**Cell line culture basics**

**CRC cell lines in the lab:**

* HT115 – MSS, low JARID2
* HT29 – MSS, low JARID2
* LS180 – MSI, high JARID2
* LS123 – MSS, high JARID2

**Splitting cells**

1. Remove old medium
2. Trypsinizing cells, usually 1ml for 2 min, LS180 for 3-5min (wash with PBS if cells are hard to detach)
3. Remove 75% cells for splitting
4. Add 10-15ml CM. Make sure final conc of trypsin is below 10%

**Freezing cells**

1. Trypsinizing cells
2. Add CM to stop Trypsinizing
3. Optional: cell counting 10 ul cells + 10 ul dye (Trypan Blue Stain 0.4%)
4. Centrifuge 900 rcf for 5min
5. Mash cells in freezing medium 1 ml
6. Print label and transfer to cryovial tube (normal tube for quick storage)
7. -80 degree freezer for 1 day
8. Liquid nitrogen for long term storage

**Freezing medium (50 ml Falcon)**

* 50% FBS – 25ml
* 40% DMEM – 20 ml
* 10% DMSO – 5 ml

**Revive cells**

1. Thaw the cells in RT or 37 degree
2. Add 12ml CM to dilute DMSO (not good for cells)
3. Remove old medium and add 8ml new CM after 1 day
4. **Culture for at least 4 days before treatment (get rid of freeze stress)**

**Freezing other stuffs:**

* Bacteria – glycerol
* Lentivirus – filter cells 0.45 um PVDF or PES

**Confluence:**

In cell culture biology, confluence refers to the percentage of the surface of a culture dish that is covered by adherent cells. **Usually, cell number doubles in incubator.**

As a general guide, from a confluent flask of cells: 1:2 split should be 70-80% confluent and ready for an experiment in 1 to 2 days. 1:5 split should be 70-80% confluent and ready for an experiment in 2 to 4 days. 1:10 split should be 70-80% confluent and ready for sub-culturing or plating in 4 to 6 days.

**Specific requirements**

LS123 cells were obtained from ATCC (CCL-255) and maintained in high-glucose (4.5 g l−1) DMEM (Gibco, catalog no. 11995073) supplemented with 4 mM L-glutamine, 1 mM sodium pyruvate and 10% heat-inactivated FBS (Axenia Biologix).

**Medium**

* 4.5 g/L glucose
* 4 mM L-glutamine
* 1 mM sodium pyruvate
* 10% heat inactivated FBS (add when use)
* Anti-anti (add when use)
* HEPES

Supplement

200 mM L-alanyl-L-glutamine for Gibco™ GlutaMAX™ Supplement.

100 mM (100x) Sodium pyruvate. The final concentration of sodium pyruvate used in most cell culture media is 1 mM (110 mg/ml).

**Different plates**

35 mm – 0.1 ml

60 mm – 0.5 ml

10 cm – 1 ml

15 cm – 3 ml

6 well plate

24 well plate

**Notice**

* Always use new gloves and clean your sleeves before using TC hood
* When you see bacteria juice (not clear) and small particles around cells, then your cells are contaminated. The check if your CM medium or trypsin are contaminated.

Transfection is the process of introducing nucleic acids into cells by non-viral methods. Transduction is the process whereby foreign DNA is introduced into another cell via a viral vector.