# DNA/RNA quantification using PicoGreen/RiboGreen

## Things to do before starting

* Wipe pipettes, outside of pipette boxes, counter etc. down with RNAase and kim wipes
* Need barrier tips of every size (10, 20 ,100, 200, 1000), remember to never reuse a tip!
* Wipe gloves with RNAase often - paper towels
* RNA samples on ice and thawed
* RNA only plate or unopened package
* Warm up Tecan

1. Prepare 1X TE buffer from the 20X stock, which is supplied in the Ribo kit (to make 50mL, add 2.5mL of 20X TE to 47.5mL NF H20). 50mL is sufficient for 250 assays.
2. Add **\_\_\_uL 1X TE** to clear tub (calculated on plate layout sheet)
3. Use 8 multi-pipette, add 99uL 1X TE to **sample wells** (according to plate layout- not to standard wells: A1-6 and B1-6)
4. Add 1X TE to **standard wells** (A1-6 and B1-6): 100, 97.5, 95, 90, 80, 70 uL
5. Discard remaining 1X TE in tub (down sink)
6. Lights off, grab PicoGreen/RiboGreen and DNA/RNA standard from fridge (PicoGreen/RiboGreen kit package), vortex and place in drawer
7. Add 140uL 1X TE + 2.86uL RNA standard (in drawer) to a 1.6mL tube (labeled RNA std) and vortex
8. Add diluted DNA/RNA standard to **standard wells**: 0, 2.5, 5, 10, 20, 30 uL
9. Add 1uL DNA/RNA to **sample wells** (follow layout)
10. Add **\_\_\_uL 1X TE** and **\_\_\_uL 200X PicoGreen/RiboGreen** (in drawer) to clear tub and mix by pipetting
11. Add 100uL of diluted Pico/Ribo in tub to **every standard and sample well** and mix by pipetting up and down (use multi-pipette and when mixing do not lift up to avoid introducing air bubbles)
12. Take 10uL pipette tips and pop any remaining bubbles in wells by moving pipette around well
13. Cover plate in tinfoil
14. Tecan - load plate in and start measurements. (Wavelength/bandwidth: Excitation: ~485nm/20nm; Emission: ~530/25nm)