Pullen Lab Protocol for Isolating Proteins from *in vitro* Cell Culture (Adherent)

Use this procedure for extracting whole, mostly cytosolic proteins from mammalian cells grown *in vitro*.

Materials:

- 1X *Cell Signaling* lysis buffer (diluted from 10X stock, which is found in the -20°C)
- 1X DPBS
- 1.5mL microcentrifuge tubes (at least two for each sample replicate)
- serological pipettes (size and number depends on your experiment)
- serological pipettor
- Container with ice
- 1mL micropipette and tips
    - The 1mL micropipette tips can also be used for cell scraping.
- 20µL-200µL micropipette and tips
- High-speed, refrigerated microcentrifuge
- Waste container (10% v/v bleach)

Procedure:

Ideally you should be performing experimental replicates in 6-well plates. The following steps are for adherent cell types (*e.g.* BHK-MKL, Vero, E3). You do not need to do this under a hood, but you should avoid all cross contamination, using fresh pipettes for each sample replicate.

1. Turn on refrigerated microcentrifuge (should be set for 4°C)
2. Thaw 10X *Cell Signaling* Lysis Buffer (keep ice cold though!) and dilute a portion to 1X in MilliQ H_2O.
    a. ex: 100µL 10X in 900µL H_2O
3. Remove culture medium and discard into waste container.
4. Place culture vessel on ice and gently rinse with room temperature DPBS to remove residual medium. Discard rinse into waste container. At the end of this your cells should not be in liquid, so don't let them dry out!
5. Add ice cold 1X *Cell Signaling* Lysis Buffer directly to the cells. The volume you add depends on the culture vessel, confluence, and type of cells: keep in mind that you want your final sample to be concentrated enough to load into a gel. Lysis buffer volumes for >80% confluent cells (most):
    a. Each well of a 6-well plate: 150µL
    b. 10cm dish: 400µL
6. Scrape the cells free using an inverted 1mL micropipette tip (sorry, actual cell scrapers are too highly priced to justify buying). A NEW TIP FOR EACH REPLICATE!
7. Tilt the plate such that the fluid collects and transfer to a 1.5mL microcentrifuge tube.
8. Vortex the tubes for 3s, and then place back on ice for 5min.
9. Repeat step 7 two more times.
10. Centrifuge at 12,000xg for 10min in the refrigerated microcentrifuge.
11. Place tubes on ice, and then remove the supernatant. **SAVE THE SUPERNATANT** as this contains most of the proteins we are usually interested in. However, many membrane proteins are left behind in the pellet, so if you are studying one of those, then you would also save the pellet, otherwise we discard the tube with the pellet.
12. Store your supernatants at -80°C. Time permitting, go ahead and measure their concentrations before freezing.