BioResearch

Pluripotent Stem Cells – From Research to Therapy

Webinar

16 June 2015 / Speaker: Mr. Andrew Winner
17 June 2015 / Speaker: Dr. Heiko Bueth

Lonza

Forward Statement

Certain matters discussed in this presentation may constitute forward-looking statements. These statements are based on current expectations and estimates of Lonza Group Ltd, although Lonza Group Ltd can give no assurance that these expectations and estimates will be achieved. Investors are cautioned that all forward-looking statements involve risks and uncertainty and are qualified in their entirety. The actual results may differ materially in the future from the forward-looking statements included in this presentation due to various factors. Furthermore, except as otherwise required by law, Lonza Group Ltd disclaims any intention or obligation to update the statements contained in this presentation.

Agenda

- Background
- Considerations for iPSC researchers with clinical aspirations
- Generation of human iPSCs from peripheral blood mononuclear cells (PBMCs)
- Xeno-free, defined culturing of human PSCs
- L7™ hiPSC Reprogramming and hPSC Culture System in action – Case studies

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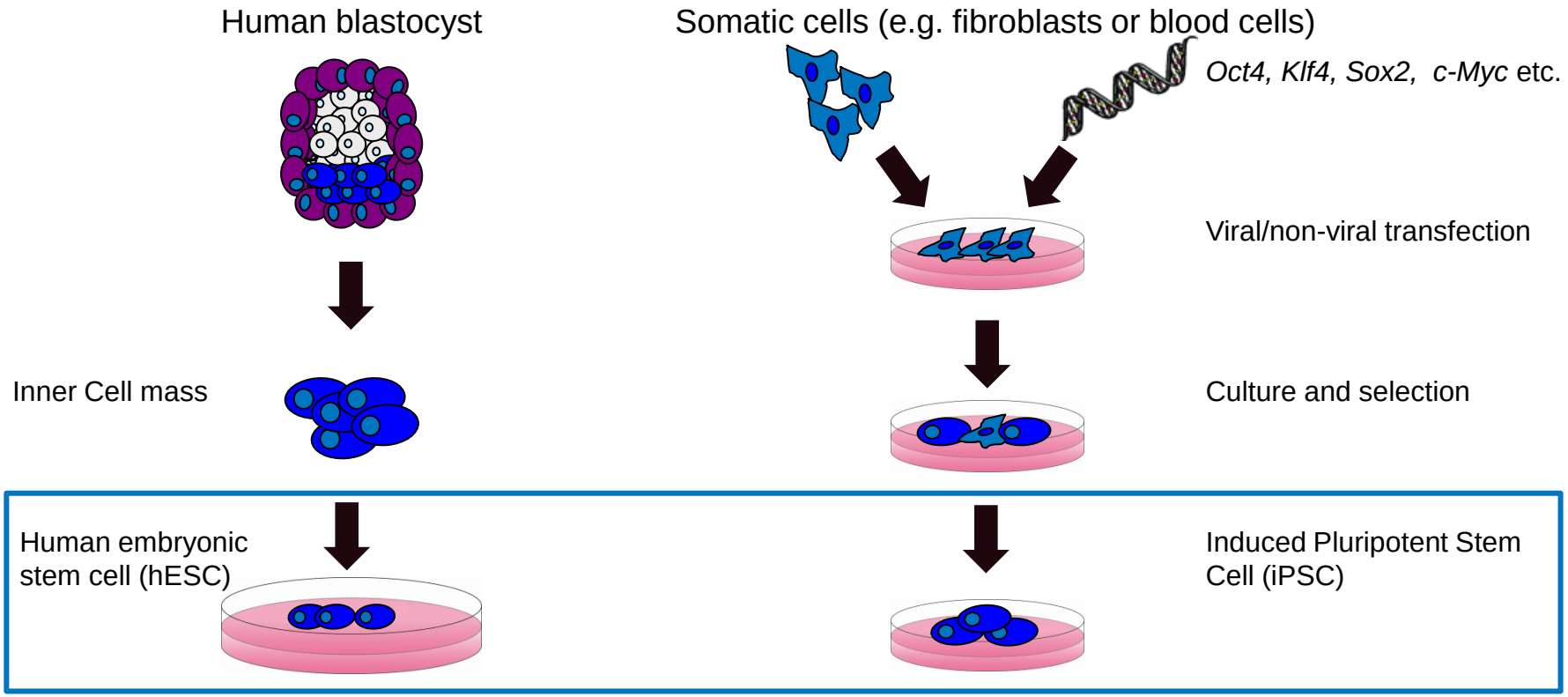
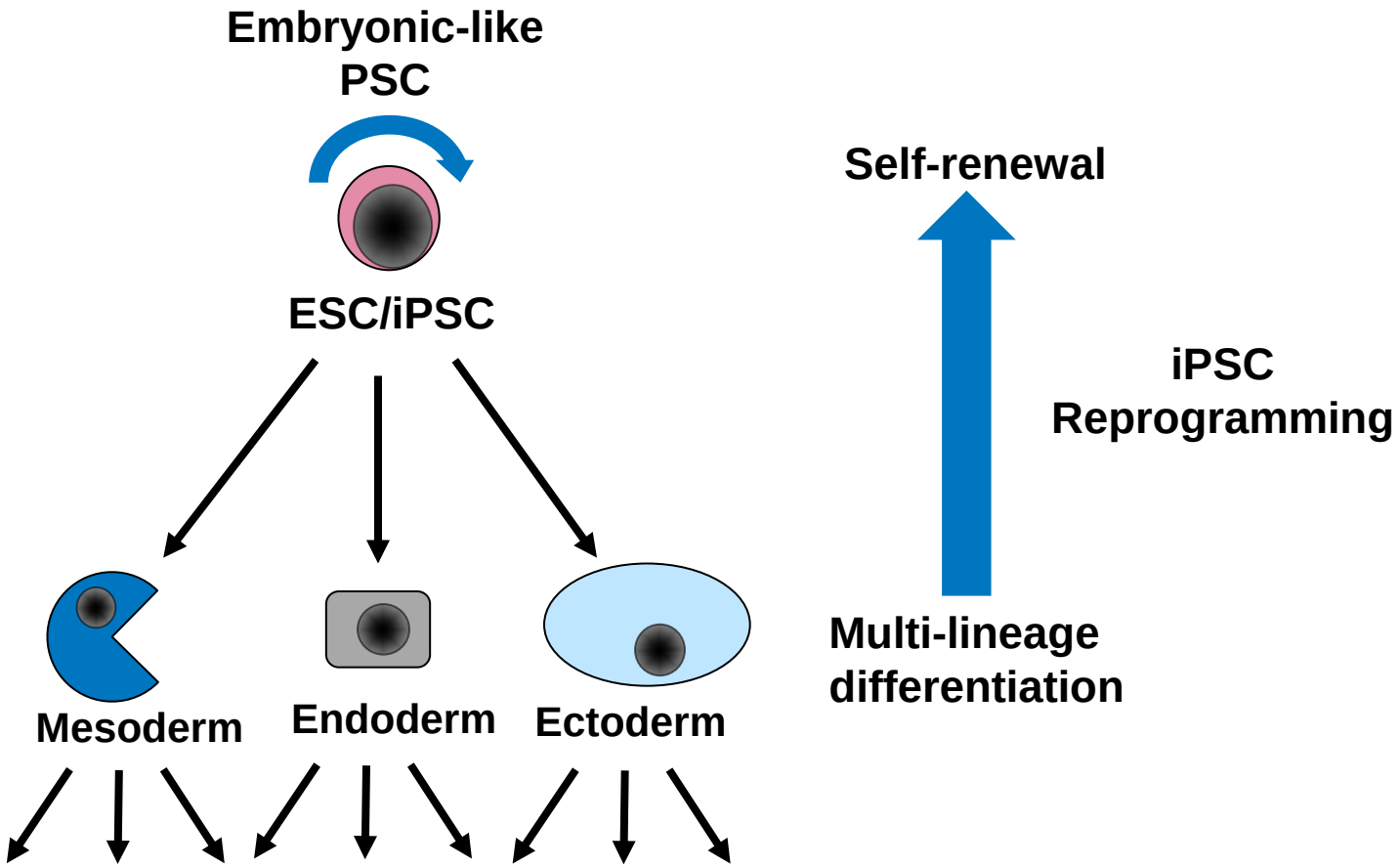
Pluripotent Stem Cells



The Hallmarks of Pluripotent Stem Cells



Characteristics of PSCs

- PSCs possess these characteristics:
  - Morphology – large nuclei, refractive edge, cobblestone spacing
  - Marker Expression – Nanog, Tra-1-60, etc
  - Karyotype Analysis – Normal through many passages
  - Teratoma Formation – Gold standard for hPSCs
  - Differentiation Potential – What do your cells do?

Human PSC Culturing

- What are the potential issues?
  - Every day feeding
  - Feeder culture
  - Cell recovery – efficient attachment after passaging
  - Differentiation bias
  - Karyotype issues
  - Viability issues
  - Clinical aspirations

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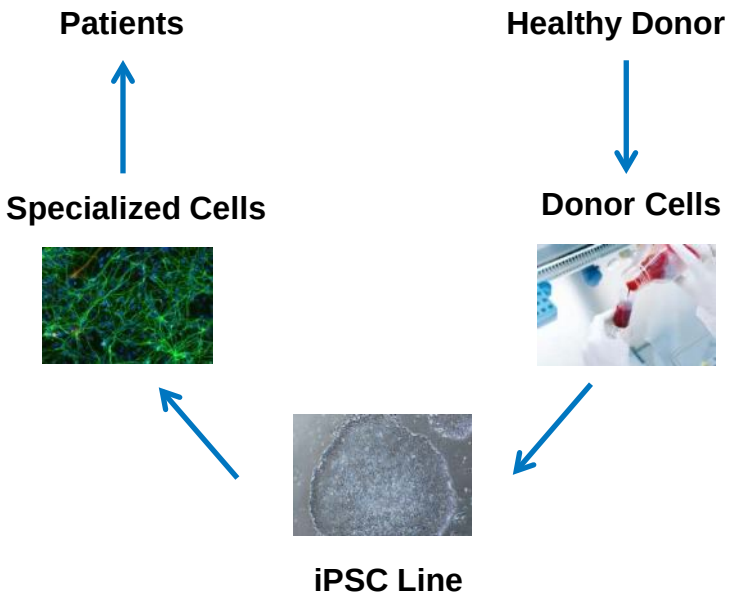
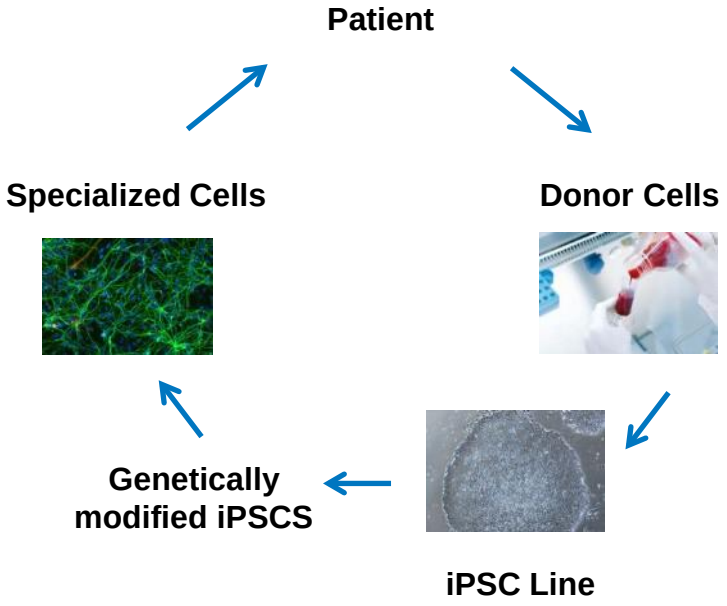
The Potential of Pluripotent Stem Cells for Clinical Applications

Allogeneic Therapy
(HLA-matched, HLA-null)



Autologous Therapy

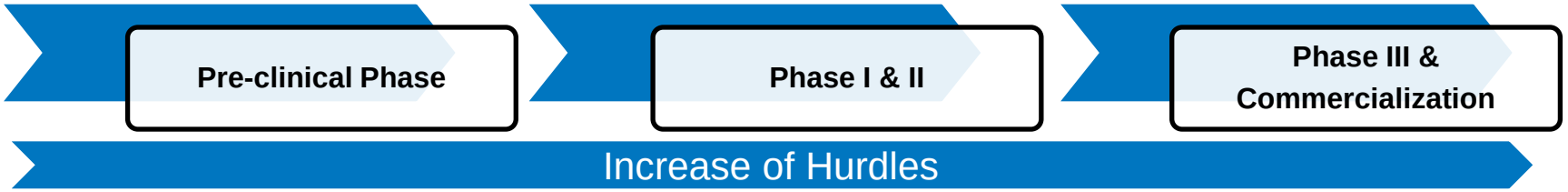


Several economic and technical hurdles need to be overcome to make iPSC-based therapies a reality.

Custom Process Development and Manufacturing of Therapeutic Products

Your Product. Our Passion.

Clinical-grade iPSCs – Technical Hurdles



Allogeneic & Autologous Therapy

- Open
- Manual
- Small-scale
- Research-grade

- Open
- Manual
- Small-scale
- Robust
- Reproducible
- cGMP-compliant

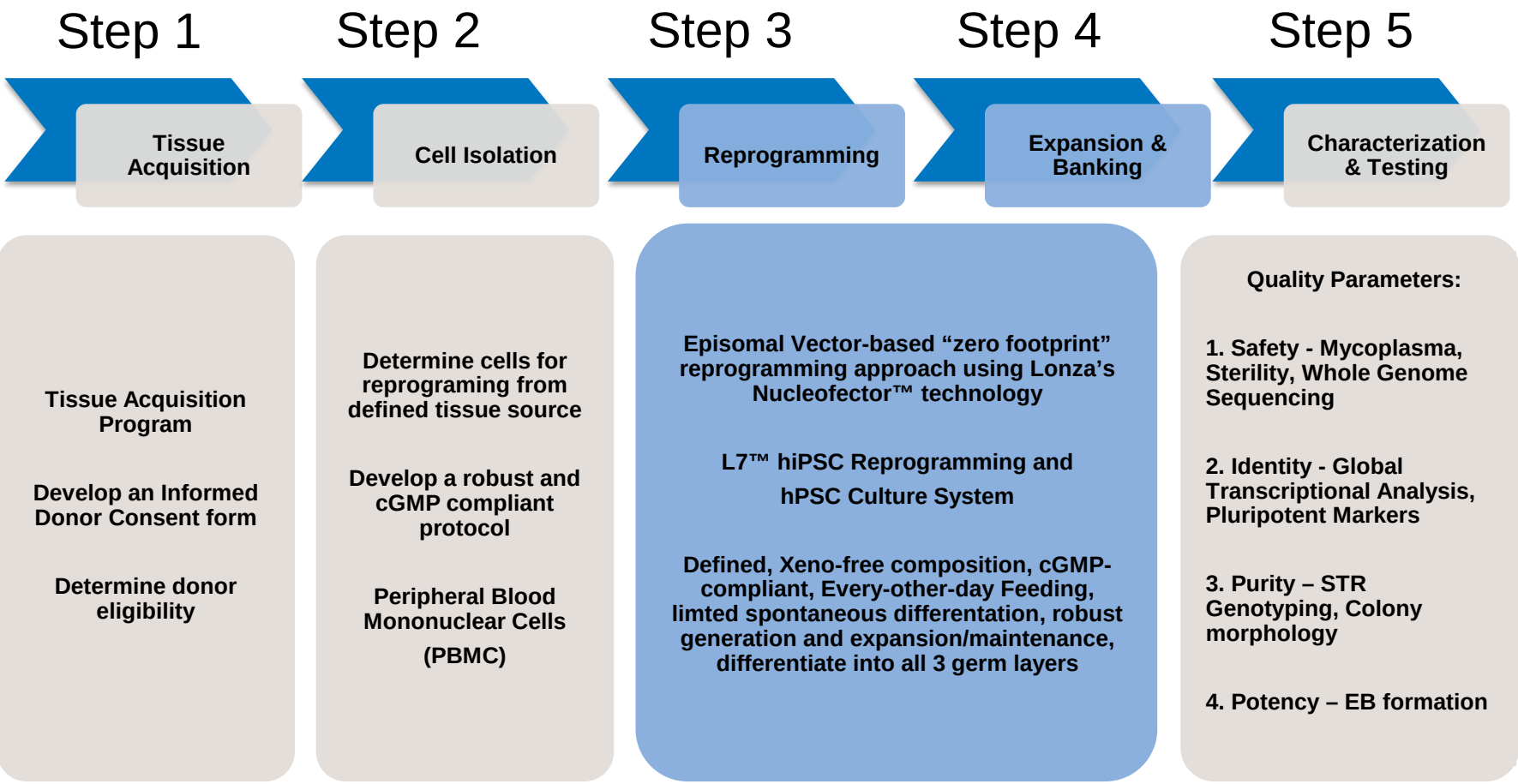
Allogeneic Therapy

- Closed
- Scaled-up
- cGMP-compliant
- Validated

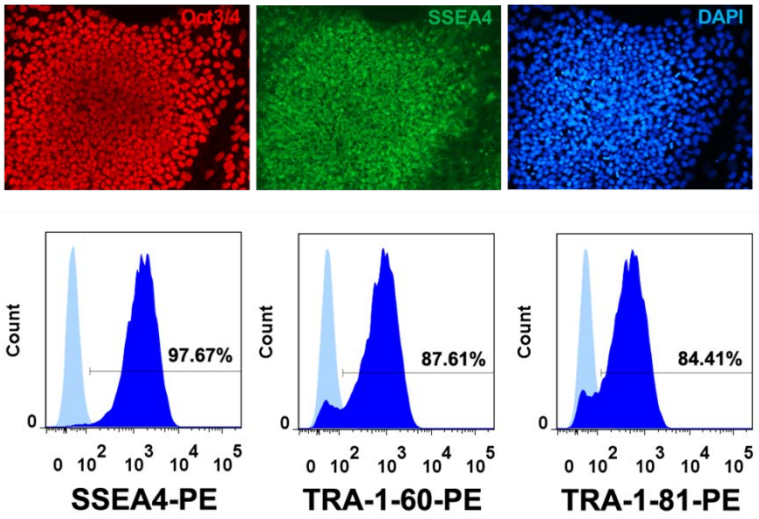
Autologous Therapy

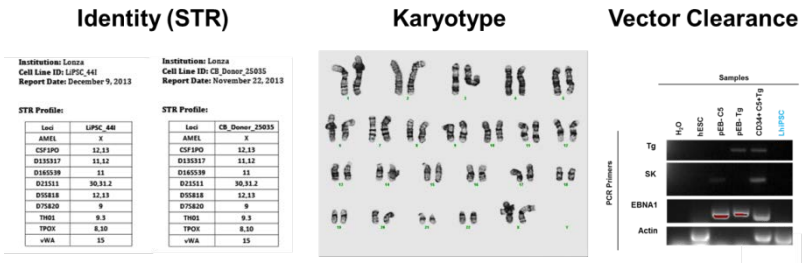
- (Semi-)automated
- Scaled-out
- cGMP-compliant
- Validated

Clinical-grade iPSCs – A Roadmap



Generation of hiPSCs under Defined, Xeno-free and cGMP-compliant Conditions





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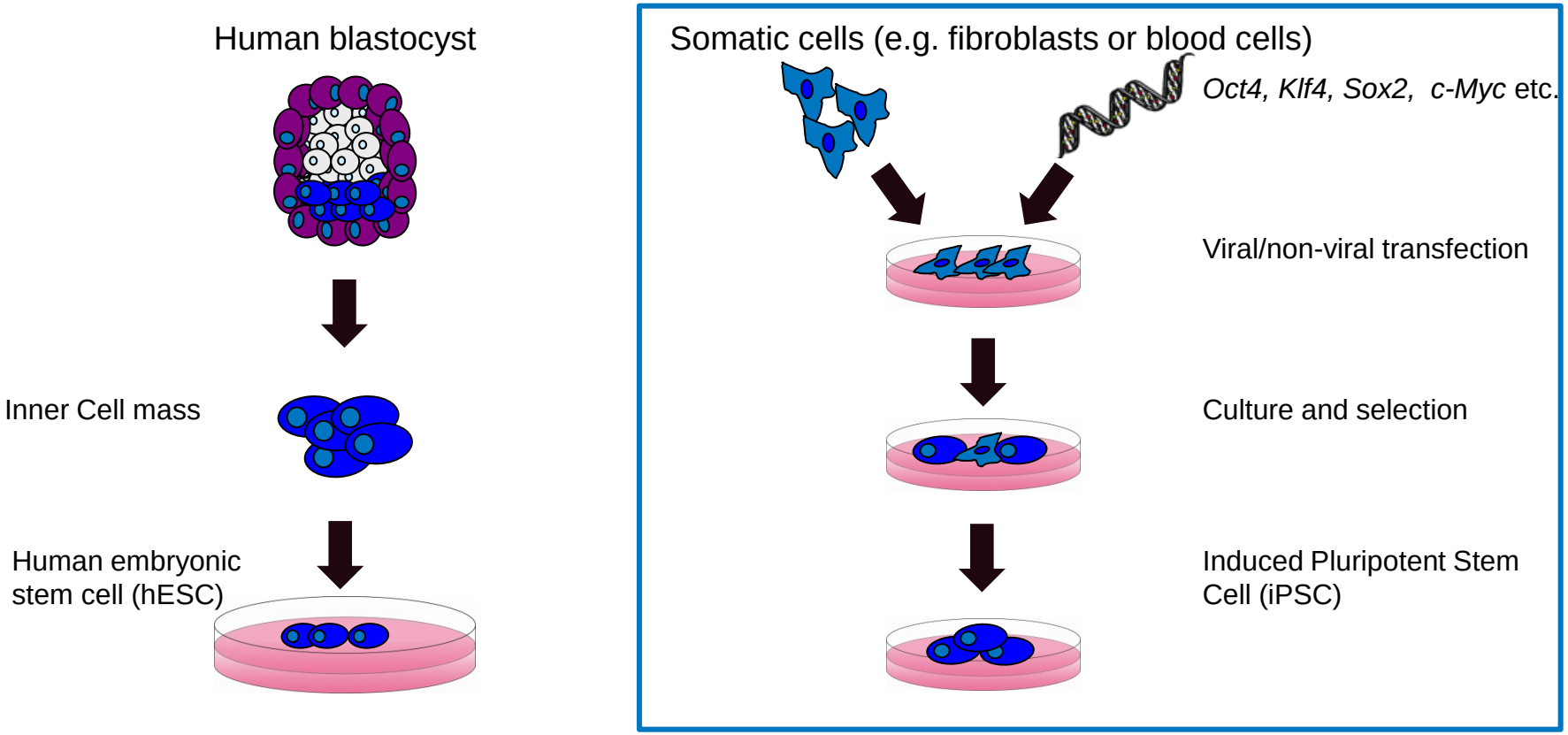
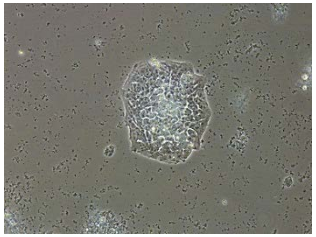
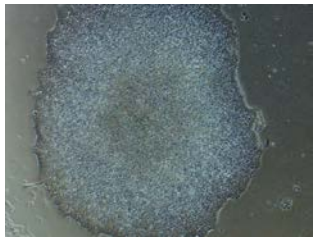
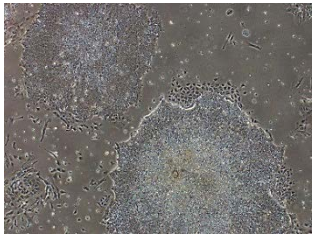
- L7™ – The Complete System in action (case studies)

Induced Pluripotent Stem Cells (iPSCs) Basics



Generation of iPS Cells via Transfection of PBMCs Using Nucleofector™ Technology

Priming → Nucleofection → Recovery → Colony appearance → Colony selection & expansion → Banking

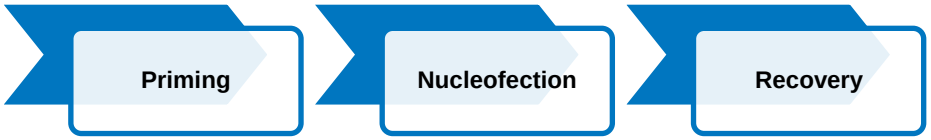
Day -9	Day 0	Day 0-4	Day 9	Day 12 - 20 (P0)	Day 76 (P5)
					

L7™ PBMC Reprogramming Bundle

L7™ hPSC Culture System

Lonza's Portfolio Overview

iPSC Reprogramming



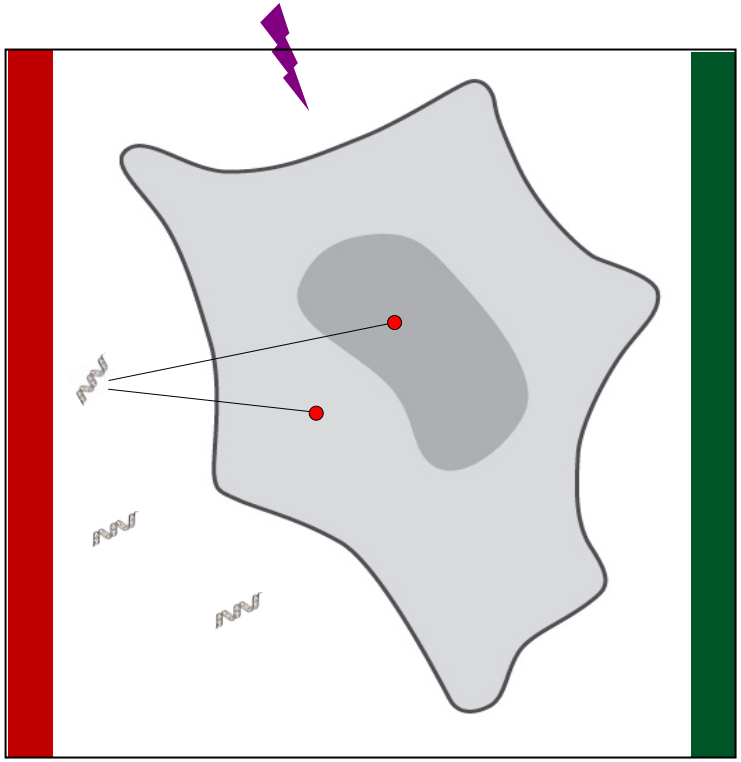
Part	Description	Size
CC-2702	Human PBMCs	50 M Cells
FP-5124	L7™ PBMC Priming-Recovery Kit	24 rxn
VAXP-3024	P3 Primary Cell 4D-Nucleofector ™ Kit (100 µl Nucleocuvette™ Vessel)	24 rxn

Cell Culture Tools

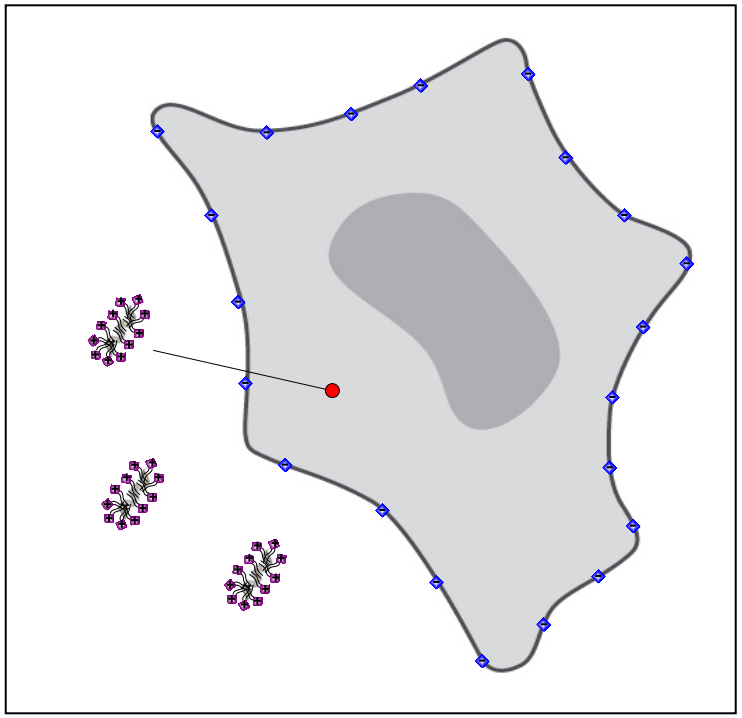
Part	Description	Size
FP-5007	L7™ hPSC Media BulletKit	500 mL + 5 mL
FP-5020	L7™ hPSC Matrix	1 mg
FP-5013	L7™ hPSC Passaging Solution	100 mL
FP-5002	L7™ hPSC Cryosolution	50 mL

Nucleofector™ Technology – The Principle

- High transfection efficiency combined with low mortality
- DNA is directed into the nucleus giving faster gene expression

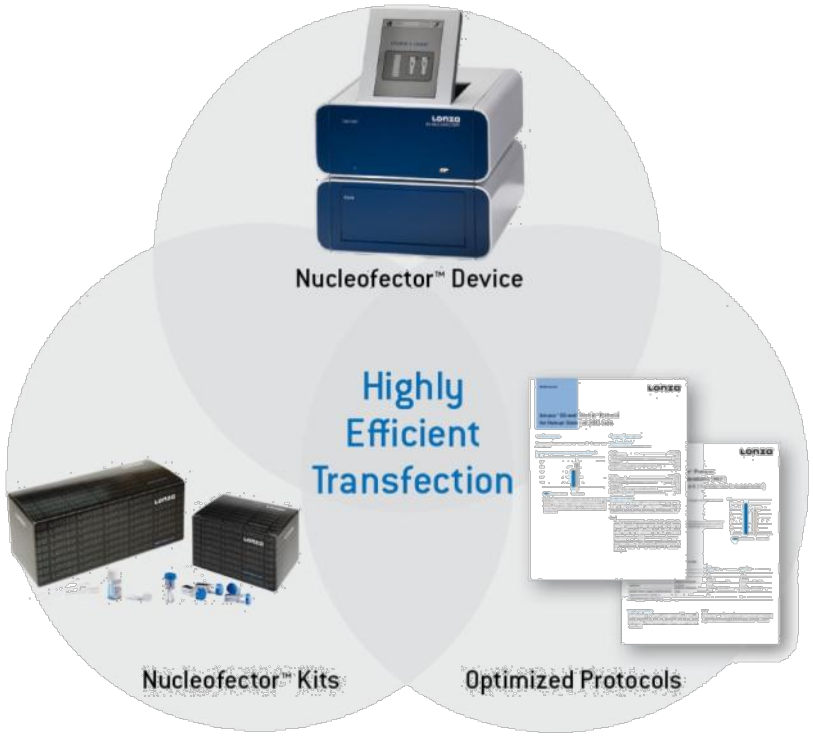


Nucleofection Process



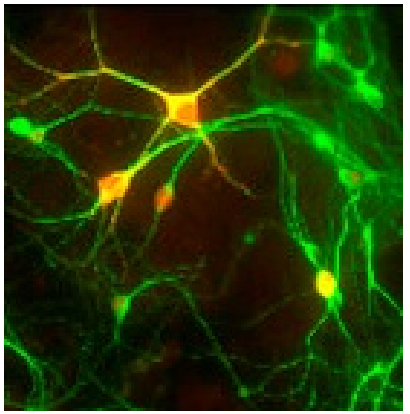
Other transfection methods

Components of Nucleofector™ Technology



A Unique Combination:

- Nucleofector™ Device
- Specific Nucleofector™ Kits
- Detailed optimized protocols
- Enabling excellent transfection performance combined with high functionality!



iPS Generation
Typically Used Human Cells Types

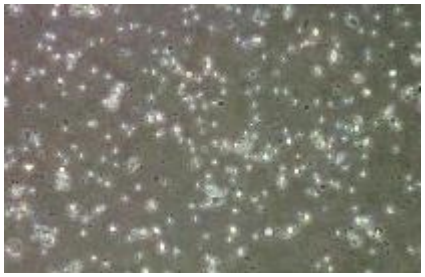
Human Cells*	Efficiency (pmaxGFP™ Vector)	References
Dermal fibroblasts	40-95%	Yu J *et al.* (2009) Science, 324(5928):797-801 Mehta A *et al.* (2011) Cardiovasc Res 91:577-86
Keratinocytes	40-80%	
Bone marrow CD34+ cells	66%	Chou BK *et al*. (2011) Cell Research, 21:518-529
Cord blood CD34+ cells	82%	Chou BK *et al.* (2011) Cell Research, 21:518-529 Mack A *et al.* (2011) PlosOne 6 (11)
Bone marrow mononuclear cells		Hu K *et al.* (2011) Blood, 117(14): e109-e119 Chou BK *et al.* (2011) Cell Research, 21:518-529
Peripheral blood mononuclear cells	30-95%	Hu K *et al.* (2011) Blood, 117(14): e109-e119 Chou BK *et al.* (2011) Cell Research, 21:518-529
Neural progenitor cells	up to 90%	
Adipose-derived stem cells	65-90%	Jia *et al.* (2010) Nature Methods, 7:197-199

***Also available from Lonza**

PBMC Reprogramming Protocol Part 1

Lonza

Blue: Lonza products
Black: required, not included



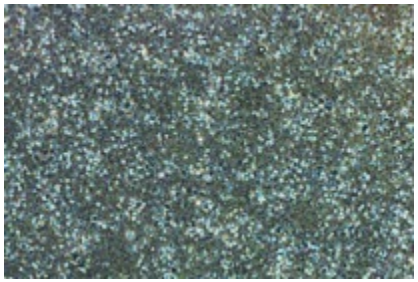
Priming
Day -8* to 0

L7™ Priming-Recovery Basal Medium
+ Supplements

***In some cases a priming phase of 6 or 10 might be recommended**

Nucleofection
Day 0

P3 4D-Nucleofector™ Kit
+ pCE-Oct4
+ pCE-Sox/KLF
+ pCE-MYC/Lin28
+ pCE-mp53DD
+ pCXB-EBNA1



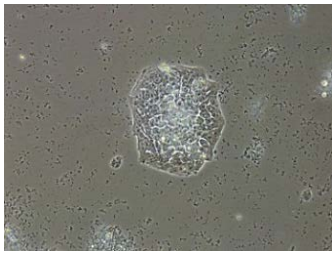
Recovery
Day 0 - 2

L7™ Priming-Recovery Basal Medium
+ Supplements
+ Enhancer A

PBMC Reprogramming Protocol Part 2

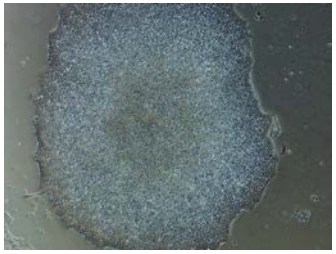
Lonza

Blue: Lonza products
Black: required, not included



Colony appearance
~Day 10

L7™ hPSC Culture Medium
L7™ hPSC Matrix
+ Enhancer B

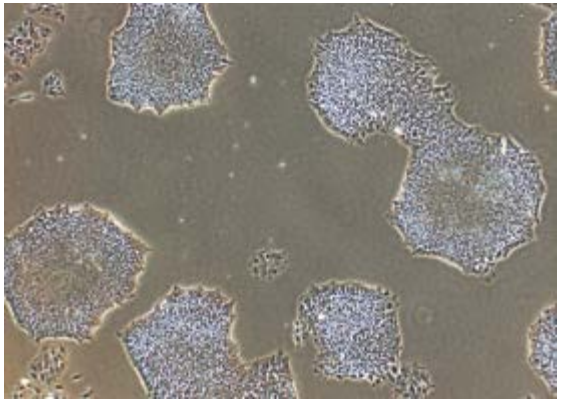


Colony selection & expansion
~Day 20 (P0)

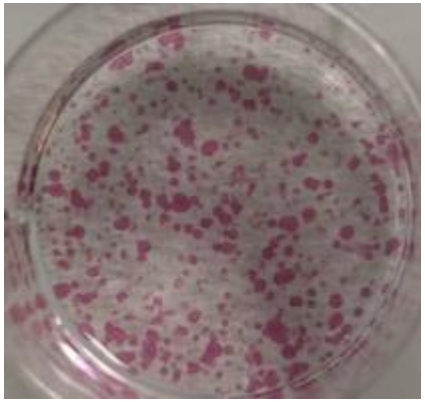
L7™ hPSC Culture System

PBMC-derived iPSC Characterization – Attachment and Pluripotency Markers

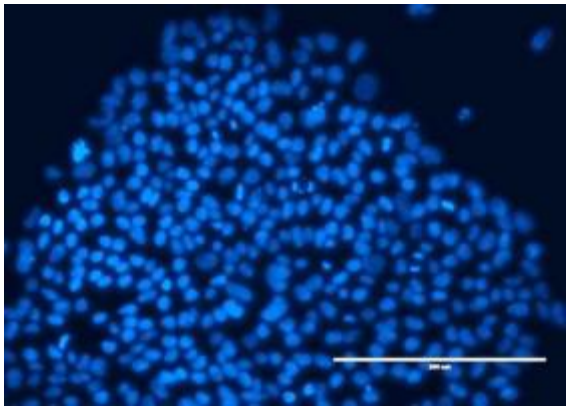
Morphology (P15, D3)



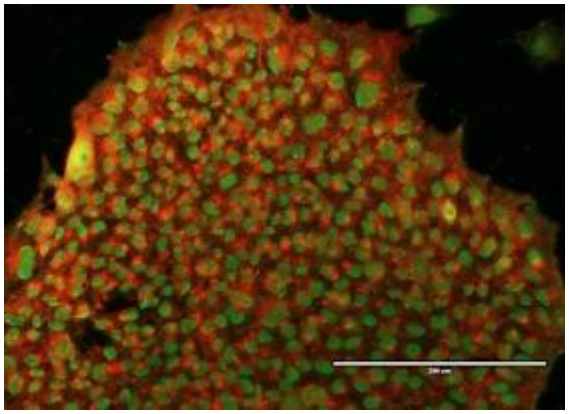
Alkaline Phosphatase (P15, D4)



DAPI



TRA-1-60/Oct4

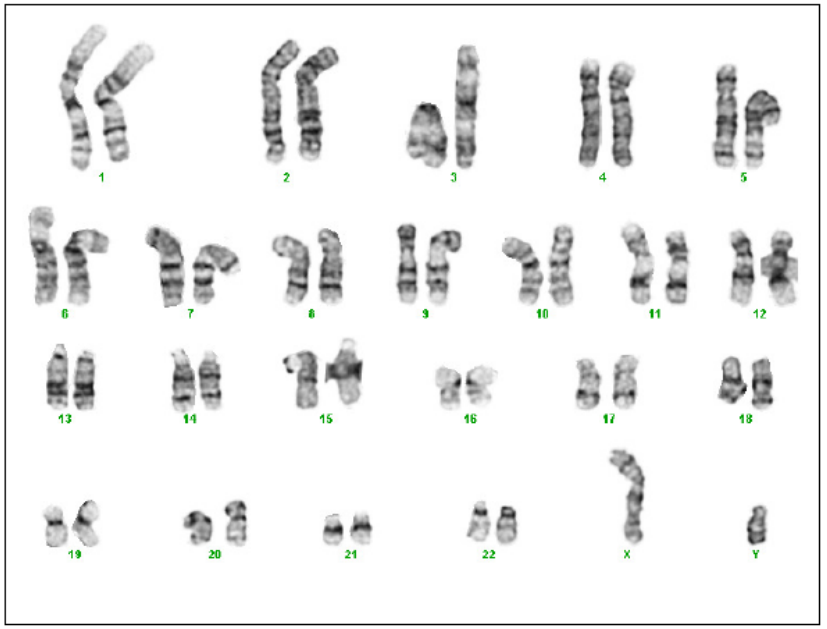


PBMC-Derived iPSC Characterization – Karyotype and Plasmid Clearance

Clear of Plasmid: LiPSC-34B (P5)

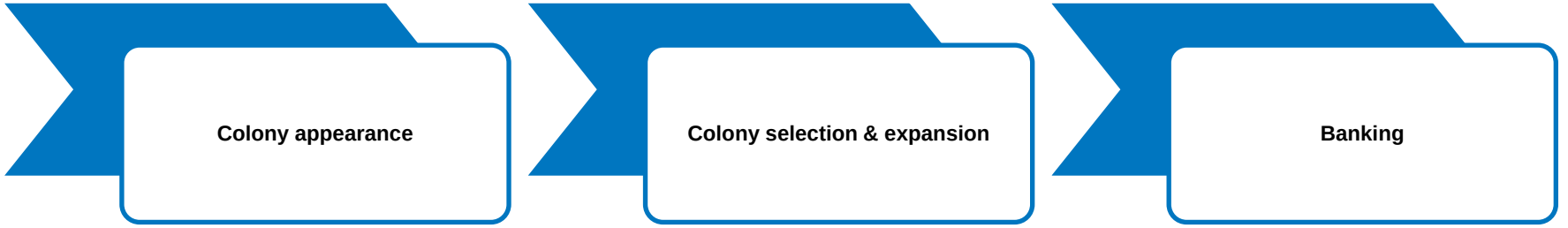
- Undetectable level of plasmid confirmed by a qPCR based assay based on the level of CT value

Normal Karyotype: LiPSC-34B (P11)



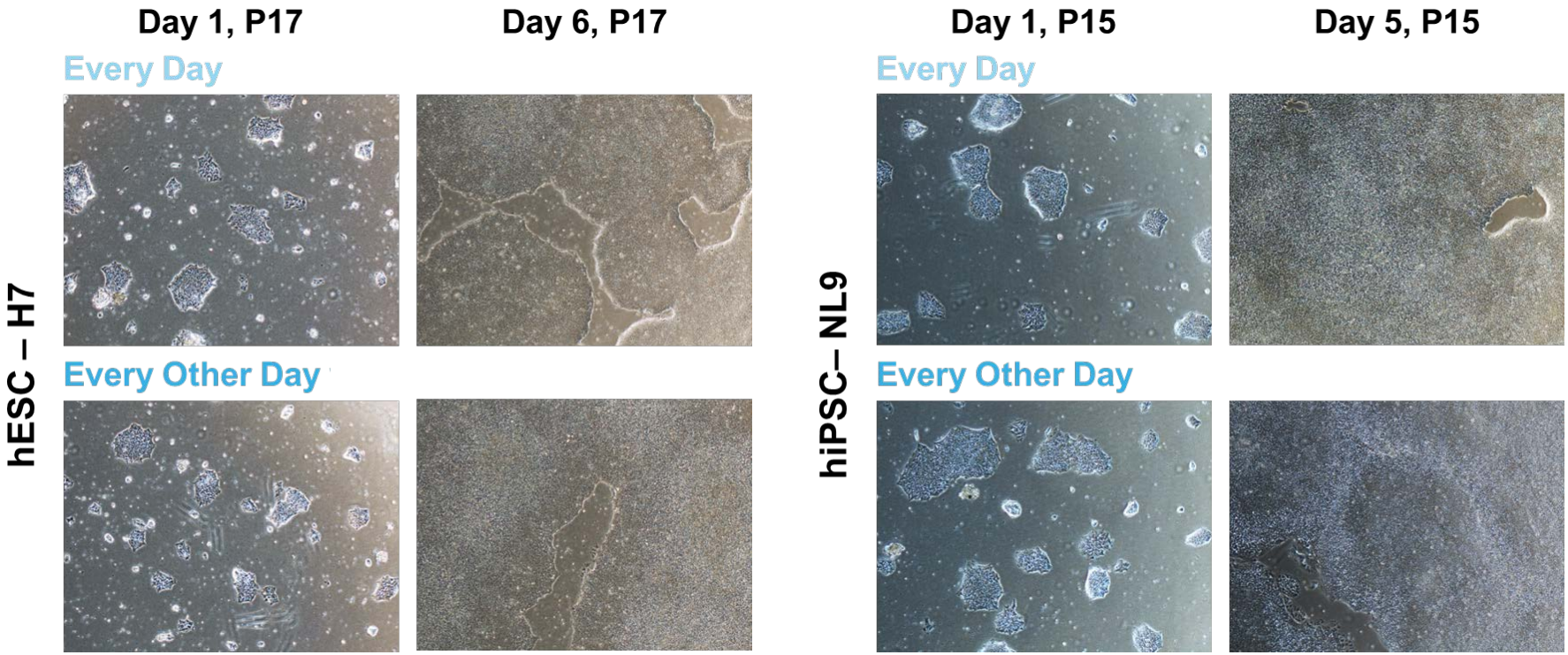
L7™ hPSC Culture System: The Components

Cell Culture Tools



- L7™ hPSC Media BulletKit
- L7™ hPSC Matrix
- L7™ hPSC Passaging Solution
- L7™ hPSC Cryosolution

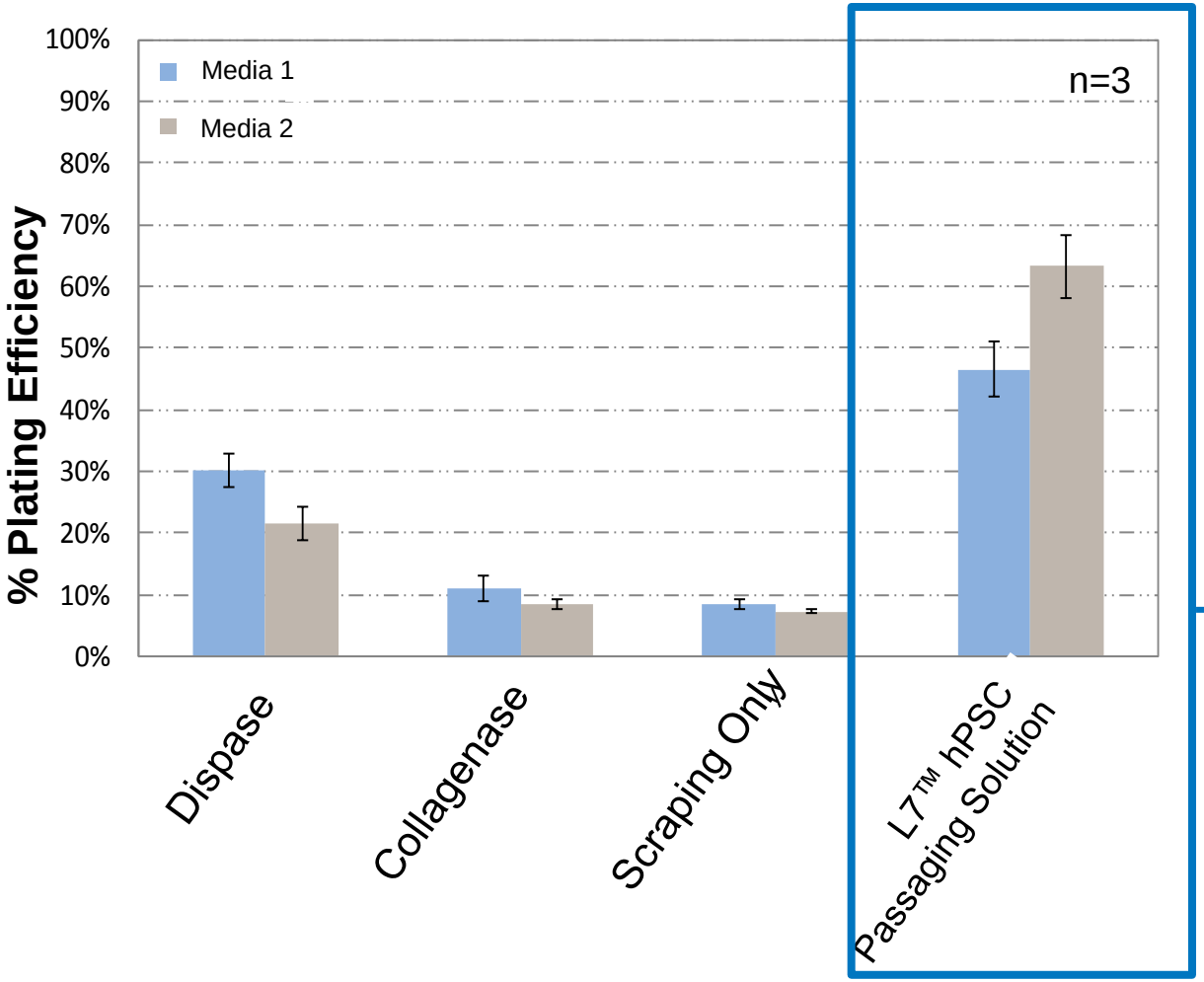
Every-Other-Day Feeding – Typical, Healthy Morphology

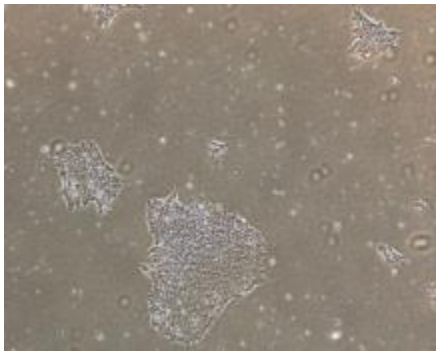


Benefits

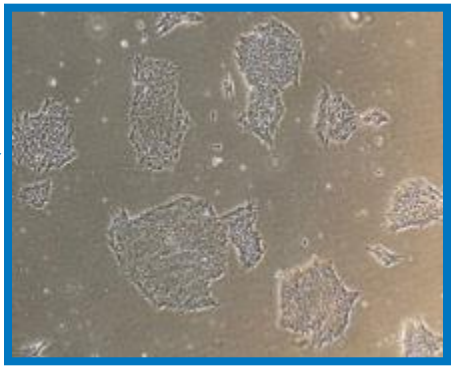
- Daily maintenance of hPSCs no longer necessary
- Similar look and growth characteristics as every-day feeding

L7™ hPSC Passaging Solution Provides High Re-Plating Efficiency



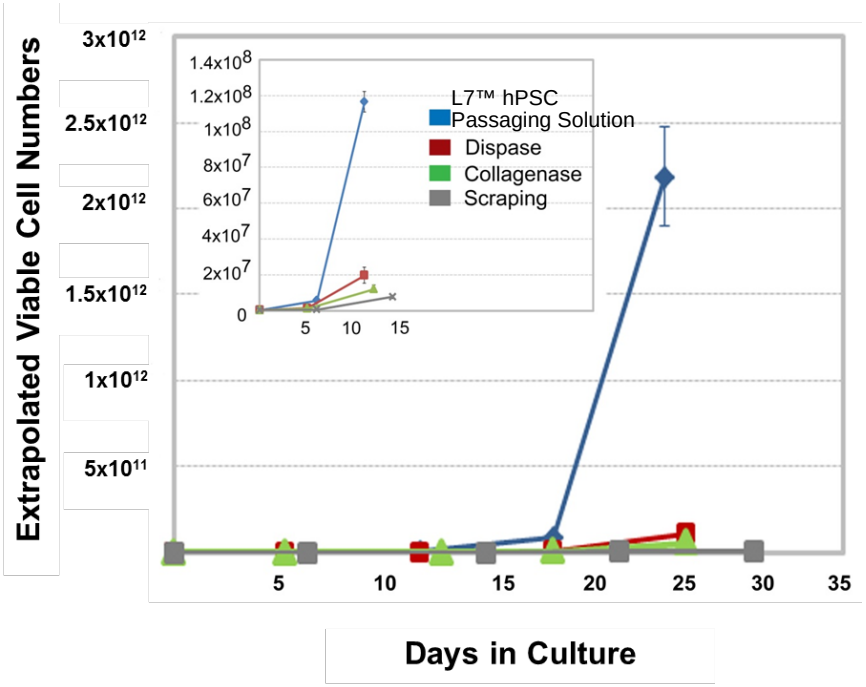


Dispase



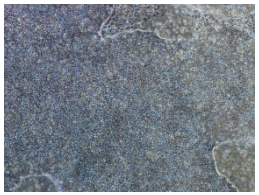
L7™ hPSC
Passaging Solution

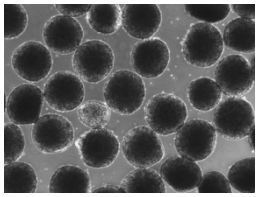
The Time Required to Expand hPSCs Using L7™ hPSC Passaging Solution is Significantly Reduced

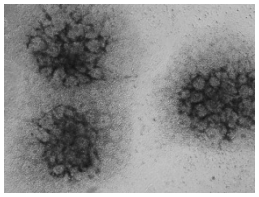


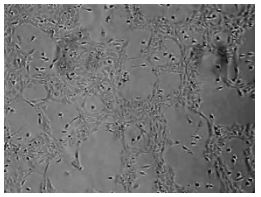
Reagent	Post-Dissociation Viability	Days to Produce 2 x 10^{12} Cells
L7™ hPSC Passaging Solution	97%	27
Dispase	53%	55
Collagenase	51%	56
Scraping	35%	97

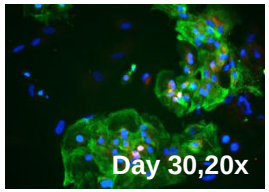
L7™ hPSC Culture System – Unaltered Directed Differentiation Capabilities


hiPSC #6.4

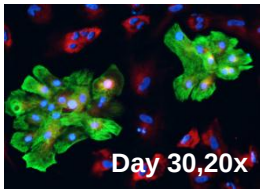

Neurospheres


Rosettes

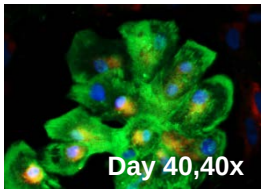

NSCs



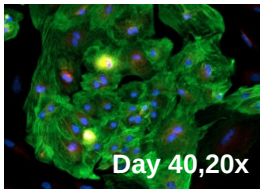
Desmin MYL2 DAPI



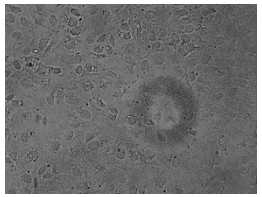
Cardiac Troponin MYL2 DAPI

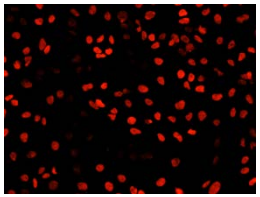


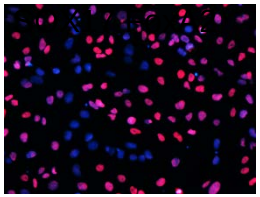
Actin MYL2 DAPI

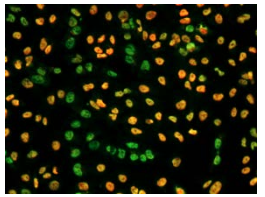


Cardiac Troponin MYL2 DAPI




FoxA2

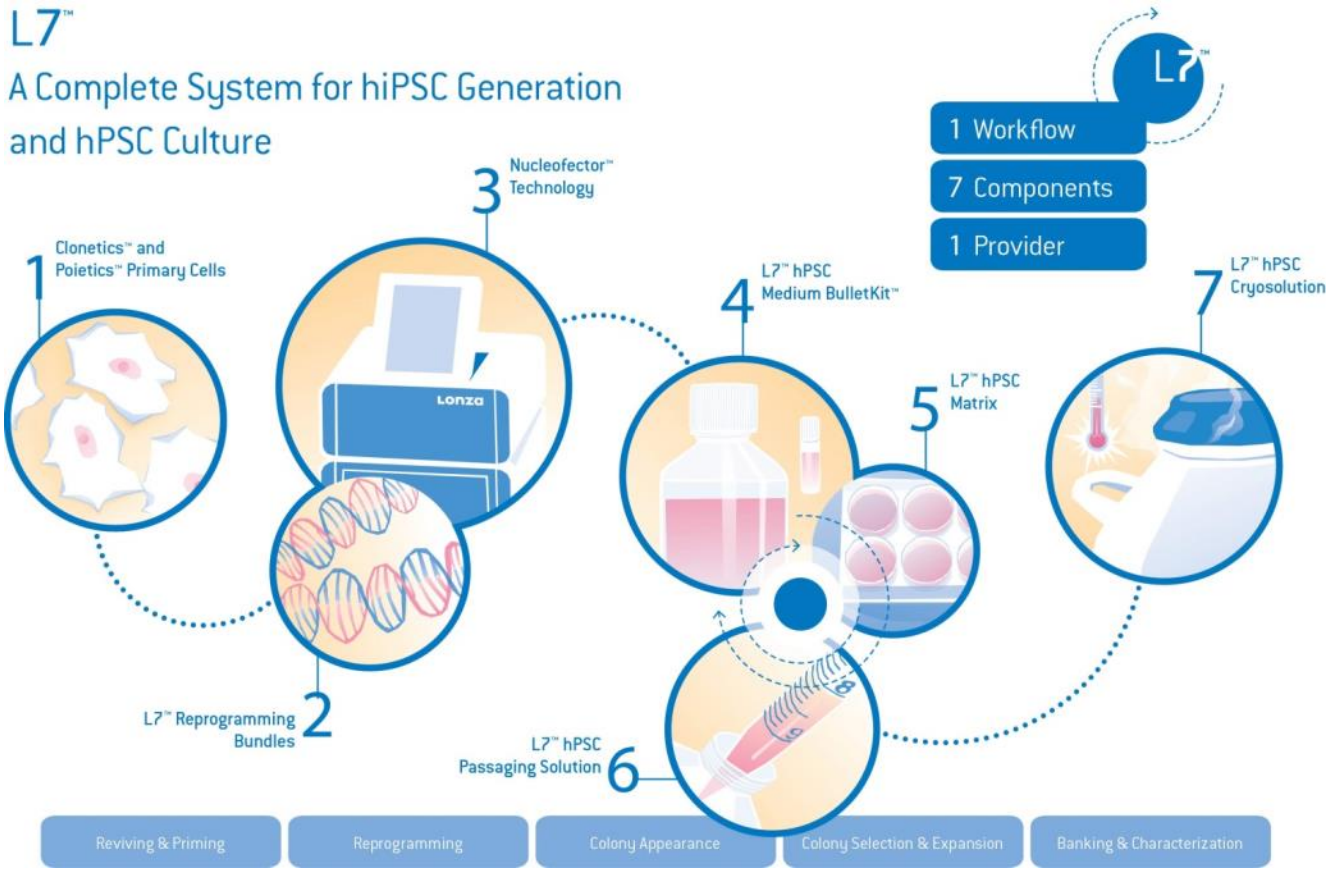

FoxA2 DAPI


SOX17 FoxA2

Lineage-specific differentiation of hPSCs

- hESCs/iPSC cultured with L7™ hPSC Culture System maintain potency for lineage-specific differentiation
- Cells are not locked in undifferentiated state by L7™ hPSC Culture System

Advantages of L7™ hPSC Culture System



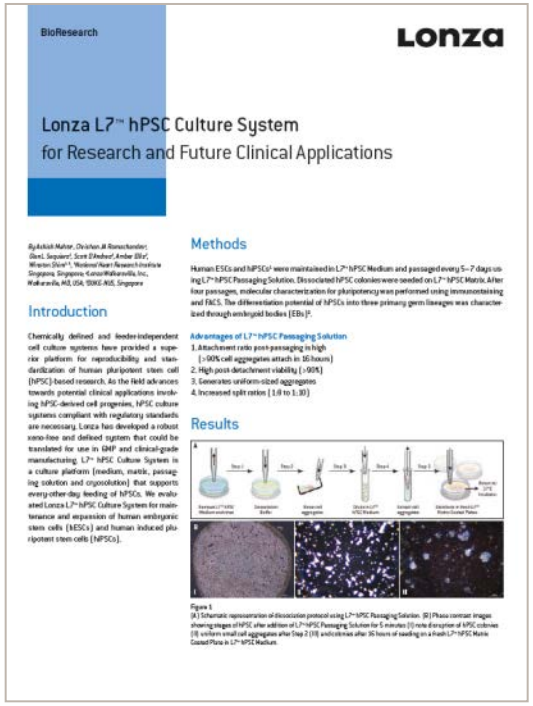
- Every-other-day feeding
- High re-plating efficiency
- Maintenance of pluripotency marker expression
- Normal karyotype
- Unaltered directed differentiation capabilities

Agenda

- Background
- Considerations for iPSC researchers with clinical aspirations
- Generation of human iPSCs from peripheral blood mononuclear cell (PBMC)
- Xeno-free, defined culturing of human PSCs
- L7™ hiPSC Reprogramming and hPSC Culture System in action – Case studies

L7™ hPSC Culture System for Research

- Dr. Winston Shim evaluated the L7™ hPSC Media BulletKit, Matrix and Passaging Solution on hESCs and hiPSCs


BioResearch

Lonza

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

Introduction

Methods

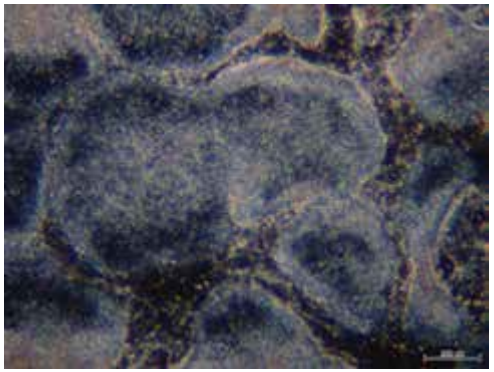
Results

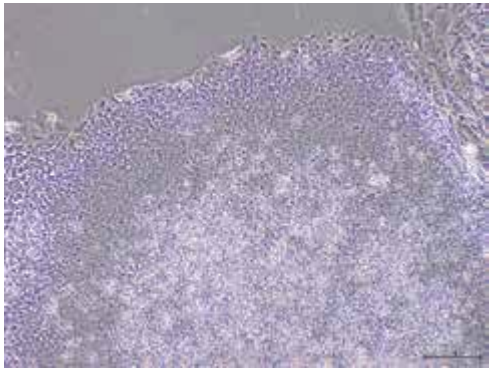
[Read the whitepaper]

hPSC Cultures on L7™ Medium to Passage 4

Low magnification

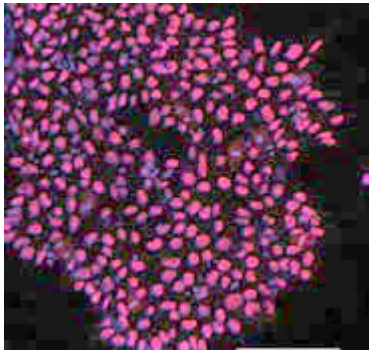
Day 6 after passaging



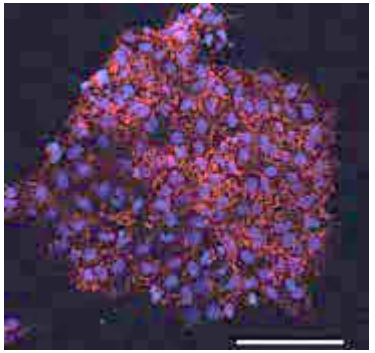


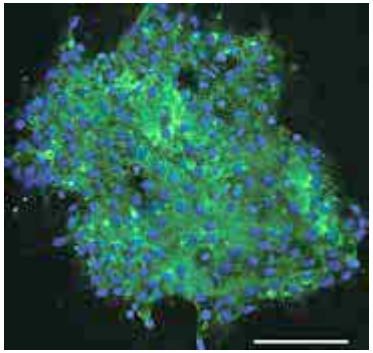
Phase contrast

Oct4/DAPI

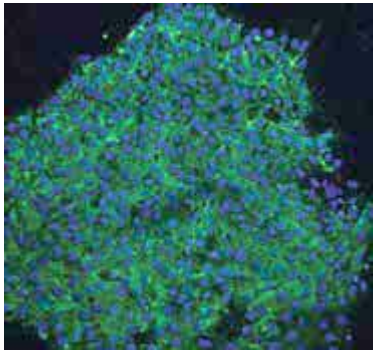


SSEA4/DAPI





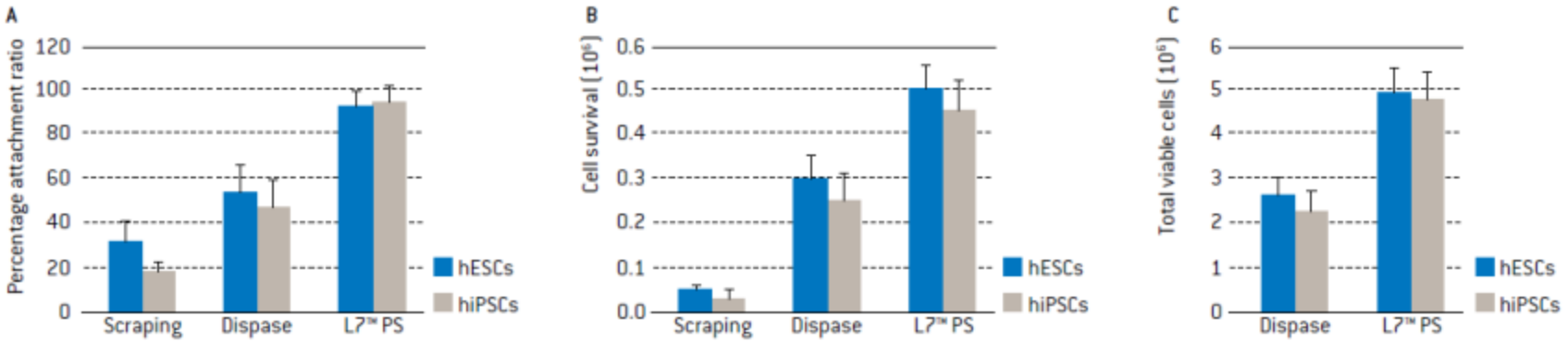
Tra-1-60/DAPI



Tra-1-81/DAPI

Immunostaining

L7™ hPSC Passaging Solution Comparisons

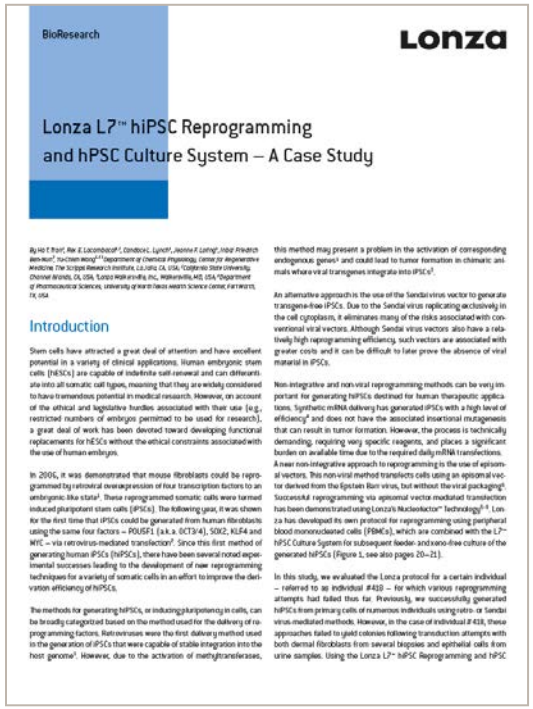


- Advantages of L7™ hPSC Passaging Solution

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

L7™ – The Complete System
The Generation of iPSCs from PBMCs Using Lonza's Protocol

- Dr. Yu-Chieh Wang evaluated the Lonza protocol for generating iPSCs from a particular individual for which retro- or Sendai virus-mediated attempts had previously failed



BioResearch

Lonza

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

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 named 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[2]. However, due to the activation of methyltransferases, this method may present a problem in the activation of corresponding endogenous genes[3] and could lead to tumor formation in chimeric animals where viral transgenes integrate into iPSCs[4].

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[5] 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.

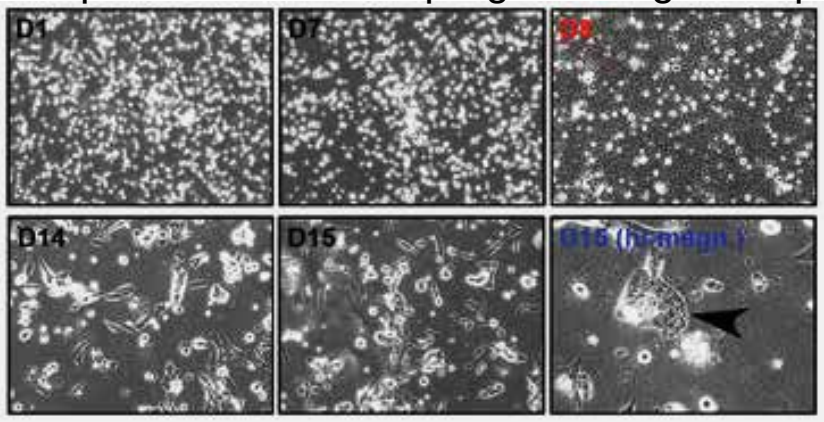
A near non-integrative approach to reprogramming is the use of episomal vectors. This non-viral method transfects cells using an episomal vector derived from the Epstein Barr virus, but without the viral packaging[6]. Successful reprogramming via episomal vector mediated transfection has been demonstrated using Lonza's Nucleofector™ technology[6,7]. 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).

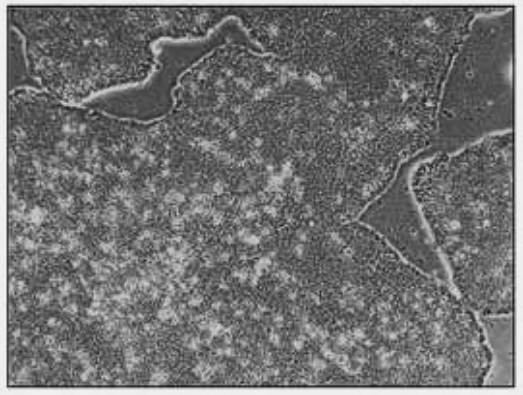
In this study, we evaluated the Lonza protocol for a certain individual – referred to as individual #418 – for which various reprogramming attempts had failed thus far. Previously, we successfully generated hiPSCs from primary cells of numerous individuals using retro- or Sendai virus-mediated methods. 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

[Read the whitepaper]

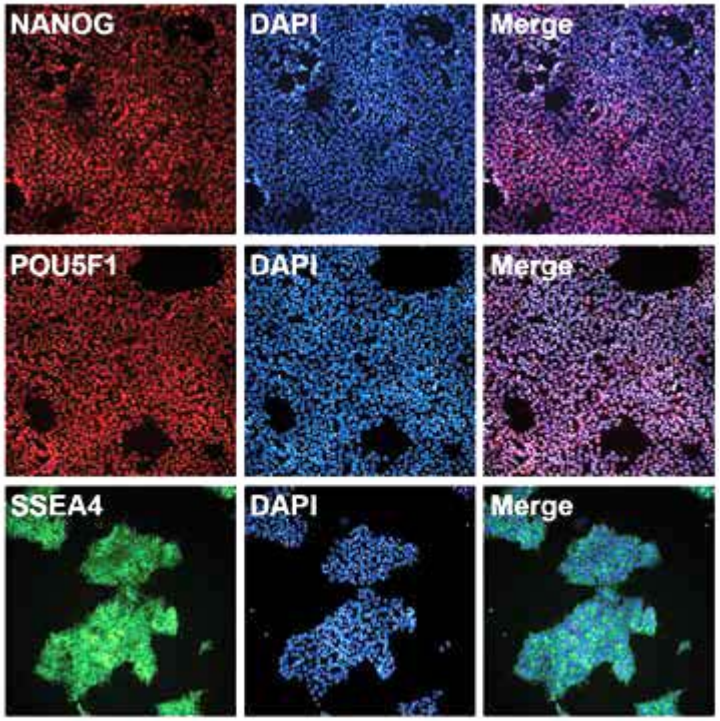
Generation and Characterization of PBMC418iPS1506 line

Snapshots from the reprogramming attempt





PBMC418iPS1506 on L7™ hPSC Culture System



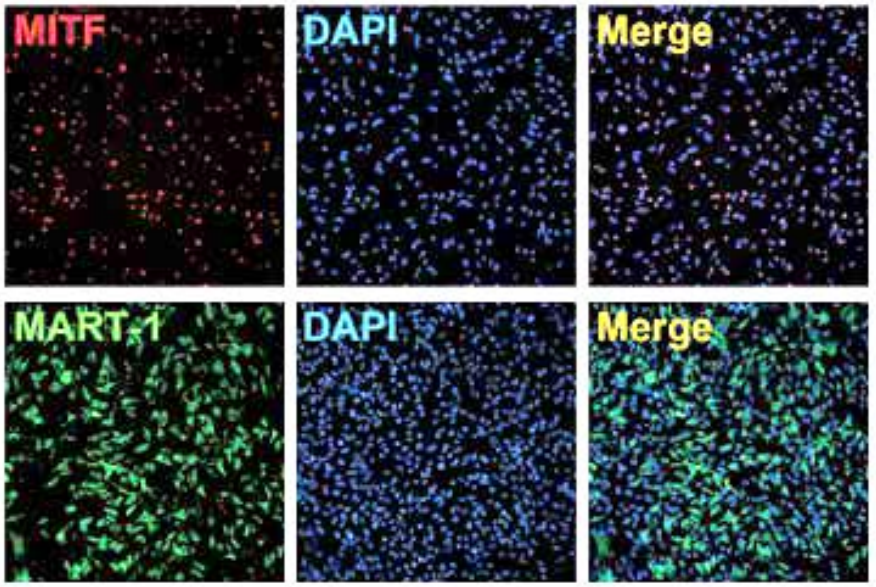
PBMC418iPS1506 cells positively stained with biomarkers for cellular pluripotency

Differentiation Potential of the PBMC418iPS1506 line

PBMC418iPS1506 cells were cultured in suspension as cell aggregates for 7 days

Aggregates were plated onto human fibronectin coated plates in MelDiff medium (ScienCell Research Laboratories, Carlsbad, CA) with the appropriate cytokines and growth factors for around 30 days

Differentiated cells were maintained on MelM medium (ScienCell Research Laboratories, Carlsbad, CA)



PBMC418iPS1506 cells directed differentiated into melanocytic cells.

Summary

- iPSC-based therapies will face different manufacturing challenges depending on the phase of development requiring new technologies and processes to overcome technical and economic hurdles.
- Lonza has created a cGMP-compliant process for the generation of clinical-grade iPSCs. From this process, L7™ hiPSC Reprogramming and hPSC Culture System was created for research use!
- L7™ – The Complete System advantages include fully defined and Xeno-free, easy to follow PBMC reprogramming kit and protocols, every-other-day feeding, high re-plating efficiency, pluripotent expansion and maintenance, and unaltered directed differentiation capabilities

Support Tools and Contact Details

Our Online Databases

- Cell Database: http://www.lonza.com/celldatabase
- Citations: http://www.lonza.com/citations

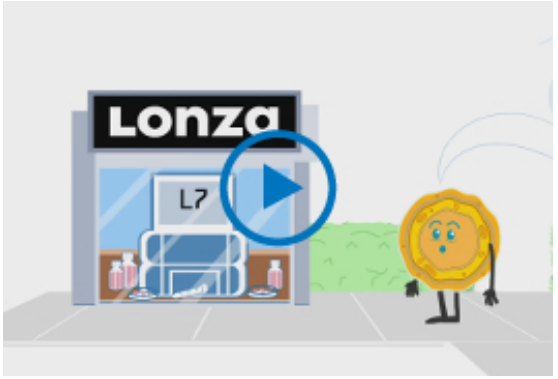
Technical Support

- Scientific Support Team US: scientific.support@lonza.com or +1 800 638 8174
- Scientific Support Team EU + ROW: scientific.support.eu@lonza.com or +32 87 321 611

Interested in Learning More?

- Meet with us at ISSCR (24-27 June 2015)
  - Stop by **Booth B04:11**
  - Or join our **Innovation Showcase**:
    "Smart Technologies for Advancing Stem Cell Research and Discoveries“
    Thursday, 25 June 2015, 11:30 am - 12:30 pm Room A4
    More on our Innovation Showcase

- More information at:
  - www.lonza.com/ISSCR
  - www.lonza.com/L7
  - www.lonza.com/stemcells



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Tuesday, 22 September 2015
11 AM PDT (Los Angeles) / 2 PM EDT (New York)
Register here

Wednesday, 23 September 2015
9 AM BST (London) / 10 AM CEST (Berlin) / 5 PM JST (Tokyo)
Register here

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Thank You!