SOP: **Propagation of PATSKI Mouse Embryonic Kidney Fibroblasts**
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Modified by: **T. Canfield, S. Hansen (UW)**

PATSKI Mouse Embryonic Kidney Fibroblasts were received from Dr. Christine Disteche, Department of Pathology, University of Washington, Seattle, WA.

As described in Lingenfelter et al., 1998 (Nat Genet. 1998 18:212-3) and Yang et al., 2010 (Genome Res. 2010 20:614-22), PATSKI is a female interspecific mouse fibroblast that was derived from the embryonic kidney of an *M.spretus* x C57BL/6J hybrid mouse such that the C57Bl/6J X chromosome (maternal) is always the inactive X. This is an adherent cell line.

Materials List

1. DMEM (Dulbecco's Modification of Eagle's Medium) with 4.5g/L glucose, L-glutamine, and sodium pyruvate (Cellgro, Cat# 10-013-CV)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. T75, T225 tissue culture flasks
4. Corning conical centrifuge tubes (15mL and 50mL)
5. Graduated pipets (1, 5, 10, 25, 50mL)
6. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
7. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
8. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
9. Freezing Medium (growth medium containing 10% DMSO)
10. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
11. Cryovials (Nunc, Cat# 368632)
12. Cryo 1^{o}C Freezing Container (Nalgene, Cat# 5100-0001)
13. Eppendorf Centrifuge 5810R
14. Revco UltimaII -80^{o}C Freezer
15. Thermolyne Locator 4 Liquid Nitrogen Freezer
16. Hemocytometer
17. Micropipet w/ P20 tips
18. Microscope

Growth Medium for PATSKI Mouse Fibroblasts

DMEM with 4.5g/L glucose, L-glutamine, and sodium pyruvate Medium
10% Characterized FBS
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1. Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2. When ready to start cell culture, quickly thaw ampoule in a 37^{o}C water bath.
3. As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, and then dispense contents of ampoule into a T75 flask with 20mL of warm growth media.
4. Allow cells to recover overnight in 37^{o}C, 5% CO_2 humidified incubator.
5. The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

B. Sub-culture

1. Propagate cells until density reaches 70-80% confluence.
2. Aspirate medium.
3. Wash cells with warm 1X PBS.
4. Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
5. Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4^oC).
6. Gently re-suspend cell pellet in warm medium.
7. Perform 1:4 to 1:5 cell split as needed.
8. Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

1. Change media the day after seeding and every 2-3 days thereafter. Use 50mL of growth medium per T225 flask.
2. Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured using Accutase as above under "Sub-culture" and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be resuspended in freezing medium.
3. Cells in freezing medium are dispensed into cryovials (2 million cells per 1 mL aliquot) and frozen at -80^oC in a Nalgene Cryo 1^oC freezing container overnight.
4. Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Harvest

1. Passage cells until the desired number of cells is reached for experimentation.
2. Remove cells from flasks according to protocol described above under "Sub-culture" section.
3. Examine viability using Trypan blue staining (SOP).