Culture and transfection of adherent cells (fibroblasts/epithelial cells)

DMEM (5 °C/cold room)	Dulbecco's Modidied Eagle Medium (500mL)	11995-065	Gibco
L-glutamine (-20 °C)	L-glutamine (200mM)	25030-081	Gibco
Pen+step (-20 °C)	Penicillin-Streptomycin	15140-122	Gibco
FBS (-20 °C)	Fetal Bovine Serum (500mL)	sh-30071.03	Hyclone
Cell/Tissue culture grade dish large	Tissue Culture dish	353025	Falcon
Cell/Tissue culture grade dish small	Nunclon TC dish	150079	Nunclon Delta
Sterile disposable pippettes 2mL	serological pippette 2mL	357507	Falcon
Sterile disposable pippettes 5mL	serological pippette 5mL		Falcon
Sterile disposable pippettes 10mL	serological pippette 10mL		Falcon
Sterile disposable pippettes 25mL	serological pippette 25mL		Falcon
Falcon 15mL tubes	15mL Polypropylene Conical tubes	352096	Falcon
Hanks Buffer	Hanks Balanced Salt Soln.	14170-112	Gibco
Trypsin	Trypsin-EDTA	25300-054	Gibco

A. Thawing and initiating cell cultures

1. Prepare cell culture medium (DMEM+) (DMEM w/ 10% FBS, 50 U Pen-Strep., and 2 mM L-glutamine): Add 5 mL Pen-Strep stock, 5 mL L-glutamine stock, and 50 mL FBS to 450mL of DMEM.

2. Pre-warm DMEM+ in 37 deg C water bath.

3. Prepare tissue culture-grade dishes for cells by adding DMEM+, 25mL/large dish or 10mL/small dish.(Perform under hood using sterile technique).

4. Prepare 15-mL Falcon tube for wash by adding 10 mL of pre-warmed DMEM+.

5. Obtain cells from liquid nitrogen tanks. (Location of cells described in liq. N2 binder) and thaw vials quickly in 37˚C water bath until tiny piece of ice remains.

6. Transfer cells into a 15mL Falcon tube containing DMEM+ and wash by centrifugation for 5min at 1000 rpm. Decant supernatant by vacuum aspiration and add fresh DMEM+ to a final volume of 2mL/large dish (i.e. if you plan on making 3 large dishes fill to a total vol. of 6mL).

7. Add 2mL of cell suspension to each dish with DMEM+. Swirl dishes slowly in a figure-8 path for 1 min.

8. Incubate in TC incubators (37 deg C, 5% CO_2).

Notes:

1. Use sterile technique: All handling in laminar flow hood with sterile disposable pipettes.

2. In our lab's current stocks, 1 tube of 1mL of cryopreserved cells may be cultured in 3 large dishes or T75 flasks (2mL/dish of washed cells). The size of the dish and the amount of cells aliquoted depends on how many cells you want and the amount of time the cells may grow before achieving the desired density.
3. Bring all soln. At least to R.T. (if not 37 deg C) before exposing to cells.
4. Avoid cells overshooting confluence or acidifaction of medium (clor changes to yellow).

B. Splitting Cells (Ideal when cells cover 60-70% of dish surface area, although this varies widely by cell type.) (Cell quality is usually maintained for 10-12 splits, or about 1.5 months in continuous culture. Beyond this interval, it's best to thaw fresh stocks.)

1. Aliqoute 20mL of DMEM+ into # of large dishes corresponding to how many times you want to divide cells. (e.g., Split 1 large dish of 293 cells @ 60% density into 5 large dishes if you wish to harvest again in ~2days).
2. Wash Cells in Hanks' Buffered Salt Solution (1XHBSS) or 1xPBS to remove serum, which acts as a protease inhibitor.
3. Treat w/ Trypsin, which detaches cells from the dish and from each other.
   - Remove wash Buffer by vacuum aspiration.
   - Add Trypsin-EDTA to cells (3mL/Large dish, 1.5mL/small dish)
   - Swirl and tap dish to detach cells (usually 2-10 min at 37˚C or longer at RT). Avoid prolonged trypsin exposure, which can be severely damaging to cells, by periodic visual monitoring.
   - After cells are fully detached, add vol.of DMEM+ so the total volume is divisible by the number of total new plates desires 2mL/plate (ie Add 7mL DMEM to large plate w/ 3ml of Trypsin so the tot. vol. is 10ml that will be divided into 5 new large plates (2ml/plate).
4. Mix plate by swirling and distribute cells into new plates w/ DMEM (2mL/plate)
5. Label dishes with type of cell, date split, and # of times split (Passage #).
6. Resume culture in 37˚C tissue-culture incubator.

C. Lipofectin Transfection (Cell density on plates should be about 60%)

1. Prepare plasmid DNA in sterile 1mL Eppendorf tubes, Vtot/tube ~600 µL (1 tube will be used for 1 large plate; 20ug of DNA is usually sufficient to transfect cells in 1 large dish or T-75 flask)

DNA stocks:	Ci	Cf	Vf (uL)
DNA	eg 2µg/µL	20 µg	10
DMEM-/-			590

2. Prepare Lipofectin stock soln. in Falcon tube
   3 µL of lipofectin is required for 1 µg of DNA.