Lab Week 7
Cell Proliferation and Estimation of Viability

Cell Split for Experiments in Week 7:
Cells will be split Tuesday for the two experiments outlined below. You will begin with a flask that is 80% confluent and you will seed 1-T25 flask with 40,000 cells or a 1:50 dilution. This cell number should yield a ~1% confluent flask upon attachment. The remaining cells will be used to seed 1-six well cluster by even distribution. The 6 well plate holds 2mL per well for a total of 12mL. The volumes you will transfer and build in your subculture and 6well are detailed below.

Trypsinize in 1.5mL. Once rounded add 3.5mL of D10 to your flask and mix well. Transfer 0.1mL to 5mL of fresh D10 in a new T25 flask. Then, with a new 10mL serological pipette, add 9mL more D10 media to the remaining cell suspension. Mix well by pipetting up and down or transfer to a 15mL falcon tube for mixing. Then transfer 2mL to each well of the 6 well. Note, this week you do not want to have coverslips in the wells.

Experiment 1: ***Cell Proliferation (Note for reference only-this will be an online only assignment and images are posted for use in the results assignment)***
Quantitation of culture growth is an important part of routine maintenance. It is a crucial element of knowing the best time for subculture as well as identifying optimum experimental conditions. Measurements of cell proliferation rates are also often used to determine the response of cells to a particular stimulus or toxin. In this experiment, you will be plating HFF cells generating a growth curve from each density.

Outline:

1. Using the skills you have already mastered (subculture) prepare T25 plates as described above.
2. Monitor and record the percent confluency of your flask every day during the next week. To record your data, snap a picture of your cells under the microscope.
3. Using these images/confluency estimations, calculate the cell number and generate a growth curve with "cell number" (y-axis) vs. days or hours after subculture (x-axis). (See analysis below)

Pointers:

- Change the media after 2-3 days for best results.
- Based on the % confluency that you observe, generate a growth curve for each cell line on the semi-log plot with cells/mL on the y-axis (log scale) and time on the x-axis (linear scale).

Cell Proliferation Analysis

- To calculate the cell number based on percent confluency, we will assume that there are 2.6×10^6 total cells in a confluent T25 flask. Therefore, a flask that is 10% confluent will have 2.6×10^5 cells.
- Determine the lag time and population doubling time for each cell line.
- What conclusions can you draw about cell proliferation and doubling time for HFF cells?

Experiment 2: ***Estimation of Viability (Use Trypan Blue Protocol)***
One of the fundamental tools in cell biology is the establishment of cellular viability under different experimental conditions. In this experiment, we will perform a viability assay using Trypan Blue dye to evaluate the cytotoxicity of ethanol.

Materials:

- 6-well plate of U2OS cells
- 5ml of 200 proof ethanol
- 50ml 1xPBS
- 6 15ml falcon tubes for solution prep
- Pipette tips and Pipettes
- Liquid and Solid Waste Containers
- Inverted (True Color) Microscope *The EVOS cannot be used

Outline:
You will be provided with a 6-well plate of >50% confluent U2OS cells. Please complete the treatment plan below with the goal of establishing the cytotoxicity of Ethanol, at varied concentrations, using the Trypan Blue viability assay. (*See the separate protocol for Trypan Blue*)

Pointers:

- In the interest of space, this experiment will be performed at the bench. Note that this is only possible because we will not be continuing these cells beyond this experiment.
- When applying the ethanol treatment solutions, you will need to remove the D10 media to make sure that your calculated concentrations are not further diluted. ***So, make your treatment solutions, then remove the D10 media and then add the treatment.***
- Cells should be incubated with the ethanol mixture for ***10 minutes*** ***at room temperature.***
- Our stock solution is at **200 proof solution.** Prepare your solutions in a tube and mix well before adding to the wells.
- This is a preliminary test, so we will troubleshoot through the experiment. Begin by choosing a large range in concentration for wells 1-3. After collecting your image data for those 3 wells, decide on a smaller range for the remaining 3. ***Do a little research before lab to see if you find any reported IC50 values for ethanol.***

Analysis:

- Based on your findings, approximate the dose of ethanol at which 50% of the treated cells are killed (IC_{50}).
- Based on your observations, develop a hypotheses about the possible mechanism of cell death with ethanol toxicity.
- What future experiments may help you to prove or disprove this hypothesis?

Cell Template: Ethanol concentration per well		
No treatment-Neg Control **200 proof ethanol: 0ml** **1xPBS: 2ml**	**_____ Ethanol** **200 proof ethanol:** **1xPBS:**	**Pos Control: 70% Ethanol** **200 proof ethanol: 1.4ml** **1xPBS: 0.6ml**
_____ Ethanol **200 proof ethanol:** **1xPBS:**	**_____ Ethanol** **200 proof ethanol:** **1xPBS:**	**_____ Ethanol** **200 proof ethanol:** **1xPBS:**

*observe the cells every few minutes to see if they are detaching from the plate. You may want to begin the Trypan Blue addition earlier than 10 minutes.