04_01_Spectralphotometer-Nanodrop

DIENSTAG, 18.5.2021

Goal-Setting

- The Nanodrop spectralphotometer is an instrument for nucleic acid and protein concentration measurements. The spectralphotometer is located in the Extension Lab on the left of our iGEM bench

Terms / abbreviations

- None

Risk areas

- None

Required materials and / or information

- Solution of blank e.g. MilliQ water or the solution without substance of interest
- 1 µL of sample
- Pipettes, Eppendorf
- Tissue

Templates, devices, software

- Nanodrop sprectralphotometer, ThermoScientific Nanodrop 2000
- Nanodrop Software

Preliminary work

- None

Operation

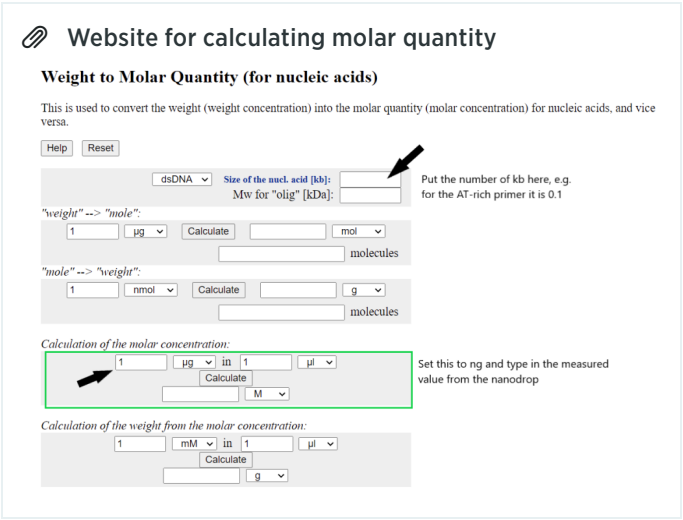
- Open the Nanodrop software on computer
- Choose the setting e.g. nucleic acid and ssDNA
- Open the lid, wipe the pedestal with a tissue to make sure the pedestal is clean and dry
- Blank the solution of the sample (whatever is in the solution, what is not the substance of interest)
- Apply 1 µL on the pedestal, close the lid and press "blank" to measure
- Once the solution is blanked, wipe the pedestal, apply 1 µL of the sample for each measurement
- Record results in following table:

Example: Recording Nanodrop results

	A	B	C	D	E	F	G	H	I	J	K
1	**Sample name and type**	**Blank**	**Nucleic acid concentration**	**Unit**	**A260**	**A280**	**A260/A280 (1.8-2.0)**	**A260/230 (≥2.0)**	**Estimated length [nt]**	**Calculated concentration [nM]**	
2	P100 1:100 (09.06.), ssDNA	MilliQ	54.1	ng/µL	1.640	0.835	1.69	3.37	100	833.59	

- Website to calculate the ng/µL into nM or µM: Weight to Molar Quantity (for nucleic acids) (bioline.com)

Website for calculating molar quantity



Troubleshooting

- The Nanodrop does not differentiate between nucleotides and nucleic acid strands
- To verify that the blank is fine or to determine the deviation, measure the blank as a sample
- For a pure DNA sample A_{260}/A_{280} = 1.8-2.0
    - Else: Contamination with proteins
- For a pure DNA sample $A_{260}/A_{230} \geq 2.0$
    - Else: Contamination with sugars, salts or phenols

Sources

- https://www.eppendorf.com/product-media/doc/de/59828/Eppendorf_Detection_Application-Note_279_BioPhotometer-D30_Detection-contamination-DNA-protein-samples-photometric-measurements.pdf

Follow-up work

- 03_04_Phusion-PCR-reaction