Cell Splitting

Protocol:

1. Let media and trypsin come to RT.
2. Turn the light on the hood. Lift the sash to the midpoint mark (the alarm will turn off). Wait until all front panel lights turn green.
3. Spray the hood down with 70% EtOH and wipe. Turn on aspirator.
4. Spray all materials down with 70% EtOH and wipe.
   1. Media (located in large 4C)
   2. Trypsin (located in large 4C)
   3. PBS (located above the microscope)
5. Remove cells from the incubator. Check confluency (% of surface covered by cells) and for contamination using the microscope.
6. Aspirate off old media.
7. Gently add PBS to wash off any residue. Aspirate off PBS.

|  | T25 | T75 | T175 |
| --- | --- | --- | --- |
| PBS (mL) | 2 | 3 | 5 |

1. Gently add trypsin to remove the cells from the flask. Place in an incubator for 5 min, or until cells are detached. Check using the microscope.

|  | T25 | T75 | T175 |
| --- | --- | --- | --- |
| Trypsin (mL) | 2 | 3 | 5 |

1. Neutralize trypsin by adding media. Pipette up and down to break up clumps. Transfer to a conical tube (located under the microscope).

|  | T25 | T75 | T175 |
| --- | --- | --- | --- |
| Media (mL) | 8 | 12 | 20 |

1. Centrifuge (300xg, 5 min). Aspirate off neutralizing media and trypsin.
2. Resuspend in new media.
3. Collect 10 uL of resuspended cells in a 1.5 mL Eppendorf tube.
4. Add 10 uL of tryphan blue (located near the microscope) and mix by pipetting. Add 10 uL to each side of the countess slide (located near the microscope). Note the live cell count (in cells/mL) and calculate the total number of cells in the resuspension.
5. Plate cells accordingly. Gently swirl to evenly distribute. Check under the microscope that the cells are floating and individually separated. Place in incubator.

|  | T25 | T75 | T175 |
| --- | --- | --- | --- |
| # cells minimum | 0.7E6 | 2.1E6 | 5.0E6 |
| Final flask volume (mL) | 7 | 15 | 25 |

1. Record cell flask information
   1. Cell line, passage #
   2. Media
   3. Where the cells came from
   4. Your initials and date
2. Dispose of any remaining biohazardous materials. Clean the aspirator with 70% EtOH and turn it off. Place reagents back where they came from.
3. Spray down the hood with 70% EtOH and wipe. Close the sash. Turn on the UV light.

Notes:

1. If you are not sure when you last sprayed your gloves, spray them again.
2. Each cell line has its own passage time and morphology, even if they are the same cancer subtype. Monitor them daily and do not wait too long to split them.
3. Media will change color from red to orange to yellow over time (pH indicator). If the media is yellow, the cells are either over-confluent, dead, or contaminated.

Cell freezing

Protocol:

1. Follow same steps until counting the number of cells
2. Determine freezing media resuspension calculation (want 10E6 cells in 1 mL of freezing media per cryovial)
   1. Example calculation:  
      2.3E6 cells/mL \* 26 mL = 59.8E6 cells total  
      Assume 60E6 cells. Divide by 10E6. Use 6 mL of freezing media
3. Centrifuge cells (300xg, 5 min). Aspirate off media.
4. Resuspend in freezing media as determined before. Pipette 1 mL of freezing media resuspension per cryovial.
5. Place vials in Mr. Frosty freezing chamber. Place in -80C.
6. Transfer cells to liquid nitrogen at least 16 h later. Update cell repo spreadsheet.

Notes:

1. Mr. Frosty freezing chambers located above the centrifuge in the TC room.
2. Exchange isopropyl alcohol in Mr. Frosty every 5x uses.
3. Cryovials located under microscope.
4. Freezing media located in 10 mL aliquots in -20C.

Cell thawing

Protocol:

1. Warm media to 37C.
2. Remove cells from liquid nitrogen. Store under dry ice.
3. Fill a 15 mL conical tube with 9 mL of media.
4. Take cryovial into the warm water bath and hold with gentle agitation until the cells are in liquid form again (30 sec - 60 sec).
5. Spray cryovial with 70% EtOH.
6. Pipette 1 mL of frozen cells into a conical vial. Gently pipette up and down a few times (do not vortex).
7. Centrifuge (300xg, 5 min). Remove supernatant. Resuspend in media.
8. Count cells and place accordingly (usually 10E6 cells in a T175 flask).
9. Remove cells from cell repo spreadsheet.

Notes:

1. Cells are not viable for experiments until at least 1x passage later.
2. Always have someone show you at least once how to take cells out of liquid nitrogen.