The University of Iowa Dezii Translational Vision Research Group

TITLE: gDNA Isolation from cultured cells

SOP Number: D-DEZ -PRO-013 Revision Number: 0

Effective Date: 27 Apr 2015

Author: _____ Date: _____

Reviewer: _____ Date: _____

QA Approval: _____ Date: _____

A. OBJECTIVE

This SOP is to provide information for the isolation of genomic (chromosomal) DNA from cultured cells in the DTVR analytical suite (4156E MERF).

B. APPLICABILITY

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

C. REFERENCES

D-DEZ-PRO-015 Evaluation of CRISPR-mediated NHEJ

D. MATERIALS AND EQUIPMENT

Hank's Balanced Salt Solution (HBSS) (Life Technologies, Order # 14170-112)
TrypLE Express Enzyme (1x), no phenol red (Life Technologies, Order # 12604-013)
NucleoSpin Tissue (Macherey-Nagel, Order # 740952, or similar)
1.5 mL eppendorf tubes (University of Iowa Biochemistry Stores)
100% EtOH (University of Iowa Biochemistry Stores)
1X PBS (Life Technologies, Order # 10010-023)
Sterile dH_2O (Life Technologies, Order # A1287301)
Centrifuge (*Thermo Fisher scientific, ST-8R*)
Digital Dry Bath heat block (Benchmark Scientific, Order # 9V392)

E. ISOLATION FROM CULTURED CELLS

1. Wash cells with sterile HBSS, add 1 mL TrypLE Express and allow cells to incubate 3 mins. Use a pipette to flush cells off plate and transfer cells into a sterile 1.5mL eppendorf tube. Centrifuge 5 min at 300g. Remaining steps will be completed in the DTVR analytical suite.

2. Aspirate off liquid and re-suspend pellet in 200 µl Buffer T1. Add 25 µl of Proteinase K (provided with NucleoSpin kit) and 200 µl Buffer B3. Vortex samples and incubate at 70 degrees Celsius for 10-15 mins.

3. After incubation, add 210 µl 100% EtOH, vortex to mix thoroughly, and transfer to a NucleoSpin tissue column in a collection tube. Centrifuge at 11,000 x g for 1 min. Discard flowthrough.

4. Add 500 µl Buffer BW to the column, centrifuge at 11,000 x g for 1 min. Discard flow through.

5. Add 600 µl Buffer B5 to the column, centrifuge at 11,000 x g for 1 min. Discard flow through.

6. Dry membrane by centrifuging the column for 1 min at 11,000 x g.

7. Transfer to a new 1.5 ml microcentrifuge tube and elute gDNA in 50 µl sterile dH2O. Centrifuge at room temperature at 11,000 x g for 1 min.

G. HISTORY

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