**CURRENT PROTOCOL Marie-Aude**

**Splitting HeLA Trex cells**

Volumes used in this protocol are for a 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 5 to 10mL of DPBS to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 mL of Trypsin-EDTA solution(1x) to the flask and observe the cells under an inverted microscope until cell layer is dispersed (usually within 5 at 37°C).  
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 10 mL of complete growth medium and aspirate cells by gently pipetting, up and down.
5. Add appropriate aliquots of the cell suspension to a new culture vessels. Generally, 1.5mL in 11mL of complete medium (from Monday to Friday, it’s depend on the number of passage).
6. Incubate cells at 37°C, 5% CO2

**Subcultivation Ratio:** A subcultivation ratio of 1:6 to 1:12 is recommended

**Medium Renewal:** 2 to 3 times per week

**Thawing a frozen HeLa cell stock**

This protocol is for thawing a frozen stock of HeLa cells and transferring the thawed cells to a T75cm² flask. Go through this protocol as quickly as possible.

Before starting the protocol: - Pre-warm 50 mL of cell media to 37°C, and place 30 mL in a 50 mL Falcon tubes just before beginning.

1. Quickly thaw the cells in a 37°C bath. Make sure the lid is tight and the cryo-tube remains upright in the bath to avoid contamination. You may want to carefully wipe the sides of the tube with 70% ethanol to avoid contamination.
2. Transfer the 1 mL of cells into the 30 mL of pre-warmed media. This dilutes out the DMSO.
3. Spin the cells down 5-10 minutes at 1500 rpm max.
4. Pull off the supernatant, and re-suspend the cells in 12 mL of prewarmed cell media.
5. Transfer to a T75cm² flask.

**The box is in the bottom 1st floor of Marie-Aude’s drawer in the -80°C**

**Take the cryotube with an orange cap, noted “Hela simple”**

**Cryopreservation procedure**

**Harvest cells in exponential growth. Not after a week end !**

**A good idea is to continue to maintain the cells in culture until the viability of the recovered cells is confirmed!**

**1 T75cm2 flask = 2 or 3 vial of 1mL**

1. Check your cell culture for contamination from bacteria, fungi, mycoplasma, and viruses immediately before cryopreservation. In most cases, the results of the contamination screen will be available some time after the cultures are cryopreserved (10 to 14 days). If contamination is confirmed, then destroy the frozen material.

2. Prepare a freeze medium consisting of complete growth medium and from 5 to 10% DMSO. Keep in the fridge 4°C.

3. Collect cells by gentle centrifugation (10 minutes at 125 x g) and resuspend them in the freeze medium 4°C at a concentration of 1 x 106 to 5 x 106 viable cells/ml.

4. Label the appropriate number of vials with the name of the cell line and the date. Then add 1ml of the cell suspension to each of the vials and seal.

5. Allow cells to equilibrate in the freeze medium at room temperature for a minimum of 15 minutes but no longer than 40. This time is usually taken up in dispensing aliquots of the cell suspension into the vials. After 40 minutes, cell viability may decline due to the DMSO. It’s not necessary to work in ice.

6. Place the vials into polystyrene rack at -20°C, or place it in the chamber in a mechanical freezer at -70°C (or colder) for at least 24 hours.

7. After 24h, quickly transfer the vials to a liquid nitrogen or -80°C freezer. Frozen material will warm up at a rate of 10°C per minute and cells will deteriorate rapidly if warmed above   
-50°C.

8. After 24 hours at -80°C, remove one vial, restore the cells in culture medium, and determine their viability and sterility.