSC. Together, these tools support a complete workflow that will allow researchers to readily progress their investigations toward translational and clinical research.

INTRODUCTION

For iPSC to be used as starting material for clinical research, it is important that the iPSC are created using relevant workflows which includes xeno-free workflows. We recently reported CTS™ CytoTune™-iPS 2.1, the first commercial reprogramming kit specifically developed for clinical and translational research. Given these changes, the new reprogramming kit was used to reprogram both fibroblasts and blood-derived cells in xeno-free workflows, and both reprogramming efficiency and quality of the resulting iPSC clones were examined. T-cell reprogramming was further optimized for the consistent derivation of iPSC. Additionally, techniques were developed for detection, elimination and quantification of SeV positive cells.

iPSC generated from human fibroblast under complete xeno-free conditions was further characterized using comprehensive characterization tools that relies on reference standards and hence serves as reliable qualification tools.

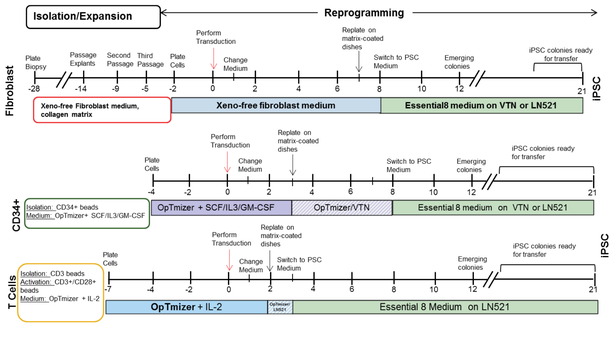
Scheme 1: Overview of iPSC generation for translational use



MATERIALS AND METHODS

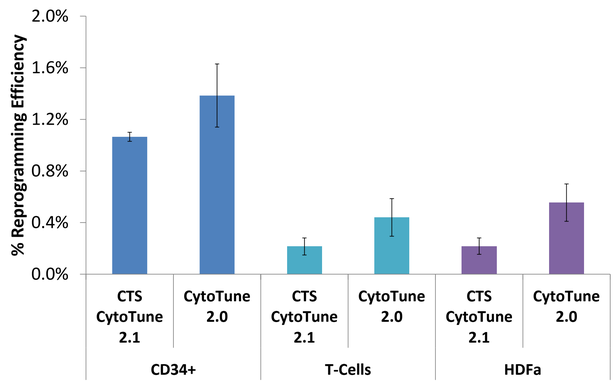
All materials are from Thermo Fisher Scientific unless specified. Basic reprogramming workflows under xeno-free culture conditions (Scheme 2) was further optimized for T-cells

Scheme 2: Reprogramming Workflows for human fibroblast & Blood cells



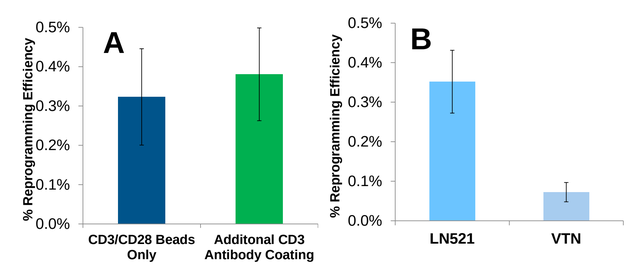
RESULTS

Figure 1. CTS CytoTune 2.1 successfully reprograms human dermal fibroblasts, T-cells, and CD34+ cells



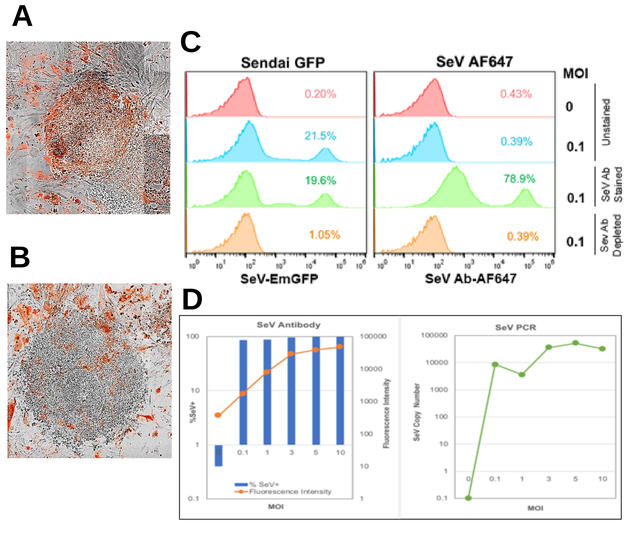
Humans dermal fibroblasts (HDFa), and two different types of blood-derived cells (CD34+ Cells and T-Cells) were reprogrammed under xeno-free conditions with either CytoTune 2.0 or CTS CytoTune 2.1, according to the schematics above. At 16 days (CD34+ cells and T-cells), or 21 days (HDFa) after transduction, cells were stained for AP, and reprogramming efficiency was calculated as the number of AP positive colonies, relative to the number of cells replated.

Figure 2. Optimization of T-Cell Reprogramming workflow



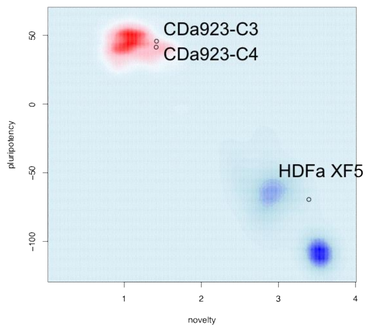
Typical T-Cell reprogramming protocols use CD3 antibody-coated plates after transduction of cells. To determine the necessity of this step, transduced T-cells were either cultured for two days in the continued presence of CD3/CD28 beads, or beads were removed and cells were cultured on CD3 antibody-coated plates **(A)**. No significant difference in reprogramming efficiency was observed between either method. Additionally, transduced T-cells were replated on either vitronectin (VTN) or Laminin-521 (LN521), and the resulting reprogramming efficiency was determined **(B)**.

Figure 3. Detection & Elimination of Sendai positive cells



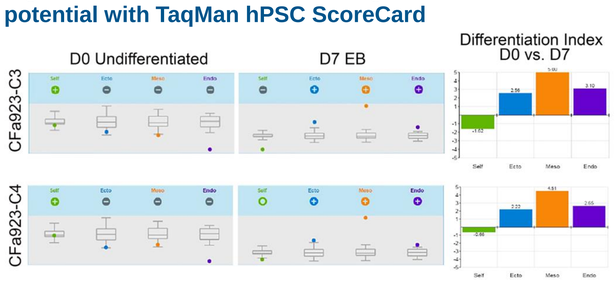
Newly formed iPSC colonies were stained with a monoclonal anti-Sendai (SeV) antibody. Immunofluoresence revealed heterogeneous distribution of SeV-positive cells among the colonies, with some colonies brightly stained **(A)**, and some partially stained **(B)**. HDFa were transduced with Sendai EmGFP and analyzed by flow cytometry before and after SeV Ab staining and depletion with IgG Dynabeads **(C)**. A TaqMan™ qPCR-based absolute quantitation assay was developed to determine the presence or absence of the Sendai viral vectors in iPSC clones **(D)**.

Figure 4. Rapid confirmation of Pluripotency with PluriTest



iPSC derived from fibroblasts under xeno-free conditions were analyzed using PluriTest, an algorithm that compares profiles to an extensive reference set with 450 profiles that includes 223 hESC and 42 iPSC lines. Results confirm high pluripotency and low novelty score for two iPSC clones and vice versa for the parental fibroblasts

Figure 5. Determination of Trilineage differentiation potential with TaqMan hPSC ScoreCard



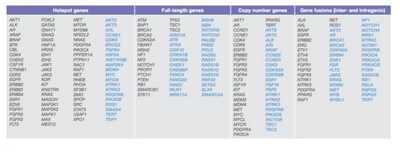
Trilineage differentiation potential was assessed using hPSC TaqMan ScoreCard, a focused gene expression array that confirm gene expression profiles by comparing data against a reference dataset derived using 13 pluripotent lines. Results indicate both iPSC clones have trilineage differentiation potential.

Figure 6. Examination of chromosomal abnormalities with array-based KaryoStat & KaryoStat HD

The two iPSC clones and parental fibroblast were also analyzed using KaryoStat or higher resolution KaryoStat HD. No gain or loss was detected with lower resolution (1-2 Mb) KaryoStat. However, one of the iPSC clones showed a mosaic gain on chromosome 12 with the higher resolution KaryoStat HD (<25-50 kb) which was not detected by G-banding.

Figure 7. Safety Assessment of iPSC with Oncomine Comprehensive Assay V3

A

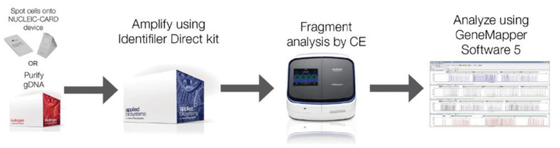


Oncomine assays, a multiple-biomarker NGS-based method, was used to further assess iPSCs for the presence of any variants associated with cancer hotspots **(A)**. Results indicate comparable number of variants between parental fibroblast and iPSC with no structural variants that are cancer hotspots. TP53 variant remains same across parental and iPSCs **(B)**

B

Sample	Variants	HotSpot Variants
HDFa XF5	182	0
CFa923-C3	181	0
CFa923-C4	181	0
H9 ESC	209	0
BG01V ESC	164	0
AOHC (Positive control)	288	37
NTC	0	0

Figure 8. Authentication of iPSC lines



	Gibco iPSC	H9 ESC	HDFa XF5	CFa923-C3	CFa923-C4
AMEL	X,X	X,X	X,X	X,X	X,X
CSF1PO	11,11	11,11	12,13	12,13	12,13
D13S317	9,11	9,9	11,12	11,12	11,12
D16S539	11,13	12,13	11,11	11,11	11,11
D18S51	15,15	OL,13	13,14	13,14	13,14
D19S433	12,14	12,15	13,14	13,14	13,14
D21S11	29,29	30,30	30,33.2	30,33.2	30,33.2
D2S1338	17,24	18,24	16,23	16,23	16,23
D3S1358	15,18	13,16	16,17	16,17	16,17
D5S818	11,12	11,12	11,11	11,11	11,11
D7S820	10,12	9,11	10,11	10,11	10,11
D8S1179	9,15	8,14	14,15	14,15	14,15
FGA	22,25	26,28	19,24	19,24	19,24
TH01	9,9.3	9.3,9.3	8,9	8,9	8,9
TPOX	8,8	10,11	8,8	8,8	8,8
vWA	17,18	17,17	15,17	15,17	15,17

Identifiler PCR Amplification Kit authenticated iPSC clones with parental identity. This assay is critical to determine cell identity-and distinguish it from other products being manufactured in the same facility. This assay ensures no cell-line mix ups and contamination. Results indicate the 2 iPSC line match with the parental fibroblast identify.

CONCLUSIONS

- CTS CytoTune 2.1 successfully reprogrammed HDF and blood-derived cells (CD34+ and T-Cells); though efficiency was about 2-fold lower than reprogramming with CytoTune 2.0.
- T-Cell reprogramming was optimized by removing additional CD3 antibody coating steps, and replating transduced cells onto LN521.
- iPSC generated from HDFa in xeno-free conditions with CTS CytoTune 2.1 were pluripotent and displayed a normal karyotype as determined using comprehensive characterization methods
  - A monoclonal anti-SeV antibody allowed for detection and depletion of Sendai positive cells and TaqMan qPCR absolute quantitation assay was developed to verify clearance of Sendai virus from iPSC clones.
  - PluriTest identifies pluripotent cells by comparing global gene expression profile with an extensive reference set of over 450 cell types including 223 hESC and 42 iPSC lines among others.
  - ScoreCard hPSC Scorecard Panel assesses pluripotency and trilineage differentiation potential using real-time qPCR assays and intuitive data analysis software.
  - KaryoStat/KaryoStat HD assay enable the detection of chromosomal gain and loss, aneuploidies, mosaic events, loss of heterozygosity and copy neutral events such as absence of heterozygosity for research (KaryoStat) and translational (KaryoStat HD) use.
  - Oncomine Comprehensive Assay V3 provides a method to detect any structural variants in the resulting iPSC clones that are associated with known cancer hotspots including TP53 which is thought to occur at a higher frequency in ESC and iPSC lines.
  - Identifiler PCR Amplification Kit was used to authenticate iPSC clones with parental identity.

TRADEMARKS/LICENSING

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