CELL CULTURE

***Check to make sure the pressure in the CO2 tanks is around 800psi and the pressure in the tank lines is around 8psi.

Making Media

For 500ml of Invitrogen DMEM high glucose media (Invitrogen Cat # 11965-092), add:
0.004g/L (0.002g/0.5L) of Ca Pantothenate
0.008g/L (0.oo4g/0.5L) of Biotin
5ml 100X Pen-Strep (Invitrogen Cat # 15140-122)
50ml Calf Serum (Preadipocytes) or Fetal Bovine Serum (Adipocytes)
Swish serum and media to eliminate a protein concentration gradient
Filter through a 0.2um filter flask in the hood and store at 4°C till ready to use.

Preparing to Plate/Split 3T3-L1 Cells:

NB: Cells are used for plating when they are 75% - 80% confluent.

1. Wash gloves and everything else going under the hood with 70% ethanol.
2. For preadipocytes, use 10% calf serum in medium for plating and feedings. To induce differentiation and post MDI when the cells are adipocytes, use 10% fetal calf serum in medium for feedings.
3. For a 10cm plate, 8ml of medium is added to the plate. When splitting cells, 1-10cm plate is usually divided to 4-10cm plates. So, 4-10cm plates would be divided to 16 plates, etc.
4. Take enough media out of the media bottle to resuspend the cells.

   Ex: You have 4-10cm plates and you are plating to 16. You will need 128ml of media, so round up to 130ml. Remove 130ml of media from the stock 10% CS or FCS bottle and put in a sterile 150 ml bottle.
5. Allow to equilibrate at 37° until you are ready to plate.

Plating of 3T3-L1 Cells:

Reagents Needed:

1X Trypsin stored in Chester; put in 37° oven so the enzyme thaws quickly and does not autodigest. If needed, dilute 10X Trypsin in a 50ml tube by adding 1X PBS.

1X PBS (Phosphate Buffered Saline) to wash the residual medium off the cells before you trypsinize.

1. Using sterile pipet, aspirate off medium from side (do not touch monolayer).
2. Wash with 1X PBS by adding about 2ml to the dish and swirl.
3. Aspirate off PBS.

4. Add 1ml of Trypsin to each plate and swirl so monolayer gets coated.
5. Leave Trypsin on for about 1 minute and aspirate.
6. Let monolayer sit for another 2 minutes and note the opaqueness or shine of the monolayer.
7. Add 4mls medium to 1-10cm plate and wash several times.
8. NB: Monolayer is an extracellular matrix of protein, so as it gets degraded, it comes off as a single cell suspension.
9. Aspirate cells, transfer to the next plate and repeat step until all 4 plates have been resuspended. Add back to cell suspension media and mix.
10. Add 8ml of cells from the resuspension to 1-10cm plate.
11. Label plates on each tray: 3T3-L1, your initials and the date plated. When you feed the plates, label with your initials, CS or FCS and the date.
12. Swirl plates on the tray forward and backward, side to side and in a figure 8 pattern.
13. After plating, feed the cells in 3 days, then every 2 days. Remember when feeding the cells to add the media to the side of the plate, not to the bottom where the monolayer of cells is attached. This could create holes in the monolayer, especially as the cells differentiate.
14. Fat cell differentiation is initiated 2 days after the cells become visually confluent.
15. Clean the aspirator and the hood surface with betadine and ethanol.
16. Empty the trash bin and leave the UV light on in the hood.