MCF-7

Cells:
Adherent. Mammary gland, adenocarcinoma. MCF-7 cells (ATCC #HTB-22) stably transfected with a plasmid containing human E2F1 fused at the N-terminus to an HA tag and the estrogen receptor ligand binding domain.

Media
500 ml DMEM High Glucose + L-Glutamine
5 mls L-Glutamine
50 ml (10%) FBS
5 ml Pen/Strep
Filter sterilize

Thawing cells

1. Add 9-10 mls warm media to a 15 ml conical tube and 5 mls each to 2, T25 flasks. Place flasks in incubator.
2. Remove one vial of cells from LN2 tank and thaw vial immediately in 37°C water bath. Keep O ring above the water surface to prevent contamination. Thaw content with slight shake until only a small amount of ice remains in vial (approx. 1 min.). Spray or submerge vial in 70% EtOH and wipe surface with clean tissue in the hood.
3. Open the vial and transfer the content to the 15 ml conical tube containing 9-10 mls of warm media. Rinse vial 2-3 times with media to ensure majority of cells have been removed.
4. Spin down at 1200 rpm for 5 min at 24°C. Return tube to hood and pour off supernatant being careful not to disturb the pellet.
5. Re-suspend cells in 10 mls fresh media and split and transfer 5 mls to each of the T25 flasks.

Check the cells under microscope.

6. Cells are cultured in 5% CO2 incubator and medium is changed approx. every 3 days.
7. It usually takes 3 days or more for cells to recover from freezing.

Passaging cells

1. After cell culture reaches 80-85% confluence, subculture is conducted.
2. Subcultivation ratio is 1:3 or 1:4
3. Observe cells to see how confluent they are, whether the cells are alive, whether the cells are contaminated, and whether the cells have the correct morphology.
4. Pour off old media.
5. Rinse 1X with warm PBS (enough to cover bottom of flask).
6. Add 0.25% warm Trypsin and trypsinize for~3min at 37°C or until cells have released from bottom of flask. If cells do not release, bang the side of

flask. You should see the cells sliding down when flask is held up to the light. (Important: never over-trypsinize the cells, so work quickly)

7. While flasks are trypsinizing, add warm fresh media to new flasks.
8. Use serologic pipette to wash down the bottom of trypsinized flask and to break up cell clumps.
9. Add appropriate amount of trypsinized cell slurry to new flasks containing media. (The serum in the media will neutralize the trypsin)
10. Place flasks in 37°C incubator with 5% CO2.

Observations

MCF-7 cells are slow growing initially but will increase in growth after 2-3 passages.

Harvesting

Crosslinked cells

Aliquot at 2.4x10^8 cells

Adherent cells:

Add formaldehyde directly to media for a final concentration of 1%, swirl gently, incubate at RT for 10 min.

Stop reaction by adding glycine for a final concentration of 0.125 M and swirl gently to mix.

Pour off media and gently rinse with cold PBS.

Pour off PBS and add 5-8 mls cold Farnham lysis buffer + protease inhibitors.

Scrape cells and transfer to 50 ml conical tubes on ice.

Pellet cells at 2000 RPM for 5 min. at 4°C.

Gently pour off supernatant and place tubes on ice. Add equal volume cold PBS and gently re-suspend cells, then centrifuge again.

Gently pour off supernatant and place tubes on dry ice. (snap freeze)

Cell Pellets

Aliquot at 6x10^7 cells or whatever amount is desired.

Pellet cells at 2000 RPM for 5 min. at 4°C.

Gently pour off supernatant and place tubes on ice. Add equal volume cold PBS and gently re-suspend cells, then centrifuge again.

Gently pour off supernatant and place tubes on dry ice. (snap freeze)

Seed stock

Aliquot at 5x10^6 cells

a. Count cells and pipette up desired amount of cells to be frozen and add to 50 ml conical tube.
b. Pellet cells at 1200 RPM for 5 min. at 4°C.

c. Pour off supernatant and re-suspend pellet in FBS + 10% DMSO. (1 ml FBS per cryo vial)
d. Aliquot into cryo vials. Make sure lids are screwed on loosely.
e. Place in isopropanol jar (max. 18 tubes) at RT. Place jar in -80°C freezer over night.
f. The following morning, remove vials from jar, fasten lids tightly, and transfer to liquid nitrogen freezer.