## The University of Iowa Dezii Translational Vision Research Group

Page 1 of 3

### TITLE: Generation of Induced Pluripotent Stem Cells (iPSC)

SOP Number:	D-DEZ-PRO-004	Revision Number:	0
		Effective Date:	28 Apr 2015
Author:		Date:	
Reviewer:		Date:	
QA Approval:		Date:	

#### A. OBJECTIVE

This SOP is to provide information for the generation of induced pluripotent stem cells (iPSCs) from human dermal fibroblasts in the DTVR cleanroom suite.

#### B. APPLICABILITY

This procedure shall be performed by a trained scientist or technician in the DTVR cleanroom suite.

#### C. REFERENCES

D-DEZ-PRO-002 Plating, Passaging and Freezing FBs from Dermal Biopsy Punch D-DEZ-PRO-003 Keratinocyte Isolation from Dermal Biopsy Punch

D-DEZ-PRO-007 iPSC Culture and Differentiation

#### D. MATERIALS, EQUIPMENT AND MEDIA

BioSpherix Xvivo system unit

Incubator/cell culture system (Biospherix)

Centrifuge (Thermo Fisher scientific)

Laminin521 Surface coated plates (Corning, Order # 354222)

Hank's Balanced Salt Solution +Mg +Ca (HBSS++) (Life Technologies, Order # 14025092)

TrypLE Express Enzyme (1x), no phenol red (Life Technologies, Order # 12604-013)

Y-27632 ROCK Inhibitor (EMD Milipore, Order # 688000)

6-well tissue culture dish (Corning, Order # 3516)

100mm Tissue Treated Cell Culture Dishes (TPP, Order# 93040)

#### Viral Transduction Media

Biopsy media (400 mL MEM-alpha (Gibco, Order # 12571-063), 50 ml KnockOut Serum Replacement (Gibco, Order # 10828-028) 1 mL Primocin (Invivogen, Order # ant-pm-2), 5ml GlutaMAX (Life Technologies, Order #35050-061) cGMP Grade bFGF (Waisman Biomanufacturing, Order # FGF))

50uM Rocki (used first 24-48hrs post-plate/thaw)

CytoTune-iPS Reprogramming Kit 2.0 at MOI of 3-5 (Invitrogen, Order # A16517)

#### Human iPSC Reprogramming Media

Essential 6 (Life Technologies, Order # A1516401)

## Human iPSC maintenance media

Essential 8 (Life Technologies, Order # A1517001)

## The University of Iowa Dezii Translational Vision Research Group

Page 2 of 3

TITLE: Generation of I	Generation of Induced Pluripotent Stem Cells (iPSC)		
SOP Number:	D-DEZ-PRO-004	Revision Number:	0
		Effective Date:	28 Apr 2015

### Human iPSC passaging media

Versene (Life Technologies, Order # 15040-066)

#### E. iPSC GENERATION PROTOCOL

- 1. Plate 250,000 fibroblasts/well of a 6-well of culture dish. The following day proceed to viral transduction/iPSC generation.
- 2. Viral transduction.
  - a. Add <u>viral transduction media</u> to fibroblast cultures and incubate overnight at 37 degrees Celsius, 5% CO2.
  - b. At 12-24 hours post-transduction wash out viral media and culture for an additional 5 days in Biopsy Media feeding cells every other day.
  - c. On day 4 prepare laminin521 coated100mm tissue culture dishes (10ug/ml laminin521 in HBSS++).
- 3. iPSC generation.
  - a. On day 5, using TrypLE Express, passage 200,000 transduced cells onto laminin521 coated 100mm dishes with Biopsy Media + Rocki (50uM).
  - b. On day 6 Feed 100mm dishes with equal parts Biopsy Media and Human iPS/ES Basal Media.
  - c. From day 7 to day 30, feed with Human iPSC Reprogramming Media.
  - d. Within 1-4 weeks iPSC colonies will appear. Once colonies reach 1-2mm in diameter, manually passage onto 12-well laminin521 coated culture plates for clonal expansion in <a href="https://maintenance.nedia">https://maintenance.nedia</a>
- 4. iPSC maintenance.
  - a. Feed iPSCs daily with fresh human iPSC maintenance media
  - b. Within 5-10 days colonies large enough to be passaged will be present. Passage at a ratio of 6-10 onto fresh laminin521 coated plates.
  - c. Expand cells for a minimum of 8 passages prior to proceeding to step 5.
- 5. Pluripotency and transgene analysis
  - a. Post-generation and expansion, analyze pluripotency via the Life Tech scorecard assav.
  - b. Use rt-PCR analysis to demonstrate endogenous pluripotency factor expression and loss of Sendai reprograming particles.

## F. History

Effective Date	Revision	Change
28 Apr 2015	0	Initial issue of SOP

# The University of Iowa Dezii Translational Vision Research Group

Page 3 of 3

TITLE: Generation	of Induced Pluripotent Sten	n Cells (iPSC)	
SOP Number:	D-DEZ-PRO-004	Revision Number:	0
		Effective Date:	28 Apr 2015