**Determining RNA Concentration Using UV Spectrophotometry**

\_\_\_Fill your ice bucket with ice from the ice machine in Room 212.

\_\_\_In a microfuge tube, make a 1:49 dilution of your RNA sample. This dilution is prepared by pipetting 1 uL of the isolated RNA into 49 uL of RNase-free water.

\_\_\_Keep the RNA dilution tube on ice.

\_\_\_Bring the tube up to room 301. This room houses both the UV spectrophotometer and the real time PCR machine. The UV lamp on the spectrophotometer should be turned on an hour ahead of use and was (hopefully) turned on prior to lab.

\_\_\_Clean the small quartz cuvette by pouring some of the Beckman Trace Clean solution (on counter next to sink) into the cuvette. Pipette the solution up and down several times and then discard the solution into the sink. We will use a 200 uL pipette with RNAse free tips for this work.

\_\_\_Irrigate the cuvette with the squirt bottle of Type I water (also on counter next to sink).

\_\_\_The cuvette is now fairly clean but still should be rinsed with some of the RNase-free water before preparing a blank of the kit water. So, pipette 50 uL of kit water into the cuvette and wash by pipetting up and down. Repeat twice more.

\_\_\_Next, pipette 50 uL of RNase-free water into the cuvette and place the cuvette into the spectrophotometer with the red dot facing towards you.

\_\_\_Use the mouse to click on the “Blank” button on the spectrophotometer.

\_\_\_Click on the “Read Sample” button three times. pausing a few seconds between each click. This will record the absorbance of the blank.

\_\_\_Next, remove the cuvette and pipette out all of the RNase-free water into a waste beaker.

\_\_\_Pipette the first 50 uL sample into the cuvette and use the mouse to click on the “Read Sample” button three times.

\_\_\_After the sample is read three times, remove the cuvette from the spectrophotometer and pipette the sample back in to the microfuge tube.

\_\_\_Wash the cuvette by placing 50 uL of kit water into the cuvette and pipette up and down several times. Then pipette the water in to the waste beaker, removing all traces of water with the pipette.

\_\_\_Repeat for all additional samples in your lab section.

\_\_\_The last sample should be a blank of RNase-free water.

\_\_\_Calculate the average of the three absorbance readings at 260 nm.

\_\_\_After spectrophotometer analysis, the concentration of the RNA can be found by the following formula:

One absorbance unit at 260 nm (OD260) corresponds to 40ug of RNA, so RNA in ug/mL is given by:

(OD260) X (dilution factor) X (40ug RNA/mL)/1 OD260 unit

For your analysis: (OD260) X 50 X 40 = concentration of RNA in ug/mL.

Formula from *At the Bench: A Laboratory Navigator* by Kathy Barker.