RNA Pipette Exercise: Serial Dilutions

Learning objectives

- Prepare a serial dilution of nucleic acids with precision and accuracy
- Analyze and interpret a spectral data from a NanoDrop
- Know when it is necessary to vortex, spin, and change tips

Nucleic acid quantification with the Nanodrop

Nucleic acid quantification is an important and necessary step prior to most downstream analysis methods. A traditional method for assessing RNA concentration and purity is UV spectroscopy. We will use the NanoDrop to quantify our RNA via UV Spectroscopy.

Follow this link to watch a 3 minute video about how the NanoDrop works. Typically the NanoDrop is housed in a communal space on the 3rd floor of Loeb.

Serial dilutions

A serial dilution is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in descending concentration in logarithmic fashion. Serial dilutions are used to accurately create highly diluted solutions or to create standard curves with a logarithmic scale. Ten-fold and two-fold serial dilutions are commonly used in molecular biology.

A **ten-fold serial dilution** reduced the concentration by a factor of 10. For instance, the final concentration of the series could be: 100 M, 10 M, 1 M, 0.1 M, 0.01 M, and 0.001 M.

A **two-fold serial dilution** reduces the concentration by one half. For instance, the final concentration of the series could be: 200 M, 100 M, 50 M, 25 M, 12.5 M, 6.25 M.

Vortex and Spin

A good serial dilution requires good mixing of the solutions. You will use the **vortex** to mix your samples. (Rayna recommends vortexing for 5 seconds at 3000 rpm.) Then, you need to **spin** the the tubes, so that all the liquid is at the bottom of the tube. (Rayna recommends spinning in the 6 place mini-centrifuge for about 3 seconds. These mini-centrifuges do not need to be balanced.) Be sure to vortex and spin after you combining the reagents for each serially diluted sample.

When to change pipette tips

It is really important keep all your reagents clean and pure. You need a clean tip every time you pipette one reagent into a tube that has another reagent. If you are pipetting into a empty tube or well, its okay to reuse the same tip because the new tube should be free of contaminants.

Keep your samples on ice

RNA can degrade rapidly. Too minimize degradation, we store RNA frozen (usually at -80C but its okay at -20 for 1 day). When working with RNA, we keeps our samples on ice as much as possible. In this exercise, place your tubes on ice when not in use.

Exercise: Prepare a 2-fold serial dilution

1. Place the 50 µL RNA sample provided by your TA on ice. This is "Standard Dilution 1 (SD1)".
2. Obtain eight 0.5 mL Eppendorf tubes and label them SD2 - SD8 and Blank.
3. Add 25 µL water two tubes SD2 - SD8 and to the Blank. (You can reuse the same tip).
4. When SD1 has thawed, vortex and spin it.
5. Transfer 25 µL of SD1 to SD2. Vortex and spin SD2. Place SD1 on ice.
6. Transfer 25 µL of SD2 to SD3. Vortex and spin SD3. Place SD2 on ice.
7. Repeat the process described above to prepare SD4 - SD8.
8. Select the "RNA" protocol on the NanoDrop.
9. Load 1.5 µL of your Blank sample onto the NanoDrop pedestal. Press the measure blank button.
10. Using a KimWipe, wipe of the pedestal and the level.
11. Load 1.5 uL of SD1 onto the NanoDrop pedestal. If it doesn't measure automatically, press the measure sample button.
12. Repeat steps 10 and 11 for all your samples.
13. From the on-screen data report, determine whether or not each samples is half as concentrated as the previous.
14. Save your results to a USB drive.
15. Optional: Plot expected concentration versus observed concentration. Calculate the R^2 value of the curve and determine with student prepared the most precise serial dilution.