Treating TK6 Cells with Lanthanide Chlorides (for use with CometChip protocol)
9/22/16

Materials:
Lanthanide chlorides (Sigma Aldrich)
DPBS
6-well plate
Complete media (RPMI with Glutamax, 10% FBS, 1% Pen strep)

Preparing lanthanide solutions:

1) Prepare 100 mM solutions by dissolving each lanthanide chloride in water. Metals should be weighed inside a chemical hood.
   Note: An iron nitrate solution can also be prepared as a control compound
2) From these 100 mM stock solutions, dilute down to 1000 μM in DPBS.
   Note: When metals are added to DPBS, you will see precipitates form.
3) Store lanthanide solutions at 4C, protected from light.
4) Immediately before use, vortex to mix solutions well.

Workflow:

1) Label a 6-well plate with each metal condition, keeping 1 well for a non-treated control. Warm approximately 35 mL of complete media for each 6-well plate.
2) Count TK6 cells. 1 million cells will be plated for each well.
   Note: For experiments, use cells at a density between 500,000 and 800,000 cells/mL. This can be achieved by seeding TK6 at 200,000 cells/mL the day before.
3) For a single 6-well plate, spin down 8 million cells for 5 minutes at 200g and 4C. Resuspend the cells in 8 mL of complete media to obtain a density of 1 million cells per mL.
4) While cells are spinning, vortex/mix the 1000 μM lanthanide solutions and add 500 μL of these 1000 μM metal solutions to each of the metal treated wells on the 6-well plate. Add 500 uL of DPBS to non-treated control.
5) Add 3.5 mL of warmed media to each well, bringing the total volume to 4 mL and the metal concentration to 125 μM.
6) Add 1 mL of the cell suspension (1 million cells) to each well, bringing the total volume of each well to 5 mL and the metal concentration to 100 μM.
7) Check each well under a microscope.
   Note: At 4x, metal precipitates are visible among cells.
8) Place 6-well plate in 37C incubator, 5% CO2, for desired amount of time. During treatment in incubator, plate can be placed on a rocker so that precipitates don't settle at bottom of dish.
9) Following treatment period, cells can be loaded onto CometChip for DNA damage measurements.