The University of Iowa Dezii Translational Vision Research Group

TITLE: Plating, Passaging and Freezing of Fibroblasts

SOP Number: D-DEZ-PRO-002 Revision Number: 0

Effective Date: 07 Apr 2015

Author: _____ Date: _____

Reviewer: _____ Date: _____

QA Approval: _____ Date: _____

A. OBJECTIVE

This SOP is to provide information for the isolation, plating, passaging and/or freezing of fibroblasts from patient skin biopsies in the cGMP-compliant Dezii Translational Vision Research facilities (DTVR).

B. APPLICABILITY

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

C. REFERENCES

D-DEZ-PRO-001 Dermal Biopsy Sample Collection
D-DEZ-PRO-004 iPSC generation

D. MATERIALS AND EQUIPMENT

Incubator/cell culture system (Biospherix)
Centrifuge (*Thermo Fisher scientific*)
-80C freezer (*Thermo Fisher scientific*)
Mr. Freeze (*Cryo-freeze container,* University of Iowa Biochem Stores)
15 mL and 50 mL Polypropylene Screw Cap Conical (Corning)
1.5mL Cryo-vials (Simport)
rhLaminin521 coated plates (Corning)
Costar 6-well TC-Treated Sterile Plates (Corning, Order # 3516)
Costar 12-well TC-Treated Sterile Plates (Corning, Order # 3513)
Sterile Forceps (FST, Order # 11251-10)
Sterile Scalpel (RPI, Order # 248180)
Nalgene Rapid-Flow Filter Units with PES Membrane – 500 mL (Thermo Scientific, Order # 5690020)
Biopsy Rinsing Solution (500 ml 1x HBSS, Life Technologies, Order # 14170-112 with 1 ml Primocin Invivogen, Order # ant-pm-2)
TrypLE Express (1x) (Life Technologies, Order # 12604-013)
Biopsy isolation media (395 mL MEM-alpha (Gibco, Order # 12571-063), 50 ml KnockOut Serum Replacement (Gibco, Order # 10828-028), 50 ml Heat-Inactivated Human Serum (Innovative Research, Order # IPLA-SERAB-HI), 5 ml GlutaMAX Supplement (Gibco, Order # 35050-061), 1 mL Primocin (Invivogen, Order # ant-pm-2), and 10ng/mL FGF2 cGMP Grade (Waisman Biomanufacturing, Order # FGF))
Freezing media (Heat-Inactivated Human Serum (Innovative Research, Order # IPLA-SERAB-HI) and 20% DMSO (Sigma, Order #D2438-50ML)
Y-27632 ROCK Inhibitor (EMD Milipore, Order # 688000)

E. DISSOCIATING AND PLATING FIBROBLASTS

1. Verify patient biopsy sample vial with paperwork. Assign DTVR number to biopsy and archive patient identifying information as per IRB.

2. Dump contents of biopsy sample vial onto petri dish. Rinse sample in biopsy rinsing solution. Transfer biopsy to a 6-well TC-treated plate and mince into fine pieces using a sterile scalpel.

3. Allow pieces to air dry ~5 mins. Carefully add 3 mL of biopsy media to each well.

4. Label plate with correct biopsy number, date, and scientist's initials.

5. Place plate in incubator. Incubator settings: 5.0% CO_2, 37 degrees Celsius and 20% O_2.

6. Feed cells Monday, Wednesday, and Friday with 3 mL of biopsy media per well.

F. PASSAGING/FREEZING FIBROBLASTS

7. When cells are confluent you can either passage or freeze depending on downstream application, i.e. reprogramming or long-term storage.

8. To passage or freeze add 1 mL TrypLE Express to each well. Incubate for 5 minutes at 37 degrees Celsius. Agitate the cells and pipette into a 15 mL conical tube with 3 mL of Biopsy media.

9. Centrifuge for 5 minutes at room temperature at 200g.

10. Aspirate supernatant leaving only the cell pellet.

11. To passage, re-suspend pellet in Biopsy media at a density of 100,000 cells/mL. Plate in 6-well TC-treated plate at a density of 250,000 cells per well. Label as indicated above with addition of passage number, i.e. P1. Feed as described above until cells are confluent and ready to be passaged and re-plated or cryo-protected and frozen.

12. To freeze, re-suspend pellet in 0.5 mL of Biopsy media/500,000 cells and add 0.5 mL to each cryo-vial. Add 0.5 mL Freezing media to each cryo-vial. Lable cryo-vials with biopsy number, passage number, date and Scientist's initials. Place vials in Mr. Freeze and place in -80 degrees Celsius freezer overnight. 24 hrs later remove vials from Mr. Freeze and place immediately into liquid nitrogen for long-term storage.

I. History

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