SOP: **Propagation of WI-38/hTERT/GFP-RAF-ER Embryonic Lung Fibroblast Cells**
Date modified: **10/18/2010**
Modified by: **T. Canfield/S. Hansen (UW)**

Ordering Information

WI-38/hTERT/GFP-RAF-ER Embryonic Lung Fibroblast Cells were received from Dr. Carl Mann, Service de Biologie Integrative et Genetique Moleculaire (SBIGeM), Gif-sur-Yvette, France.

Notes:
This is an adherent cell line immortalized with an hTERT-expressing viral construct [pLXSN(neo)-hTERT]. The line also contains an integrated construct for inducing senescence (pBabe-puro-GFP-ΔRAF1DD-ER; provided by Dr. Martin McMahon at UCSD) as described in *Genes and Development* 12, 2997-3007 (1998) for senescence induction in IMR90 cells. Blasticidin may be added to a final concentration of 2.5 μg/mL to select for expression of the GFP-ΔRaf1DD-ER integrated construct, if necessary. However, more than 95% of the cells stably express GFP-ΔRaf1DD-ER over long numbers of passages even without blasticidin. Cells can be senesced after exposure for 72 hours with 4-hydroxytamoxifen prior to harvesting.

Materials List

1. MEM, 1X, with Earle's salts and 2mM L-glutamine (Cellgro, Cat# 10-010-CM)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH-30071-03)
3. Non-essential Amino Acids, 100X solution (Invitrogen, Cat# 11140-050)
4. Sodium Pyruvate, 100mM (Cellgro, Cat# 25-000-CI)
5. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
6. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
7. 4-Hydroxytamoxifen (Sigma-Aldrich, Cat# H7904)
8. Alcohol, 200 proof (Decon Laboratories Inc., Cat# 2716)
9. Blasticidin S HCl, 10mg/mL (Invitrogen, Cat# A11139-03)
10. T75, T225 tissue culture flasks
11. Corning conical centrifuge tubes (15mL and 50mL)
12. Graduated serological pipets (1, 5, 10, 25, 50mL)
13. Freezing Medium (growth medium containing 10% DMSO)
14. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
15. CryoVials (Nunc, Cat# 368632)
16. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
17. Eppendorf Centrifuge 5810R
18. Revco UltimaII -80°C Freezer
19. Thermolyne Locator 4 Liquid Nitrogen Freezer
20. Hemocytometer
21. Micropipet w/ P20 tips
22. Microscope

Growth Medium for WI-38/hTERT/GFP-RAF-ER Cells

MEM, 1X, with Earle's salts and 2mM L-glutamine
10% FBS
Non-essential Amino Acids (1X)
Sodium Pyruvate (1mM)
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Cultures

1. Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2. When ready to start cell culture, quickly thaw ampoule in 37^{o}C water bath until ice crystals disappear.
3. Swab outside surface of the ampoule with 70% ethanol and then dispense contents of ampoule into a T75 tissue culture flask with 20mL of warm growth medium.
4. Allow cells to recover overnight in a 37°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 cell layer with warm 1X PBS.
4. Add 15mL of Accutase and return flask to the incubator for 10-15 minutes, or until cells detach.
5. Immediately remove detached cells to a centrifuge tube, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
6. Gently re-suspend cell pellet in warm growth medium.
7. Perform no greater than 1:3 cell split as needed.
8. Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

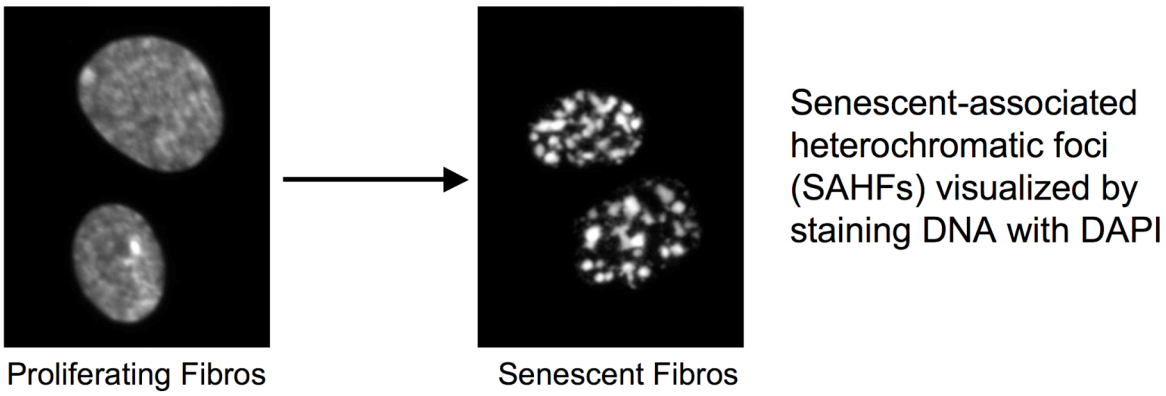
1. Change medium the day after seeding and every 2-3 days thereafter. Use 50mL of growth medium per T225 flask.
2. Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured 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 1mL aliquot) and frozen at -80°C in a Nalgene Cryo 1°C 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 at confluency.
2. Remove cells from flasks as described above under “Sub-culture.”
3. Examine viability using Trypan blue staining (SOP TP-7).

E. Senescence

1. To activate the GFP-ΔRAF1DD-ER fusion protein and induce senescence, cells at 50-80% confluency are treated for 72 hours with 20nM 4-hydroxytamoxifen in growth medium (from a 1000X working stock in absolute ethanol). At harvest, greater than 95% of DNA synthesis is inhibited and the cells have an induced fusiform/round morphology with greater than 95% showing dramatic senescence-associated heterochromatic foci (SAHF; see below).


Proliferating Fibros
Senescent Fibros
Senescent-associated heterochromatic foci (SAHFs) visualized by staining DNA with DAPI