Pullen Lab Protocol for Determining Prtoein Concentration Using Nanodrop

The Nanodrop is shared equipment among research and teaching labs, so be mindful of other users' time, and only use consumables from our lab (*e.g.* tips).

Materials:

- Protein samples
- Nanodrop device
- Container with ice
- 10µL micropipette with tips
- Kimwipes
- Vortex mixer *
- Single-speed minicentrifuge *

Procedure:

1. If samples are frozen, start here: thaw them and keep on ice. If you are continuing directly from protein isolation, proceed to step 4.
2. Briefly vortex thawed samples.
3. Briefly centrifuge the samples in the single-speed minicentrifuge, then place on ice.
4. Turn on the PC next to the Nanodrop, and open the "NanoDrop 2000/2000c" software.
    a. There will be a series of clicks from the Nanodrop if it is operational.
5. Click the "Protein A280" button.
6. **CAUTION**: do not immediately click "OK" at the first prompt: first pipette 2µL of MilliQH_2O onto the Nanodrop detector, lower the arm, then click "OK".
7. After some clicking, the instrument is normalized. Lift the arm and gently wipe away the water with a kimwipe.
8. Place a new 2µL aliquot of MilliQH_2O onto the detector, lower the arm, and click "Blank" (in the upper left corner of the screen).
9. After the instrument is done, lift the arm and gently wipe away the water with a kimwipe.
10. Pipette 2µL of sample onto the detector, lower the arm, and click "Measure" (upper left corner of screen).
11. After the instrument has made the measurement, record the value in µg/µL.
12. Lift the arm, wipe away the sample.
13. Repeat steps 10-12 for each sample (make sure to change tips!)[1]
14. When finished, make sure the detector is clean and close the software.

*only necessary if your samples are frozen

[1] You can re-blank the device at any point, especially if results are not consistent from replicates of the same sample.