* **Cell Passage** (RPMI for suspension; DMEM for adherent)
  + Check confluency to decide on split ratio
  + Warm up media in hot bath (42 C)
  + Sanitize with 80% EtOH spray
  + Aspirate old media from old dish
  + Detach adherent cells with 0.25% Trypsin-EDTA (4 mL for big 15-cm dish and 2 mL for small 10-cm dish) 🡪 Evenly distribute in the plate 🡪 Wait 5 minutes 🡪 Check confluency
  + Quench trypsinization with media (2x more than trypsin: 8 mL if 4 mL trypsin; 4 mL if 2 mL trypsin)
  + 1:2 split: Transfer 6 mL suspension to a new 15-cm dish
  + 1:3 split:
  + Top off new and old dish with media until 25-30 mL final total volume.
* **Preparing Fresh Media**
  + 1 new bottle of RPMI/DMEM
  + Add 50 mL of FBS into new media bottle
  + Add 6 mL pyruvate
  + Add 6 mL Anti-Anti (anti-fungal, anti-biotic)
  + Add 6 mL NEAA (non-essential amino acids)
  + Mix and shake
  + Store at 4 C
* **Cell Counter with Invitrogen CellCountess 3FL:**
  + Trypsinize with 0.4% Trypsin-EDTA 🡪 Check if detached after 5 minutes
  + In PCR tube: 10 uL 2X Trypan Blue + 10 uL cell suspension to count
  + In glass slide: Transfer from PCR tube 10 uL mixture
  + Insert glass slide inside machine
  + Adjust focus such that plane is in the center layer of cell.
* **Cell Plating in 96-well plate**
  + Goal: Distribute 1,000 cells per well
  + Desired Volume per well: 100 uL
  + Desired Cell Density per well: 10,000 cells / 100 uL
  + (10,000 cells / 100 uL) x (96 -> 150 wells/plate) x (1 plate) =   
    1,500,000 cells / 15, 000 uL 🡪 15 mL final volume
  + Suppose Cell Counter read is 1,000,000 cells/mL alive cells
  + In Falcon Tube: (15 – x) mL media + (?) mL suspension = 15 mL where
  + Steps:
    - Trypsinize cells with 4 mL Trypsin-EDTA
    - Quench with 8 mL media
    - Remove volume that has the desired number of cells
    - Centrifuge cell suspension @ 180 g for 2 min
    - Aspirate/ Decant supernatant
      * Resuspend with 10 mL media
* **Freezing cells**
  + Prepare 10 mL of freezing media
    - 0.5 mL DMSO
    - 1 mL FBS
    - 8.5 mL media (already has 10% FBS)
  + Trypsinize cells with 4 mL Trypsin-EDTA
  + Quench with 8 mL media
  + Remove volume that has the desired number of cells
  + Centrifuge cell suspension @ 180 g for 2 min
  + Aspirate/ Decant supernatant
  + Resuspend with 5 mL media = 6 mL Final Volume
  + Divide into 4 cryo-vials 🡪 1.5 mL mixture per cryo-vial
  + Place cryo-vials into Mr. Frosty container filled with 250 mL isopropanol
  + Close the cap on Mr. Frosty but not too tight.
  + Store in -80 C freezer.
* **Thawing cells**
* **Drug Treatment**
  + Plate 5,000 cells / 100 uL for each well on a non-transparent 96-well plate