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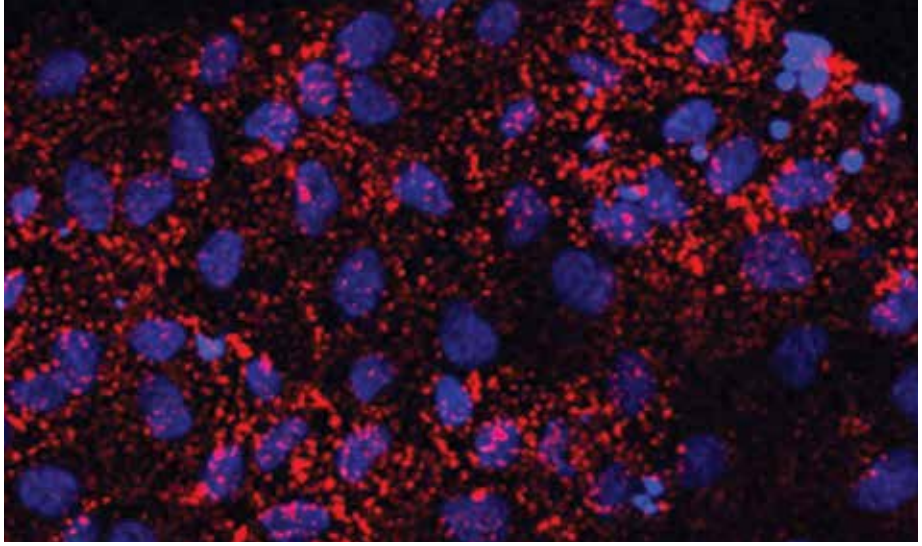
Fall 2014

The Newsletter for Life Science Researchers



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It All Stems from Here

In 2007, both Yamanaka and Thomson, individually, showed that normal adult cells can be reprogrammed into induced pluripotent stem cells (iPSCs). Since then, stem cell research has been on the rise. The potential to generate a required cell type *in vitro* from patient-derived tissue offers virtually unlimited opportunities for modern cell therapy. However, current practices for iPSC-generation rely primarily on literature recommendations.

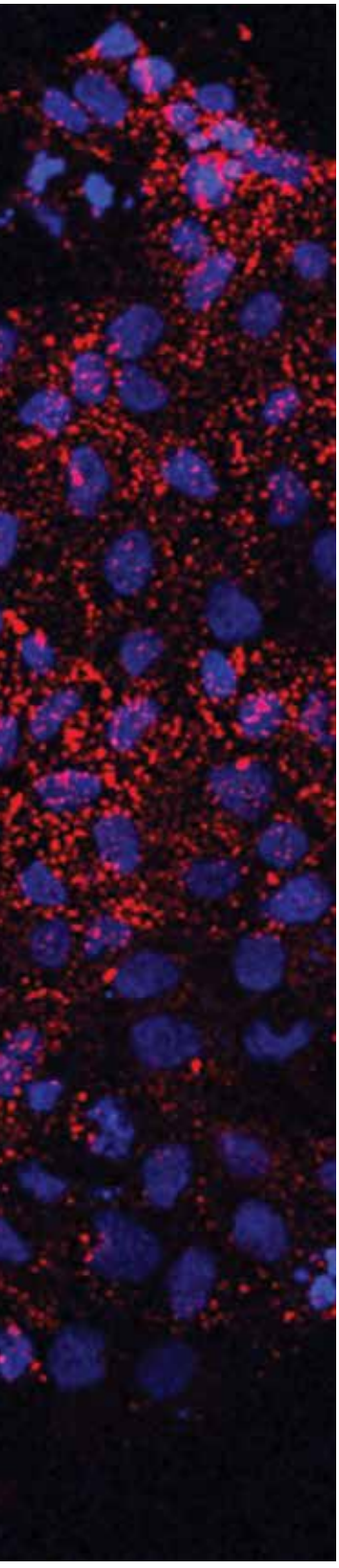
Our novel L7™ hiPSC Reprogramming and hPSC Culture System has been developed for generation of human iPSCs as well as the maintenance of human pluripotent stem cells (hPSCs). The seven individual key components are designed to work together as a robust iPSC-generation workflow. Dedicated reprogramming protocols for our Clonetics™ Primary Cells and Poietics™ Stem Cells, together with the Nucleofector™ Technology, are available as well as the various components of the L7™ hPSC Culture System for iPSC cell culture and cryopreservation. The L7™ hPSC Medium allows for every-other-day feeding of hESCs and iPSCs under defined, xeno-free conditions, thus providing the basis for a seamless transition from the lab bench to clinical development applications.

Genome editing is currently gaining large momentum in the scientific community in that it enables researchers to precisely remove, insert or replace DNA in a specific genome location. The Nucleofector™ Technology allows efficient co-transfection of various substrates into iPSCs or hESCs, and has been successfully used to perform genome editing with zinc fingers (ZFN), TALENs and CRISPR/Cas technology.

Don't miss the opportunity to learn more about our new CytoSMART™ System. This easy-to-use and affordable live cell imaging system will truly support you in your stem cell research, whether it is for cell culture documentation purposes or to remotely view your cell culture, e.g., under hypoxic conditions.

Enjoy reading!

Your Lonza Team



News

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18–21 October 2014	**9th Biennial Meeting of the Chinese Society of Immunology (CSI)**	–	Jinan City, China
16–19 November 2014	**Society for Neuroscience**	1937	Washington, DC, USA
03–05 November 2014	**German Stem Cell Network**	–	Heidelberg, Germany
01–04 December 2014	**British Society for Immunology**	13	Brighton, UK

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Webinars

Date	Webinar
28 October 2014	Genome Editing – Transfecting ZFNs, TALENs or CRISPR/Cas Using Nucleofector™ Technology
29 October 2014	Genome Editing – Transfecting ZFNs, TALENs or CRISPR/Cas Using Nucleofector™ Technology

For details and registration visit www.lonza.com/webinars

Lonza L7™ hPSC Culture System for Research and Future Clinical Applications

By Ashish Mehta[1], Chrishan JA Ramachandra[1], Glen L Sequiera[1], Scott D'Andrea[2], Amber Ellis[2], Winston Shim[1,3]

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Introduction

Chemically defined and feeder-independent cell culture systems have provided a superior platform for reproducibility and standardization of human pluripotent stem cell (hPSC)-based research. As the field advances towards potential clinical applications involving hPSC-derived cell progenies, hPSC culture systems compliant with regulatory standards are necessary. Lonza has developed a robust xeno-free and defined system that could be translated for use in GMP and clinical-grade manufacturing. L7™ hPSC Culture System is a culture platform (medium, matrix, passaging solution and cryosolution) that supports every-other-day feeding of hPSCs. We evaluated Lonza L7™ hPSC Culture System for maintenance and expansion of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs).

Results

Methods

Human ESCs and hiPSCs[1] were maintained in L7™ hPSC Medium and passaged every 5–7 days using L7™ hPSC Passaging Solution. Dissociated hPSC colonies were seeded on L7™ hPSC Matrix. After four passages, molecular characterization for pluripotency was performed using immunostaining and FACS. The differentiation potential of hPSCs into three primary germ lineages was characterized through embryoid bodies (EBs)[2].

Advantages of L7™ hPSC Passaging Solution

1. Attachment ratio post-passaging is high (>90% cell aggregates attach in 16 hours)
2. High post-detachment viability (>90%)
3. Generates uniform-sized aggregates
4. Increased split ratios (1:8 to 1:10)

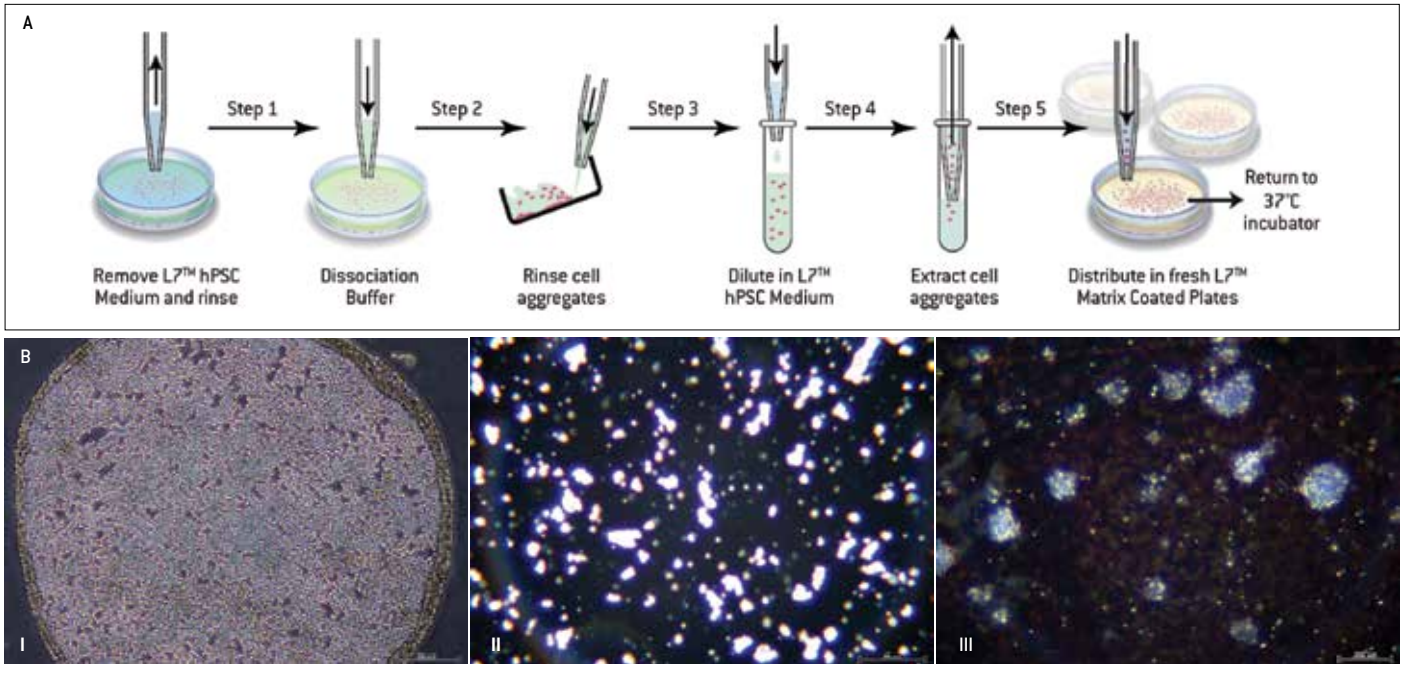

Figure 1
(A) Schematic representation of dissociation protocol using L7™ hPSC Passaging Solution. (B) Phase contrast images showing stages of hPSC after addition of L7™ hPSC Passaging Solution for 5 minutes (I) note disruption of hPSC colonies (II) uniform small cell aggregates after Step 2 (III) and colonies after 16 hours of seeding on a fresh L7™ hPSC Matrix Coated Plate in L7™ hPSC Medium.

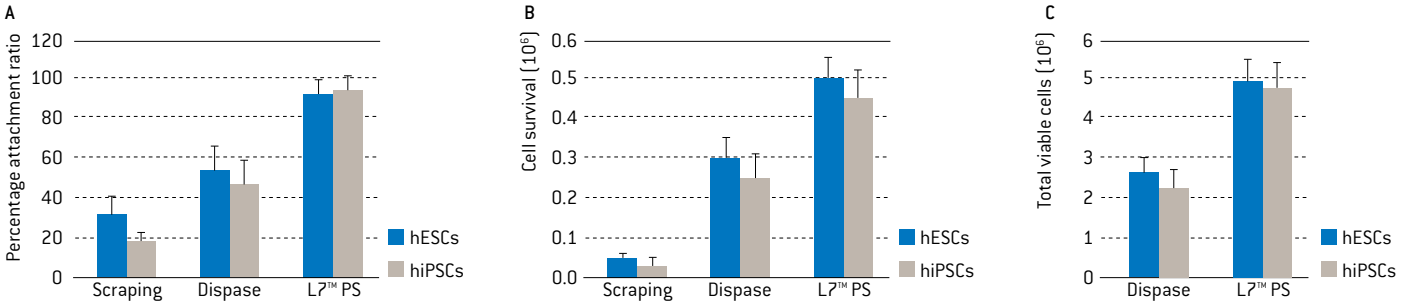

Figure 2
(A) Graph shows the percentage attachment ratio of cell aggregates that attach at 16 hours post passaging using various methods. Note the marked difference between the three methods. (B) Graph represents number of viable cells after 24 hours of passaging with various methods. (C) Graph shows the total viable cells after 6 days of culture, comparing two different passaging methods. Data presented are mean ± s.e.m. of three experiments. Abbreviation: L7™ PS = L7™ hPSC Passaging Solution.

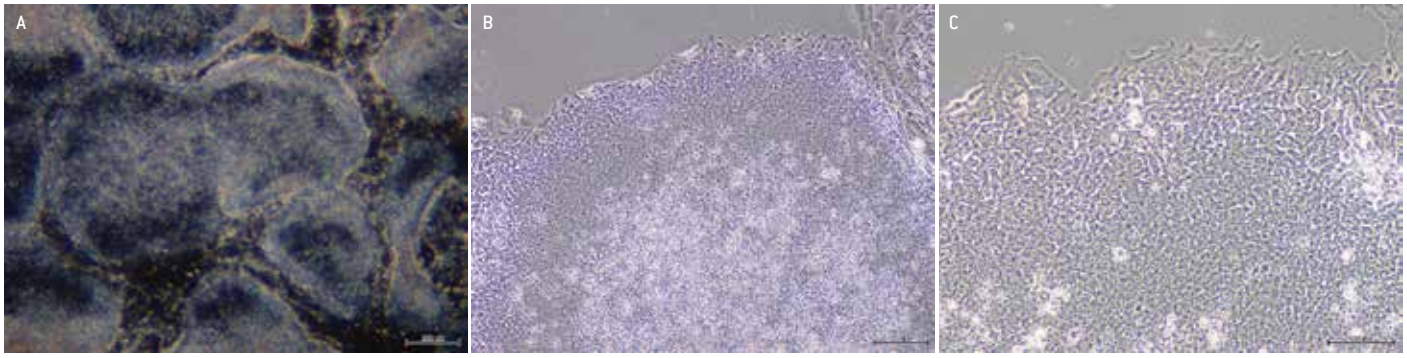

Figure 3
(A) Low magnification of hPSC culture on day 6, ready for passaging. (B) Phase contrast image of an hPSC colony showing compactness. (C) Magnified phase contrast image of hPSCs maintained in L7™ hPSC Medium. Note hPSCs show high nucleus to cytoplasm ratio and are highly compact in morphology.

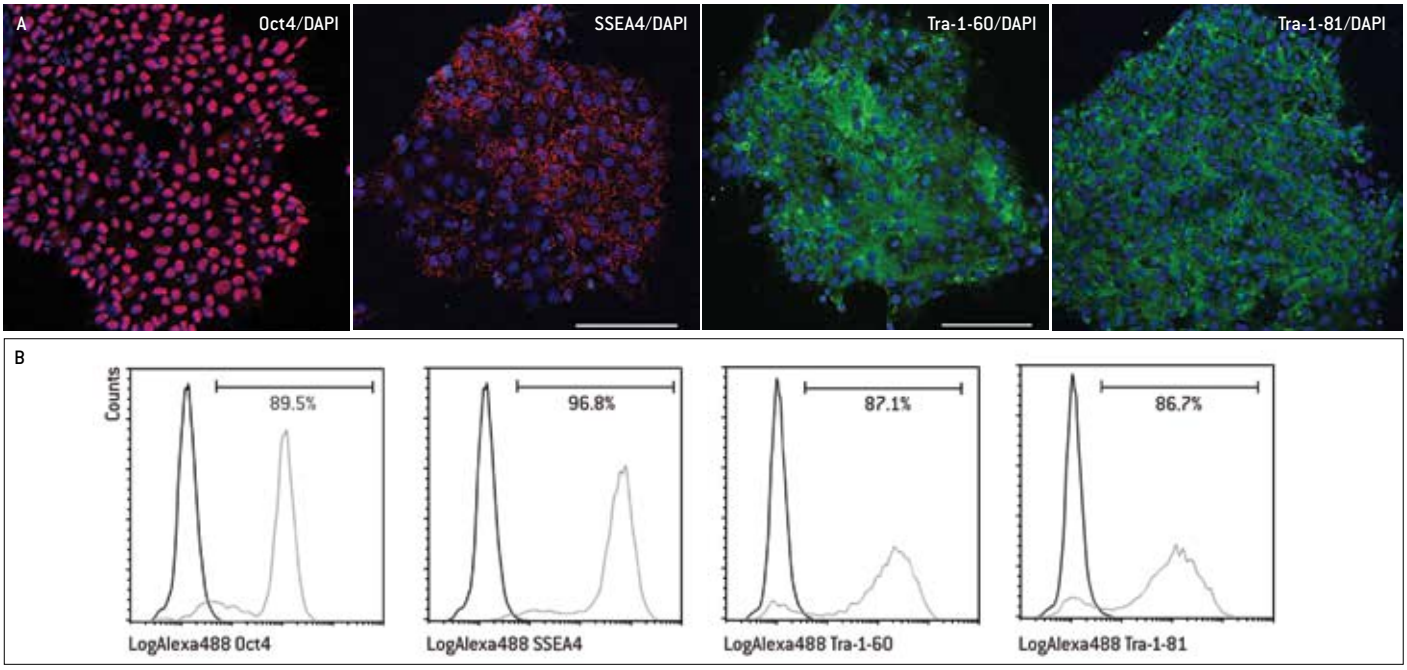

Figure 4
(A) Immunostaining of hPSC colonies grown on L7™ hPSC Medium in combination with L7™ hPSC Matrix exhibiting pluripotency markers, Oct4, SSEA4, Tra-1-60 and Tra-1-81. Scale bar: 100 µm. (B) Flow cytometric analyses demonstrate more than 85% cells express pluripotency markers. A total of 10,000 events were counted and evaluated.

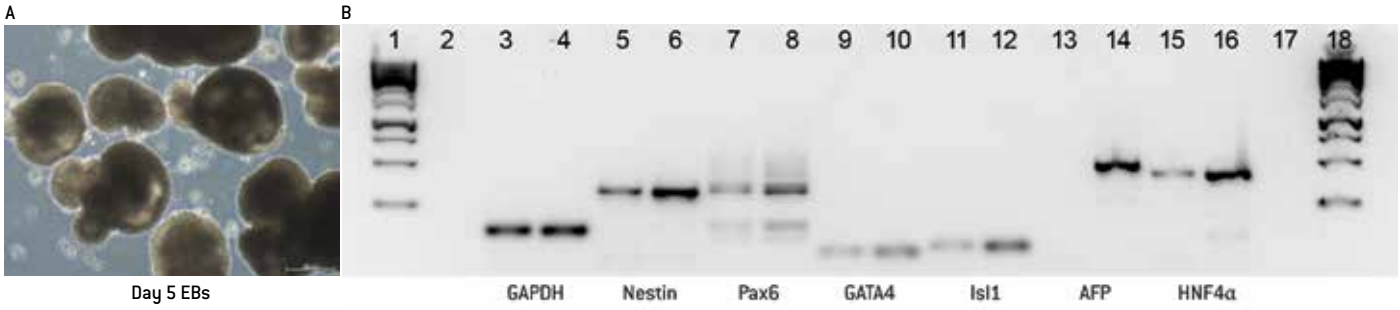

Figure 5
(A) Phase contrast image of 5-day-old EBs showing ability of hPSCs grown in L7™ hPSC Culture System for differentiation. (B) Semi-quantitative RT-PCR for two lines showing the ability of the cells to differentiate into three germ layers, ectoderm (Nestin-lane 5, 6; Pax6-lane 7, 8), mesoderm (GATA4-lane 9, 10; Isl1-lane 11, 12) and endoderm (AFP-lane 13, 14; HNF4α-lane 15, 16), with GAPDH (lane 3, 4) as housekeeping gene. hiPSC line (lane 3, 5, 7, 9, 11, 13, 15) and hESC line (lane 4, 6, 8, 10, 12, 14, 16). Molecular weight ladder (lane 1, 18).

Conclusion

We evaluated L7™ hPSC Culture System to passage 4 and found it to be a robust and efficient system for expansion and growth of hPSCs in a xeno-free, regulatory-compliant environment for use in both research and clinical applications involving hPSC-derived cell derivatives. For long-term data, please see ISSCR poster # T-2173, "Robust Generation and Maintenance of Human Induced Pluripotent Stem Cells Under Defined Conditions".

References

1. Mehta A *et al.*, *Cardiovasc Res.* 2011; 91: 577–586.
2. Mehta A *et al.*, *Toxicol Sci.* 2013; 131: 458–469.

Acknowledgements

A.M., W.S., C.R., G.S., are funded by the National Research Foundation Singapore (NRF-CRP-2008-02), National Medical Research Council (NMRC/BNIG/1074/2012), Goh Foundation Gift (Singapore)/Duke-NUS Graduate Medical School (GCR/2013/0008 and GCR/2013/011) and Biomedical Research Council Singapore (BMRC 13/1/96/686).

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Lonza L7™ hiPSC Reprogramming and hPSC Culture System – A Case Study

By Ha T. Tran[1], Rex E. Lacambacal[1,2], Candace L. Lynch[1], Jeanne F. Loring[1], Inbar Friedrich Ben-Nun[3], Yu-Chieh Wang[1,4]

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Introduction

Stem cells have attracted a great deal of attention and have excellent potential in a variety of clinical applications. Human embryonic stem cells (hESCs) are capable of indefinite self-renewal and can differentiate into all somatic cell types, meaning that they are widely considered to have tremendous potential in medical research. However, on account of the ethical and legislative hurdles associated with their use (e.g., restricted numbers of embryos permitted to be used for research), a great deal of work has been devoted toward developing functional replacements for hESCs without the ethical constraints associated with the use of human embryos.

In 2006, it was demonstrated that mouse fibroblasts could be reprogrammed by retroviral overexpression of four transcription factors to an embryonic-like state[1]. These reprogrammed somatic cells were termed induced pluripotent stem cells (iPSCs). The following year, it was shown for the first time that iPSCs could be generated from human fibroblasts using the same four factors – POU5F1 (a.k.a. OCT3/4), SOX2, KLF4 and MYC – via retrovirus-mediated transfection[2]. Since this first method of generating human iPSCs (hiPSCs), there have been several noted experimental successes leading to the development of new reprogramming techniques for a variety of somatic cells in an effort to improve the derivation efficiency of hiPSCs.

The methods for generating hiPSCs, or inducing pluripotency in cells, can be broadly categorized based on the method used for the delivery of reprogramming factors. Retroviruses were the first delivery method used in the generation of iPSCs that were capable of stable integration into the host genome[1]. However, due to the activation of methyltransferases, this method may present a problem in the activation of corresponding endogenous genes[1] and could lead to tumor formation in chimeric animals where viral transgenes integrate into iPSCs[3].

An alternative approach is the use of the Sendai virus vector to generate transgene-free iPSCs. Due to the Sendai virus replicating exclusively in the cell cytoplasm, it eliminates many of the risks associated with conventional viral vectors. Although Sendai virus vectors also have a relatively high reprogramming efficiency, such vectors are associated with greater costs and it can be difficult to later prove the absence of viral material in iPSCs.

Non-integrative and non-viral reprogramming methods can be very important for generating hiPSCs destined for human therapeutic applications. Synthetic mRNA delivery has generated iPSCs with a high level of efficiency[4] and does not have the associated insertional mutagenesis that can result in tumor formation. However, the process is technically demanding, requiring very specific reagents, and places a significant burden on available time due to the required daily mRNA transfections.

A near non-integrative approach to reprogramming is the use of episomal vectors. This non-viral method transfects cells using an episomal vector derived from the Epstein Barr virus, but without the viral packaging[5]. Successful reprogramming via episomal vector-mediated transfection has been demonstrated using Lonza's Nucleofector™ Technology[5–9]. Lonza has developed its own protocol for reprogramming using peripheral blood mononucleated cells (PBMCs), which are combined with the L7™ hPSC Culture System for subsequent feeder- and xeno-free culture of the generated hiPSCs (Figure 1, see also pages 20–21).

In this study, we evaluated the Lonza protocol for a certain individual – referred to as individual #418 – for which various reprogramming attempts had failed thus far. Previously, we successfully generated hiPSCs from primary cells of numerous individuals using retro- or Sendai virus-mediated methods. However, in the case of individual #418, these approaches failed to yield colonies following transduction attempts with both dermal fibroblasts from several biopsies and epithelial cells from urine samples. Using the Lonza L7™ hiPSC Reprogramming and hPSC Culture System, we have generated hiPSCs from PBMCs of individual #418. The data presented here demonstrate that the Lonza protocol can be used for the generation of high quality hiPSCs, capable of maintaining their differentiation potential.

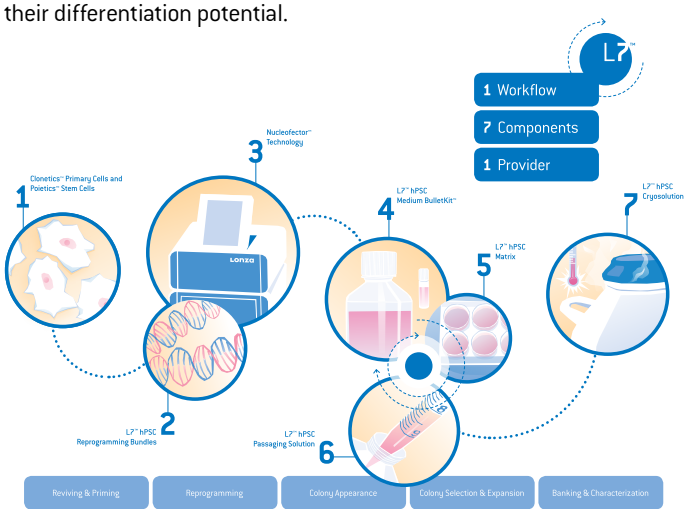

Figure 1
Schematic of Lonza's L7™ hiPSC Reprogramming and hPSC Culture System.

Materials and Methods

Reprogramming of PBMCs

PBMCs were cultured over 6–8 days in a priming medium (provided by Lonza) that supports differentiation of the mixed PBMC population toward erythroblasts. After this priming phase, 1 million cells/sample were co-transfected with 3 µg of the vector cocktail (provided by Lonza) using the P3 4D-Nucleofector™ Kit (Lonza, cat. no. V4XP-3012) and program EO-115. Transfected cells were plated on 6-well plates coated with L7™ hPSC Matrix (Lonza, cat. no. FP-5020) and cultured in an optimized recovery medium containing a reprogramming enhancer (both provided by Lonza) for 2 days. At day 2, post transfection, the recovery medium was mixed 1:1 with fresh L7™ hPSC Medium (Lonza, cat. no. FP-5007) before replacing it completely at day 4 with L7™ hPSC Medium. Typically, iPSC colonies appear at days 9–10, post transfection, with some colonies being large enough for picking from day 12 onwards.

Culture of hiPSCs

After transfer into L7™ hPSC Medium, the medium was replaced every other day with 2 ml freshly supplemented media until colonies were large enough to subculture. The initial colonies were manually passaged (P1) into separate 12-well plates coated with L7™ hPSC Matrix (Lonza, cat. no. FP-5020) and containing supplemented L7™ hPSC Medium. Cells were cultured in a humidified 37°C incubator under normoxic conditions (20.9% O_2; 5% CO_2). To subculture colonies during expansion at P3 and later passages, L7™ hPSC Passaging Solution (Lonza, cat. no. FP-5013) was used according to the product instructions. Some cells (P2) were also manually passaged onto radiation-inactivated mouse embryonic fibroblast (MEF) feeders and continuously cultured in DMEM/F12 media with L-glutamine containing 20% KnockOut™ Serum Replacement, 100 µM non-essential amino acids, 100 µM ß-mercaptoethanol (all from Life Technologies, Carlsbad, CA), and 12 ng/ml FGF2 (Stemgent, Cambridge, MA).

Embryoid Body Formation

For differentiation into each germ layer by embryoid body (EB) formation, we used our previously reported protocol[10]. Pluripotent cell colonies were harvested and cultured in ultra-low attachment plates with FGF-deficient DMEM/F12 medium with L-glutamine containing 20% KnockOut™ Serum Replacement, 100 µM non-essential amino acids, and 100 µM ß-mercaptoethanol (all from Life Technologies, Carlsbad, CA) for 7 days. On day 8, EBs were transferred onto gelatin-coated coverslips and cultured in the same medium for an additional 7 days. EBs were then fixed, permeabilized and incubated with antibodies against biomarkers relevant to the three germ layers.

Melanocyte Differentiation

To differentiate hiPSCs into melanocytic derivatives, we used a directed differentiation protocol previously developed by our group[11]. In brief, hiPSC colonies were harvested and cultured in suspension as cell aggregates for 7 days in bFGF-deficient DMEM/F12 medium with L-glutamine containing 20% KnockOut™ Serum Replacement, 100 µM non-essential amino acids, and 100 µM ß-mercaptoethanol (bFGF-deficient hESC medium; all components from Life Technologies, Carlsbad, CA). Cell aggregates were then plated onto plates coated with PBS containing 10 µg/ml human fibronectin (BD Biosciences, San Jose, CA) and cultured in the MelDiff medium which consists of MelM basal medium (ScienCell Research Laboratories, Carlsbad, CA), 4 ng/ml bFGF (Stemgent, San Diego, CA), 20 pM cholera toxin (Enzo Life Sciences, Plymouth Meeting, PA), 50 ng/ml human Wnt3a (R&D Systems, Minneapolis, MN), 50 ng/ml human stem cell factor (SCF; R&D Systems, Minneapolis, MN), 0.1 µM endothelin-3 (Sigma-Aldrich, St. Louis, MO), 100 µM L-ascorbic acid (Sigma-Aldrich, St. Louis, MO), 1x melanocyte growth supplement (ScienCell Research Laboratories, Carlsbad, CA) and 1x insulin-transferrin-selenium solution (Life Technologies, Carlsbad, CA) for 7 additional days. On day 15, the cells were further propagated and matured by culturing them in MelDiff medium containing 1 µM α-MSH (Sigma-Aldrich, St. Louis, MO) for 14 more days. At the end of differentiation (around day 30), cells were maintained in MelM medium (ScienCell Research Laboratories, Carlsbad, CA).

Fluorescence Staining

For staining hiPSCs, EBs and melanocyte differentiation derivatives, cells were rinsed with PBS and then fixed in PBS containing 4% paraformaldehyde for 25 minutes, permeabilized in PBS containing 0.2% Triton X-100 and incubated with antibodies against specific biomarkers. The primary antibodies used here were purchased from Cell Signaling (POU5F1), Millipore (NANOG and SMA), R&D Systems (SSEA4 and SOX17), Covance (TUBB3), Thermo Scientific (MITF), and Santa Cruz (MART-1/Melan-A).

Results and Discussion

Prior investigations attempting to reprogram either dermal fibroblasts or epithelial cells from individual #418, using traditional virus-mediated transductions (Sendai virus and retrovirus), were unsuccessful.
The Lonza L7™ hiPSC Reprogramming and hPSC Culture System was proved a useful tool in overcoming certain reprogramming hurdles during the generation of hiPSCs for individual #418. The system yielded hiPSC-like colonies 6 days after transfection using Nucleofector™ Technology. These hiPSC-like cells displayed typical morphologies of hPSCs in both feeder and feeder-free culture (Figure 2). These cells were positive of many biomarkers for cellular pluripotency including NANOG, POU5F1 and SSEA4 (Figure 3). In addition, the hiPSCs (PBMC418iPS1506 cells) generated from individual #418 PBMCs can be differentiated into cell types belonging to all three germ layers (Figure 4) and also successfully differentiated into melanocytes (Figure 5).

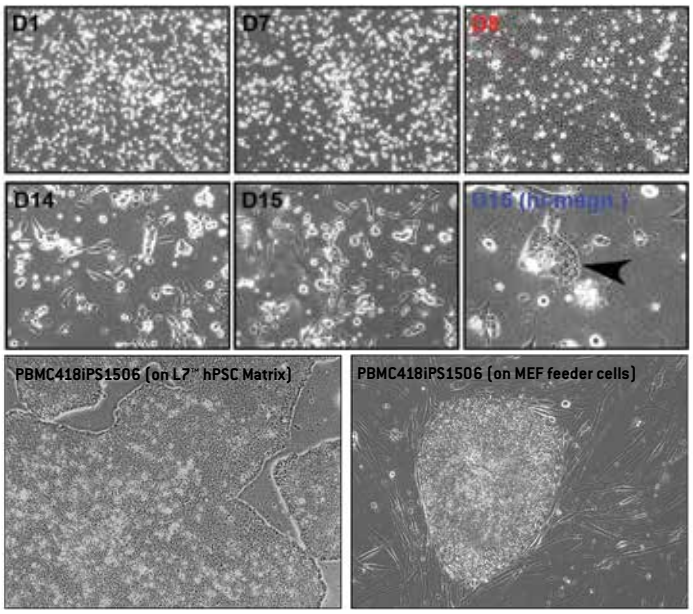

Figure 2. Reprogramming PBMCs isolated from the blood sample of individual #418. 7 days after Nucleofection (D15), small but iPSC-like cell colonies (indicated by an arrowhead) can be observed. PBMC418iPS1506 hiPSCs showed typical morphologies of hPSCs in feeder and feeder-free culture.

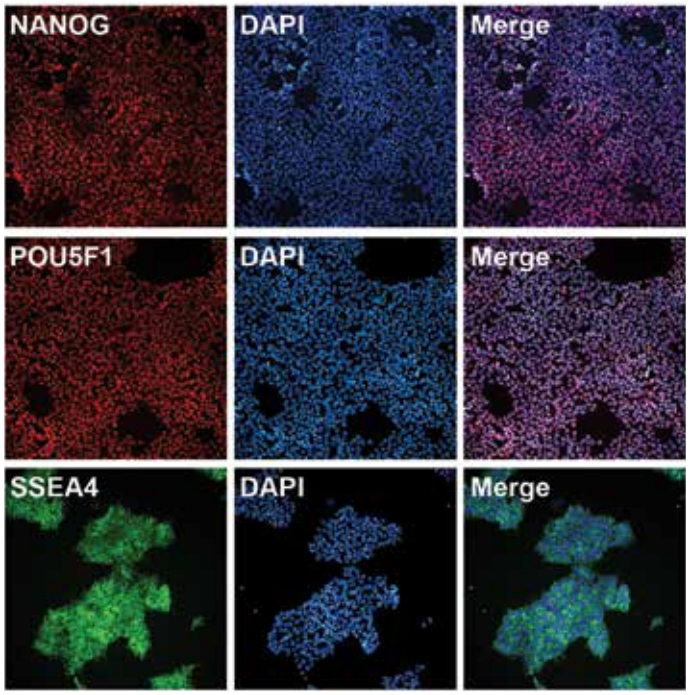

Figure 3. PBMC418iPS1506 cells were positively stained with biomarkers (NANOG, POU5F1 and SSEA4) for cellular pluripotency.

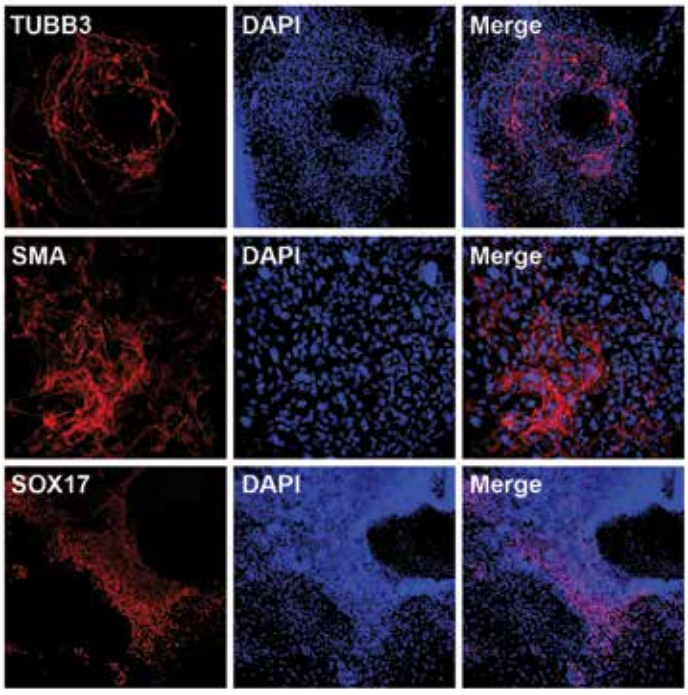

Figure 4. Differentiation of PBMC418iPS1506 cells into cell types relevant to three germ layer lineages through embryoid body formation. Distinct cell types were clearly differentiated as demonstrated by germ layer-specific gene expression: TUBB3 – ectoderm, SMA – mesoderm, SOX17 – endoderm.

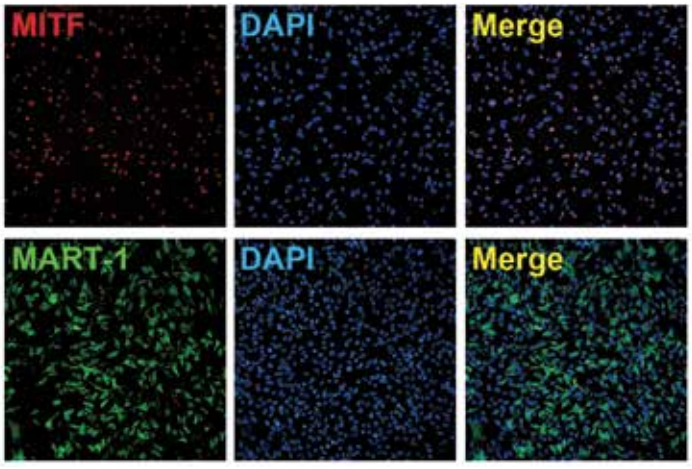

Figure 5. Directed differentiation of PBMC418iPS1506 hiPSCs into melanocytic cells. The differentiated derivatives (PBMC418iPS1506_Mel Diff) expressed melanocytic biomarkers MITF and MART-1.

Conclusion

Here we have shown that the system devised by Lonza effectively reprogrammed PBMCs via episomal vector-mediated transfection, leading to the generation of viable hiPSCs. Pluripotency of the hiPSCs was maintained as demonstrated by subsequent non-directed differentiation (EB formation) and directed differentiation (melanocyte differentiation). Regenerative medicine and the reprogramming of somatic cells are research fields still very much in their infancy, and the generation and culture of consistently robust and high quality hiPSCs represents an ongoing challenge to stem cell biologists. However, human pluripotent stem cells show great promise in numerous research and clinical applications, and thus new developments in technology continue to drive the research forward. For example, our recent work has shown that functional melanocytes can be successfully differentiated from transgene-free hiPSCs[10], providing a reliable protocol for generating unlimited numbers of melanocytes that could be used for modeling physio-pathological development of melanocytes and for providing material for transplantation.

hiPSCs open up multiple opportunities for the development of novel therapies through the exploration of disease mechanisms and novel therapeutic targets, as well as the development of drug screening platforms. The Lonza L7™ hiPSC Reprogramming and hPSC Culture System represents a reliable and robust tool for the generation of hiPSCs, which successfully overcame the "reprogramming-resistance" challenge from a specific case (individual #418) presented in our study. The L7™ hiPSC Reprogramming and hPSC Culture System is likely to be a complete and inclusive approach for hiPSC-generation that streamlines the process of somatic cell reprogramming.

References

1. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–76 (2006).
2. Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–72 (2007).
3. Okita, K. & Yamanaka, S. Induced pluripotent stem cells: opportunities and challenges. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **366**, 2198–207 (2011).
4. Warren, L. *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* **7**, 618–630 (2010).
5. Yu, J. *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. *Science* **324**, 797–801 (2009).
6. Reichman, S. *et al.* From confluent human iPS cells to self-forming neural retina and retinal pigmented epithelium. *Proc. Natl. Acad. Sci.* (2014). doi:10.1073/pnas.1324212111.
7. Chen, J. *et al.* Transcriptome Comparison of Human Neurons Generated Using Induced Pluripotent Stem Cells Derived from Dental Pulp and Skin Fibroblasts. *PLoS One* **8**, (2013).
8. Yu, J., Chau, K. F., Vodyanik, M. A., Jiang, J. & Jiang, Y. Efficient feeder-free episomal reprogramming with small molecules. *PLoS One* **6**, (2011).
9. Chou, B.-K. *et al.* Efficient human iPS cell derivation by a non-integrating plasmid from blood cells with unique epigenetic and gene expression signatures. *Cell Res.* **21**, 518–529 (2011).
10. Wang, Y.-C. *et al.* Specific lectin biomarkers for isolation of human pluripotent stem cells identified through array-based glycomic analysis. *Cell Res.* 21, 1551–1563 (2011).
11. Jones, J. C. *et al.* Melanocytes derived from transgene-free human induced pluripotent stem cells. *J. Invest. Dermatol.* **133**, 2104–8 (2013).

Optimization of Rat Oligodendrocyte Precursor Cell Transfection with the 4D-Nucleofector™ System

By Sabrina Schröder[1,2] and Alexander von Holst[1,2]

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Abstract

Transfection of neural cells comprising neurons and glial cells, as well as their progenitor cells, is limited by their general resistance to commonly used methods such as lipofection or electroporation. The situation improved remarkably when the Nucleofector™ Technology became available, thus enabling transfection of neural cells with high efficiency and reasonable viability. However, limitations were still encountered due to the need of high cell numbers for each transfection sample. Here, we demonstrate that rat oligodendrocyte precursor cells (OPCs) can be transfected with reasonable efficiency and remarkably improved survival rates by using the 4D-Nucleofector™ System. Compared to former systems, the cell number used per sample has been reduced roughly 20-fold when using the strip format. This illustrates a clear improvement over existing transfection capabilities.

Introduction

For several years, transfection has been a widely used approach to demonstrate the functional importance of genes. Allowing for the overexpression or the knockdown of genes, selective information about the respective gene functioning can be obtained. Depending on the cell type used, the transfection process can cause substantial damage. In particular, primary rat OPCs turned out to be rather sensitive with regard to the transfection process. Although the Nucleofector™ Technology made the transfection of OPCs feasible (Czopka *et al.*, 2010)[1], it was limited by the high amount of 5 x 10^6 cells needed for each transfection and a considerable loss of OPCs caused by cell death during the procedure.

To overcome these two disadvantages, we investigated the new 4D-Nucleofector™ System in comparison to previous data obtained for OPCs isolated from early postnatal rat cortices. In comparison to the Nucleofector™ II Device, the 4D-Nucleofector™ System proved advantageous in handling, with a reduction of the cell number per sample as well as the ease of optimization regarding embryonic murine cortical neural stem cells (Bertram *et al.*, 2012)[2]. Having these advantages in mind, an optimization of the transfection conditions for OPCs seemed to be achievable.

The high amount of cells needed for one electroporation process is especially problematic, due to the time-consuming and low-yield isolation of OPCs. With the new 4D-Nucleofector™ X Unit, a minimum of 2 x 10^5 cells is required for each transfection when using the strip format. The new system provides 16-well Nucleocuvette™ Strips for low cell amounts or 100 µl Nucleocuvette™ Vessels allowing the transfection with cell numbers ranging from 2 x 10^5 cells up to 5 x 10^6 cells per cuvette.

In order to establish a protocol for the transfection of OPCs with the new 4D-Nucleofector™ System, we used the pEGFP-C1 plasmid to transfect 2 x 10^5 OPCs isolated by a shaking protocol after one week of culture of cells derived from early postnatal rat cortices (Czopka *et al.*, 2010; Milner and Ffrench-Constant, 1994)[1, 3]. Transfected cells were recovered shortly after various pulses and plated directly on poly-L-ornithine-coated 4-well dishes. The number of successfully transfected cells was determined by imunocytochemical stainings against EGFP, the fluorescent tracer protein of the construct. Based on the already established program for the transfection of cortical mouse neural stem cells, similar programs were tested for the transfection of OPCs.

Materials and Methods

Isolation of Cortical Rat OPCs

Single cell suspension, obtained from early postnatal rodent brains (P2-3) by trypsination, was cultured for 7 days in mixed glial culture medium (MGC), which consisted of Dulbecco's modified minimal essential medium (DMEM) with 10% fetal calf serum (FCS) and 1% penicillin/streptavidin. The separation of astrocytes, OPCs and microglia in two layers is exploited to remove the OPCs and the microglia from the bottom layer of astrocytes by shaking on an orbital shaker overnight. The separation of microglia and OPCs was executed by a short pre-plating on a bacterial dish where microglia attach to the bottom of the vessel and the OPCs remain in the supernatant. The resulting cell suspension consists mainly of OPCs and immature oligodendrocytes that can be either expanded in chemically-defined medium (CDM), consisting of DMEM with 1% N2-supplement, 1% penicillin/streptavidin and 100 µg/ml BSA V in the presence of PDGFα and FGF-2, or differentiated by the addition of 1% FCS and T3 instead of the aforementioned growth factors.

Vector

Our routinely used antibody against green fluorescent protein (GFP) bypassed the use of the pmaxGFP™ Vector (Lonza) provided with the Nucleofector™ Kit due to limited detection ability. In order to determine the transfection rates as accurately as possible, we used the pEGFP-C1 plasmid (Clontech) instead.

Transfection Using the 4D-Nucleofector™ System

Due to the low yield isolation of OPCs, 16-well Nucleocuvette™ Strips (20 µl per sample) were used for the optimization phase, as they allow for the transfection of lower cell numbers. 2 x 10^5–5 x 10^5 cells, derived from one week old glial cultures obtained from rat cortices, were suspended after centrifugation (220 x g, 5 minutes) in 20 µl P3 Primary Cell 4D-Nucleofector™ Solution containing 0.5 µg plasmid DNA. Transfection was performed according to the guidelines provided by Lonza. In a pulse screening Nucleofector™ Program, CA-138 was determined as being the optimal program for rat OPCs. After transfection, 180 µl prewarmed CDM was added to the transfected OPCs. The OPCs were gently suspended and transferred to a sterile 1.5 ml tube. Cells were kept for 15 minutes at 37°C for recovery. Depending on the further approach, more medium was added and cells were either plated directly on poly-L-ornithine-coated 4-well dishes for differentiation or were expanded in CDM containing FGF-2 and PDGFα. Optionally, for a reduction of possible debris, the medium was replaced by fresh CDM after the cells were attached.

The transfection of higher cell numbers (2–5 x 10^6) was conducted in 100 µl Nucleocuvette™ Vessels, after the resuspension of OPCs with 100 µl P3 solution with 5 µg of plasmid DNA. The resuspension after transfection occurred with 500 µl prewarmed CDM. After the recovery step, cells were plated on poly-L-ornithine-coated dishes. Again, replacing the medium after attachment of the cells minimized debris.

Immunocytochemical Staining

Successfully transfected OPCs were detected with an antibody against EGFP. Antibodies against PDGF receptor α (PDGFRα; Santa Cruz), O4 and Myelin Basic Protein (MBP, both Sigma Aldrich) were used as markers for OPCs, immature and mature oligodendrocytes, respectively. The transfection efficiency was determined 24–48 hours post transfection by counting the number of GFP-positive cells with regard to the total cell number determined by Hoechst staining of cell nuclei.

Results

Evaluation of an Optimized Nucleofector™ Program for Rat OPCs

Our group already established a protocol for the transfection of sensitive mouse neural stem cells (Bertram *et al.*, 2012)[2]. The efficient electroporation of mouse neural stem cells utilizing the 4D-Nucleofector™ System was the starting point for improving the transfection conditions for rat OPCs. Since we previously determined the program DS-113 as the best pulse for transfecting neural stem cells (Bertram *et al.*, 2012)[2], we started the first experiments with freshly isolated OPCs with this protocol. It turned out that this pulse was not suitable for the transfection of OPCs, because both the transfection rate and, in particular, the viability (survival of cells after transfection) were insufficient (Figure 1).

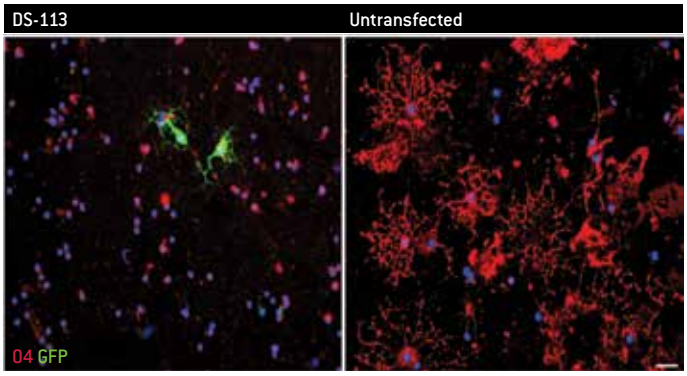


Figure 1. Transfection results with suboptimal conditions. Photomicrographs of transfected OPCs with P3 solution and program DS-113 are shown on the left side. EGFP is visualized with the fluorochrome Cy2 (green). O4 labels immature oligodendrocytes and is visualized with Cy3. Cell nuclei are Hoechst-stained (blue). For pointing out the suboptimal transfection conditions, untransfected OPCs are depicted as well. Scale Bar: 30 µm

To optimize conditions, we tested various parameters (see Table 1) including further pulses recommended by Lonza. These pulses were tested in parallel on P2 rat cortical OPCs with P3 primary cell solution using 16-well Nucleocuvette™ Strips. Due to the sensitivity of OPCs toward the electroporation process, cell viability was initially chosen as a parameter to evaluate the tested pulse protocol. The first programs tested showed a comparatively low viability, and the surviving cells failed to differentiate into oligodendrocytes. Therefore, a meaningful quantification of the transfection efficiency was impossible and revealed that these pulses were also affecting the cells too strongly. Based on these results, Lonza provided 26 additional pulses that were tested for the viability of OPCs after Nucleofection. The cell viability increased dramatically (Figure 2). Among the many different pulses tested, we found the pulse protocol CA-138 with a cell viability of 90 ± 21% (n = 10) of OPCs well suited and allowed for a quantification of the transfection efficiency.

Pre-transfection	Transfection	Post transfection
– Coating of 4-well dishes (poly-L-ornithine, poly-D-lysine, pre-incubation with medium) – Centrifugation steps (duration, RPM) – Amount of cells per transfection (2.5–9 x 10^5)	– Solutions (P1, P3) – Amount of plasmid (0.1 µg, 0.25 µg, 0.5 µg, 1 µg) – Different plasmids (pmaxGFP™, pEGFP-C1, pEGFP-N1) – 26 pulses tested (weak pulse, e.g. CA-140 → high viability/ low transfection rate; strong pulse, e.g. DS-113 → low viability/ high transfection rate)	– Recovery steps (e.g. recovery over night under several conditions, or for max. 30 minutes at 37°C after transfection) – Start of cell culture (either direct plating of cells with transfection solution, or previous resuspension of cells in fresh medium – Amount of plated cells (2–5 x 10^4)

Table 1
Parameters changed during the optimization procedure, prior to, during and post transfection.

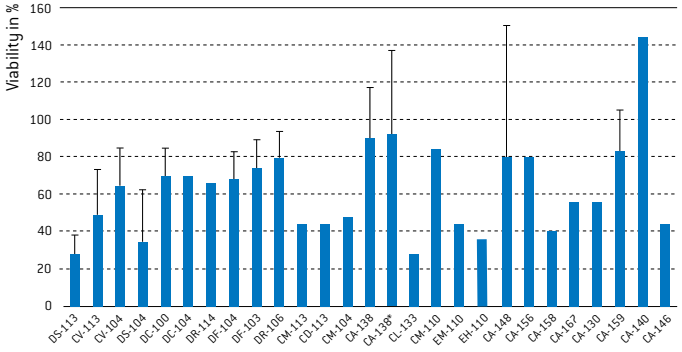


Figure 2. Pulse screening for best viability of OPCs. OPCs were transfected without DNA using 26 different programs (exception: CA-138* = CA-138 with 1 µg pEGFP-C1). OPC viability was determined directly post transfection and is expressed as mean ± SD. Bars with SD represent at least 3–10 independent experiments. Those without error bars were performed once or twice. Counting low cell numbers with the Neubauer cell chamber (around 250,000 cells) can cause variation, explaining viability rates above 100%.

To do so, we compared the pulse CA-138 and five other pulse protocols with reasonable cell survival rates for the quantification of the transfection efficiency. The latter was determined by counting the number of 04-positive and EGFP-positive immature oligodendrocytes as determined by double-immunocytochemisty (Figure 3). We found that the program CA-138 resulted in 17 ± 7% (n = 10) of transfected oligodendrocytes, which was the best transfection rate we obtained (Figure 3).

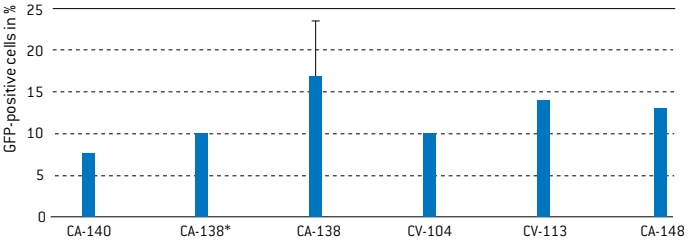


Figure 3. Transfection efficiency for OPCs with pre-selected pulses. OPCs were transfected with 0.5 µg pEGFP-C1 (CA-138*: 1 µg) using a set of pre-selected pulses based on cell viability and morphology. EGFP-positive cells were quantified 24 hours post transfection.

We wondered if Nucleofection™ Program CA-138 was not only compatible with cell survival and successful transfection of OPC, but if it also allows for the differentiation and characteristic morphological maturation of cultured OPCs (Zhang, 2001)[4]. Since the morphological maturation of OPCs along the lineage toward mature oligodendrocytes is characterized by the expression of specific proteins, we performed double-immunostainings against EGFP and defined lineage markers of OPCs that were allowed to differentiate for 48 hours after Nucleofection with pulse CA-138. We observed a normal pattern of differentiation and maturation because we recorded transfected OPCs, identified by the expression of the PDGFRα, transfected immature oligodendrocytes expressing 04, and mature oligodendrocytes expressing the myelin basic protein MBP (Figure 4).

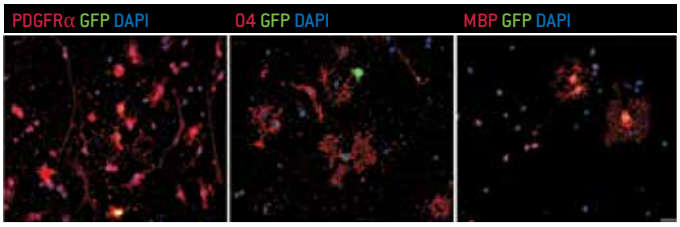


Figure 4. Maturation of OPCs post Nucleofection. Immunocytochemical stainings of cultured OPCs 48 hours after transfection with the program CA-138. In the above photomicrographs, the transfected EGFP-positive cells are visualized with the fluorochrome Cy2 (green). Left: Co-staining against the PDGFRα (Cy3, red) that marks OPCs. Middle: Co-staining against 04, which labels immature oligodendrocytes Cy3 (Cy3, red). Right: Co-staining against MBP, which becomes expressed in mature oligodendrocytes. Cell nuclei are Hoechst-stained (blue) in the entire panel. Scale bar 30 µm.

Therefore, the pulse protocol CA-138 is well suited for transfection of OPCs using the 4D-Nucleofector™ System and compatible with normal differentiation and maturation.

Conclusion

The 4D-Nucleofector™ System represents an improved transfection technology enabling the electroporation of sensitive cell types. Here, the 4D-Nucleofector™ System was tested for the transfection of primary rat cortical oligodendrocyte precursor cells. These cells are well known to be very sensitive toward transfection. The transfection of adherent mouse oligodendrocytes is already satisfactorily working (Fruhbeis *et al.*, 2013)[5], whereas the transfection of non-adherent OPCs was so far rather inefficient.

In this extensive study of different pulse protocols, we demonstrated that transfection of non-adherent OPCs is now reproducibly possible with a high viability, normal differentiation and an acceptable transfection efficiency of up to 25%. In combination with the huge advantage that a low cell number of only 2 x 10^5 cells is needed for the single electroporation process with the 4D-Nucleofector™ System, we decided not to further attempt additional improvements.

References

1. Czopka TA *et al.* (2010) *J Neurosci.* 30:12310–12322.
2. Bertram BS *et al.* (2012) *J Neurosci Methods.* 209:420–427.
3. Milner R and Ffrench-Constant C (1994) *Development.* 120:3497–3506.
4. Zhang SC (2001) *Nat Rev Neuroscience.* 2:840–843.
5. Frühbeis CD *et al.* (2013) *PLoS Biol.* 11:e1001604.

New The CytoSMART™ System

Your Personal Live Cell Imaging Device

The era of expensive and laborious live cell imaging is over. Experience the CytoSMART™ System – your personal cell culture assistant.

The CytoSMART™ Device, roughly the size of a T-75 flask, fits snuggly into any incubator. It is operated via a tablet outside the incubator, which transmits the captured data to a German hosted cloud. This means you can monitor your cell culture conveniently outside the lab from any browser capable system, be it computer, laptop, smartphone or your personal tablet device.

Applications

Scratch assays – perform and record the results of your scratch assays automatically

Document your cell culture – conveniently record cell morphology, growth and temperature

Monitor your cell culture from outside – while the cells remain in the incubator

Monitor iPSC-generation or remotely view your MSC culture under hypoxic conditions

Get an automatic alert when your cell culture has obtained a desired confluency

Retrieve your cell culture data anytime – anywhere with the secure CytoSMART™ Cloud System

Experience your personal CytoSMART™ System now!

www.lonza.com/cytosmart

Product Highlights

ZFN = Zinc Finger Nuclease

TALEN = Transcriptional Activator-like Effector Nuclease

CRISPR/Cas9 = Clustered Regularly Interspaced Palindromic Repeats and CRISPR-associated Nuclease 9

Genome Editing Using Nucleofector™ Technology

Successful Transfection of CRISPR & Co.

For genome editing, engineered nucleases are transferred into cells to delete, insert or replace a gene at a targeted genomic location. Those nucleases are fused or interact with sequence-specific DNA-binding components directing the nuclease to the target sequence.

Lonza's non-viral Nucleofector™ Technology has been shown to work as a reliable and sufficient method for transferring the required DNA- or RNA-based components into various cell lines and primary or stem cells, e.g., primary T cells, human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSCs).

ZFN or TALEN: When using ZFN or TALEN, two plasmids or mRNAs are transfected expressing a fusion protein consisting of Fok1 nuclease fused to a sequence-specific ZF or TALE protein.

CRISPR/Cas9: For CRISPR-based genome editing, various transfection scenarios are possible. This includes transfer of one plasmid carrying both the guide RNA (gRNA) and Cas9 nuclease or co-transfection of two separate plasmids (one for the gRNA and one for Cas9). Alternatively, the gRNA can be expressed via PCR cassette (Ran FA *et al.* 2013).

For all systems, an additional donor/repair plasmid or a single-strand oligonucleotide (ssODN) has to be co-transfected when aiming for insertion or replacement.

Benefits of the Nucleofector™ Technology for Genome Editing

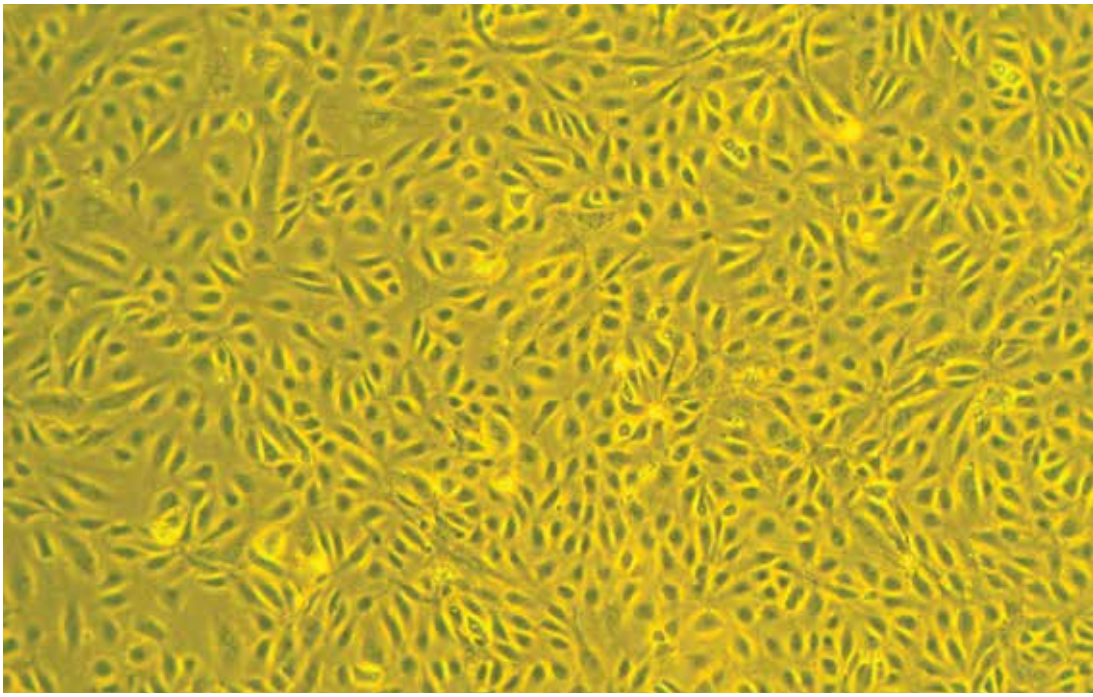
- High transfection efficiencies for a broad range of cell types, including iPSCs
- Efficient co-transfection of various substrates
- Same conditions for transfecting plasmids, DNA, mRNA or PCR cassettes, ssODN
- Proven for ZFN, TALEN and CRISPR by more than 30 publications, including high ranking journals

www.lonza.com/genome-editing

Genome Editing Nucleofection™ Publications

Authors	Citation	Year	Tool	Nucleofector™ System	Cell Type
Fung H *et al.*	PLOS ONE May 2011, e.0020514	2011	ZFN	Nucleofector™ I/II/2b Device	hESC
Zou J *et al.*	Blood **117**:5561–5572	2011	ZFN	Nucleofector™ I/II/2b Device	iPSC
Zou J *et al.*	Blood **118**:4599–4608	2011	ZFN	Nucleofector™ I/II/2b Device	iPSC
Torikai H *et al.*	Blood **119(24)**:5697–705	2012	ZFN	Nucleofector™ I/II/2b Device	Human T cells
Liu X *et al.*	PLOS ONE May 2012, e.0037071	2012	ZFN	Nucleofector™ I/II/2b Device	hES
Schjoldager K	PNAS **109**:9893–9898	2012	ZFN	Nucleofector™ I/II/2b Device	HepG2
Wang J *et al.*	Genome Res **22**:1316–1326	2012	ZFN	Nucleofector™ I/II/2b Device and 96-well Shuttle™ Add-on	K562
Ou W *et al.*	PLOS ONE Nov 2013, e0081131	2013	ZFN	Nucleofector™ I/II/2b Device	iPSC
Qu X *et al.*	Nucleic Acids Res **41**:7771–7782	2013	ZFN	Nucleofector™ I/II/2b Device	HIV-infected PBL + CD4 T cells
Richter S *et al.*	PLOS ONE Jun 2013, e0065267	2013	ZFN	Nucleofector™ I/II/2b Device	HTC116 + H460
Robbez-Masson LJ *et al.*	PLOS ONE **8(11)**:e78839	2013	ZFN	Nucleofector™ I/II/2b Device	MCF7
Samsonov A *et al.*	PLOS ONE July 2013, e0068391	2013	ZFN	Nucleofector™ I/II/2b Device	A549
Toscano MG *et al.*	Dis Model Mech **6**:544–554	2013	ZFN	Nucleofector™ I/II/2b Device	K562
Genovese P *et al.*	Nature **510**:235ff	2014	ZFN	4D-Nucleofector™ System	hCD34
Piganeau M *et al.*	Genome Res **23**:1182–1193	2013	TALEN/ZFN	Nucleofector™ I/II/2b Device	hESC and Jurkat cells
Yang L *et al.*	Nucleic Acids Res **41**:9049–9061	2013	TALEN/Cas9	4D-Nucleofector™ System	iPSC
Zhu F *et al.*	Nucleic Acids Res **10**:1093/nar/gkt1290	2014	TALEN/DICE	4D-Nucleofector™ System	iPSC + H9 hES
Petit Cs *et al.*	J Cell Biol **202**:1107–1122	2013	CRISPR	Nucleofector™ I/II/2b Device	HeLa
Ran FA *et al.*	Cell **154**:1380–1389	2013	CRISPR	4D-Nucleofector™ System	Various cell lines, e.g., HEK293FT
Ran FA *et al.**	Nat Prot **8(11)**:2281–2308	2013	CRISPR	4D-Nucleofector™ System	HUES62 + HEK293

*Provides a comprehensive protocol for use of the 4D-Nucleofector™ with CRISPR/Cas9



Studying VEGF's Role in Tumor Progression

New Primary HUVECs Cultured Without Additive VEGF Will Advance Your Research

Vascular endothelial growth factor (VEGF) is a protein that is involved in the creation of new blood vessels (angiogenesis). The overexpression of this gene has been linked to solid tumor progression and retinal vascular diseases. Human umbilical vein endothelial cells (HUVECs) cultured without additive VEGF offer expanded applications in cancer research, angiogenesis studies, cardiovascular disease research, and wound healing. These cells provide flexible options to researchers who can use them to understand the implications of the presence and absence of VEGF.

Lonza supports advances in vascular research by introducing umbilical vein endothelial cells cultured in the absence of additive vascular endothelial growth factor (VEGF).

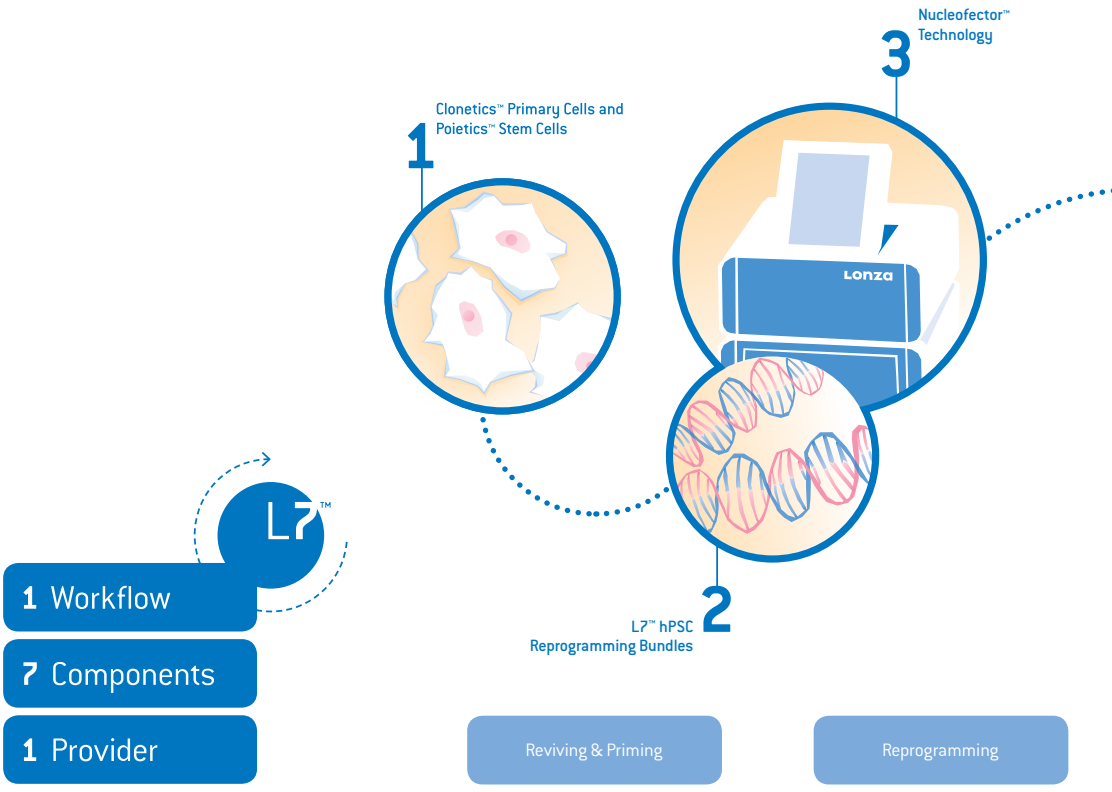
Our new EGM™-Plus Growth Media Kit is now available to support these cells and yields much higher cell proliferation rates.

Lonza's new HUVECs and Media without additive VEGF are an improved version of our existing HUVECs cultured in EGM™ Media. HUVECs cultured in EGM™-Plus Media offer better proliferation rates while maintaining the same high quality characterization as HUVECs in EGM™ Media.

Other Lonza endothelial products include EGM-2™ Growth Media, which sets the industry standard for high quality. Our pre-screened HUVECs that express the VEGFR2 receptor offer more characterization with additional vascular cell health receptors. With this new addition of HUVECs in EGM™-Plus Media, we now provide a more complete solution to our customers who rely upon Lonza for their vascular research cell culture needs.

Lonza currently offers more than 30 different types of primary human endothelial cells including cells from normal, diabetic type I and II donors, with tissue sources ranging from heart, lung, skin, and reproductive organs. Endothelial growth media systems have been specifically developed and optimized for all cell systems.

www.lonza.com/huvec



Coming Soon – Reliable iPSC-generation from PBMCs Using the L7™ Reprogramming Bundle

The Nucleofector™ Technology has been demonstrated to be an efficient and cost-effective non-viral alternative for iPSC-generation and is being used by 2012 Nobel prize winner Dr. Shinya Yamanaka (Kyoto University) and other leading scientists around the world.

Using the 4D-Nucleofector™ System, Lonza's pluripotent stem cell innovation team has developed an optimized protocol for reprogramming of PBMCs (erythrocyte population) and is continuing with further cell types (e.g., fibroblasts).

Components of the L7™ hiPSC Reprogramming and hPSC Culture System

Coming soon! For PBMC reprogramming:

1. L7™ PBMC Priming-Recovery Medium BulletKit™ – containing optimized supplements and reprogramming enhancers
2. L7™ Episomal Vector Kit I
3. 4D-Nucleofector™ System – now coming with a fully-defined protocol for PBMC reprogramming

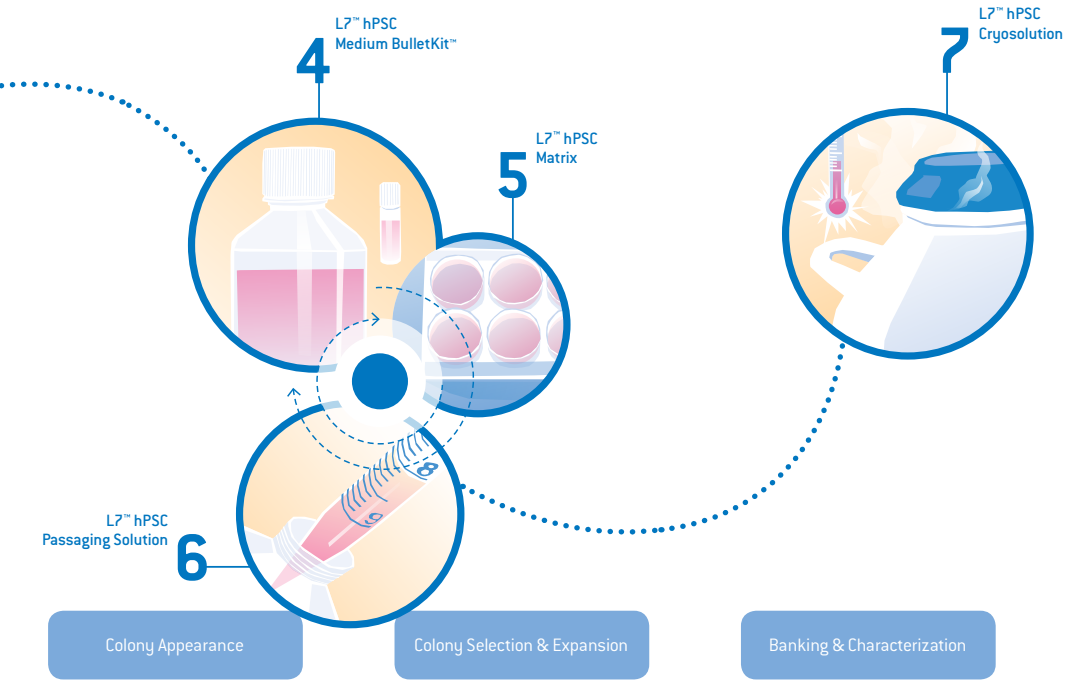
For iPSC culture:

4. L7™ hPSC Medium BulletKit™
5. L7™ hPSC Matrix
6. L7™ hPSC Passaging Solution
7. L7™ hPSC Cryosolution

Benefits

- Efficient, non-viral and xeno-free reprogramming – suited for clinical applications
- Optimized PBMC priming and recovery media improving reprogramming efficiency
- iPSC colonies ready for picking in less than 2 weeks
- Guarantees seamless workflow in combination with our L7™ hPSC Culture System for feeder- and xeno-free iPSC culture

www.lonza.com/L7



New Xeno-free, Defined Culturing of Human Pluripotent Stem Cells

Lonza has developed the new L7™ hPSC Culture System, which contains a medium, matrix, passaging solution and cryosolution for the culturing of human embryonic stem cells and human induced pluripotent stem cells.

The L7™ hPSC Culture System allows for medium changes every other day and has been proven to maintain karyotype and pluripotent marker expression for over 40+ passages.

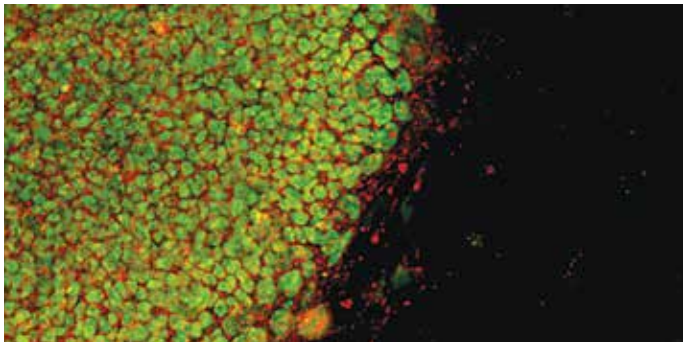
Benefits

- Every-other-day feeding – daily maintenance of hPSCs no longer necessary
- Typical, healthy morphology – similar look and growth characteristics as every-day feeding
- Maintained pluripotency – cells cultured for 40+ passages in L7™ hPSC Medium show high level of pluripotency marker expression

www.lonza.com/L7

Let's Talk About Culture and Generation of Human Induced Pluripotent Stem Cells (hiPSCs)

In this issue of our **TechTalk**, we will provide you with guidelines for feeder-free, xeno-free culture of iPSCs and efficient generation and modification of iPSCs.



Q. How can I use the Nucleofector™ Technology for the generation of hiPSCs?

A. Lonza offers ready-to-use Nucleofection™ Protocols for virtually all somatic cell types (e.g., fibroblasts, keratinocytes, $CD34^+$ hematopoietic stem cells, PBMCs, etc.) that are typically used for reprogramming into iPSCs. It has been proven by various publications that the Nucleofector™ Technology offers a reliable tool for generating iPSCs in combination with episomal vectors. You would use the standard transfection conditions provided in the respective protocol, but may have to optimize the amount and ratio of the used vectors and the culture of cells prior to and post Nucleofection. For the most frequently used cell types, we will soon offer a ready-to-use reprogramming protocol, together with the required accessory products, to simplify your reprogramming approach. Watch for our L7™ Reprogramming Protocols.

Q. How many colonies can I achieve when reprogramming PBMCs with the Nucleofector™ Technology?

A. This is hard to predict. It strongly depends on the quality of the PBMC donor and how you culture the cells before and after reprogramming. It has been shown that the use of a hypoxic incubator could be advantageous when working with a bad donor to increase colony numbers.

Q. Can I also use the Nucleofector™ Technology for transfecting human iPSCs, e.g., for genome editing with CRISPR?

A. Yes. It has been proven by more than 30 publications that the Nucleofector™ Technology provides an efficient tool for genome editing, whether you want to use ZFNs, TALENs or CRISPR. As each iPSC clone might be slightly different, we would recommend to follow our basic protocols for human stem cells to determine the optimal Nucleofection™ Conditions for your clone. Once defined, you can use those conditions for your substrates of interest. When conducting co-transfection of multiple plasmids or combinations of plasmids and mRNA, you may have to fine-tune substrate amounts and ratios. Please refer to existing Nucleofection™ Publications for your genome editing tool of interest (e.g., Ran *et al. Nature Protocols* 8(11):2281–2308, 2013).

Q. Does Lonza offer a xeno-free, defined system for culturing human pluripotent stem cells (hPSCs)?

A. Yes. In April 2014, Lonza launched the L7™ hPSC Culture System. The components of the culture system include:

- L7™ hPSC Medium BulletKit™ (FP-5007)
- L7™ hPSC Matrix (FP-5020)
- L7™ hPSC Passaging Solution (FP-5013)
- L7™ hPSC Cryosolution (FP-5002)

Q. Can these products be used with only human cells or other species as well?

A. The L7™ hPSC Culture System was developed to work with human cells, and we have not conducted in-house studies with mouse or rat cells. **Human pluripotent stem cells only!**

Q. Can I use just one product of the system?

A. The system was designed as a whole. For optimal results, you should use all components of the L7™ hPSC Culture System.

Q. What are the major features of this system over the competition?

A. The L7™ hPSC Culture System allows for every-other-day feeding of human pluripotent stem cells, maintains high cell viability and efficiency after serial passaging, maintains normal karyotype and allows for spontaneous and directed differentiation of human pluripotent stem cells even after 40+ passages of culturing.

Q. What is the optimized feeding schedule?

A. The L7™ hPSC Medium is designed to feed the hPSCs every other day. For the best performance, we recommend changing the medium the day after passaging and the day before passaging for long-term maintaining of hPSCs. However, if you prefer to split the cells at 80% or less confluence, there is no need to feed the cells the day before passaging if it does not fall on the feeding schedule. Morphologically, the hPSCs may seem a bit stressed without feeding for 2 days when the culture is at 70% confluence or above. Once fed with fresh medium, the hPSCs will recover quickly.

Q. Can you skip feeding over the weekend?

A. If the cells are at a lower confluence (20%), they can sustain the weekend without feeding. If the cells are above 50% confluence on Friday, a media change would be needed Sunday or else the cells could be stressed and you would have problems passaging on Monday.

Q. Is there variability in the L7™ hPSC Culture System?

A. All hESC and hiPSC lines will behave differently, and while we have tested quite a few, we have not tested them all, so it is possible you will see some variation.

Q. Is there any adaptation required when switching to the L7™ hPSC Culture System?

A. There could be. Please check **www.lonza.com/L7** for specific protocols depending on the products currently used.

General FAQs

Q. How do pluripotent stem cells differ from adult stem cells?

A. Adult stem cells are partially differentiated stem cells present in adult tissues. They are multipotent, i.e., capable of forming a limited number of specialized cell types. Pluripotent stem cells, such as embryonic stem cells (ESCs) or iPSCs, can give rise to any of the 200+ fetal or adult cell types and have the ability to self-renew indefinitely.

Q. What are iPSCs and how are they generated?

A. They are pluripotent stem cells that were artificially derived (reprogrammed) from non-pluripotent somatic cells by inducing a "forced" expression of stem-cell-associated genes using transfection. They are believed to be identical to embryonic stem cells in many respects, e.g., they can self-renew in culture and have the potential to differentiate into all human cell types, but the full extent of their relation to natural pluripotent stem cells is still being assessed. As with ESCs, they could be differentiated into various human cells for studying human development or disease pathogenesis. Those iPSC-derived cells can also serve as novel human disease models for performing *in vitro* drug screening and toxicity studies. In addition, they might be a powerful tool to develop cell transplantation therapies for the treatment of degenerative diseases, including diabetes, Parkinson's and a number of cardiovascular diseases. Stem-cell-specific transcription factors are transferred into primary somatic cells by either viral transduction or non-viral transfection. Expression of these factors has been shown to be able to revert a somatic cell back into a pluripotent stem cell. Typical reprogramming factors used in different combinations are Oct4, Sox2, Klf4, c-Myc, Nanog and Lin28.

Q. What potential advantages do human iPSCs have over ESCs?

A. iPSCs can be generated in the lab by a simple method with no ethical controversy. Since hiPSCs can be made from the patient's own skin or blood, reprogramming can provide a source of patient-specific (genetically identical) specialized cells that would be recognized by the patient's body and would not be rejected. iPSCs also offer scientists an opportunity to model a disease *in vitro* on a patient-by-patient basis, enabling screening for individual genomic differences that may aid in disease progression, or even for pharmacologic screens to determine which pharmacologic agents are ideal for each individual.

Q. What is an episomal vector and why is this unique?

A. Episomal vectors are oriP/EBNA1-based vectors that replicate independently in eukaryotic cells. They do not integrate into the genome, but stay as extrachromosomal elements in the nucleus. By using episomal vectors, undesired side effects can be avoided, such as insertional mutagenesis that can occur with viral vectors. Introduction of episomal plasmids to generate integration-free hiPSCs with a non-viral transfection has resulted in successful reprogramming of multiple cell types and made iPSC-generation easy to establish in the research and clinical laboratories. Moreover, the episomal vectors disappear completely after serial passaging, leaving iPSCs with a "zero footprint" genome.

Q. What is the advantage of using serum-free media?

A. Use of culture media containing undefined components limits the development of downstream applications for pluripotent stem cells (PSCs) that could lead to unknown responses. Some responses of using undefined components with PSCs could be impact on self-renewal, differentiation, and lineage selection. Overall, a defined serum-free medium for PSCs could contribute to advances in the field and thus is highly recommended.

Q. What does xeno-free mean?

A. Xeno-free is a term that means a product can contain components from human origin, but no other species. Many researchers concerned about cross-contamination choose to use xeno-free cell culture products.

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