Assessment of the Extracted Nucleic Acid

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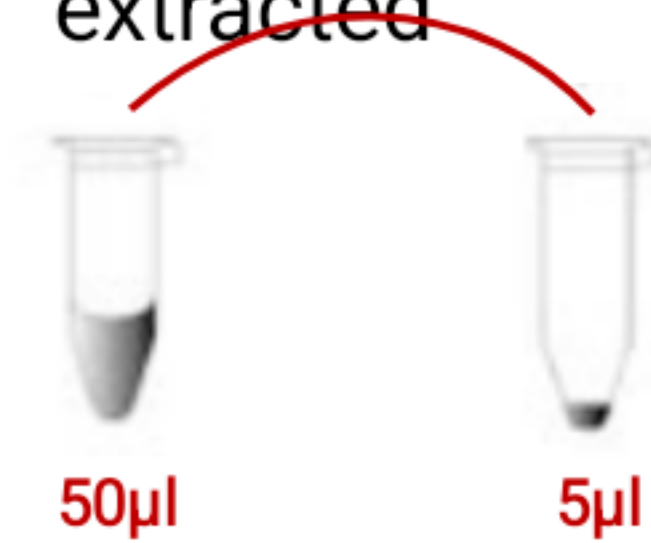
Assessment of Extracted Nucleic Acid

- Three routine tests can be performed to check for quantity (concentration/ amount / yield) and quality (purity and integrity) of the extracted product
- Take an aliquot of the sample (5µl out of 50µl)



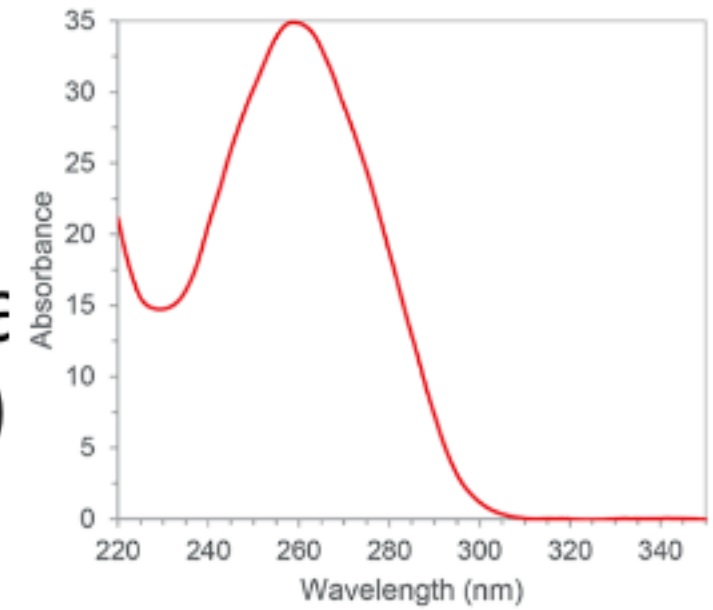
1. Measurement of concentration

Assessment of Extracted Nucleic Acid

- UV-Vis spectrophotometer (ultraviolet-visible spectrophotometer) instrument is used to measure the concentration and the yield of the extracted nucleic acid (DNA or RNA)

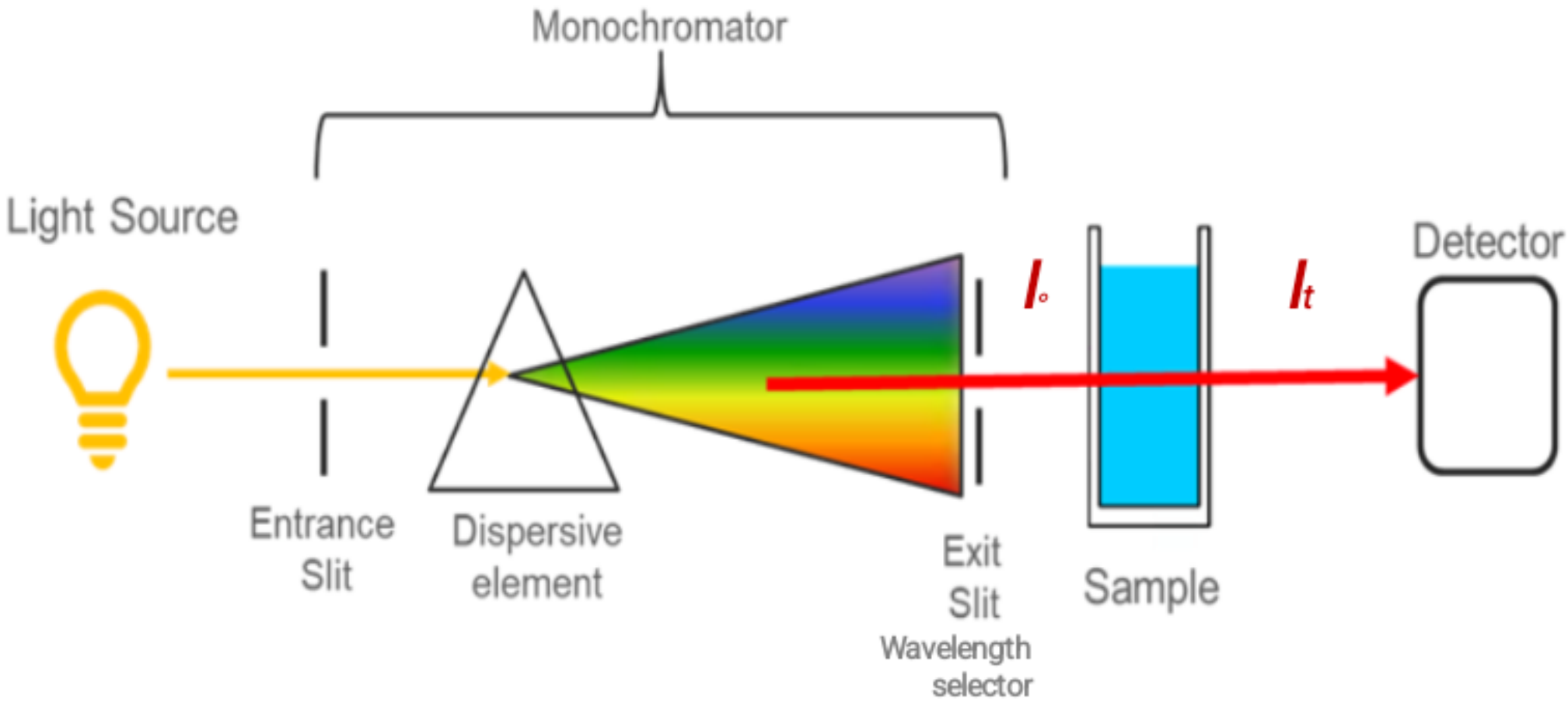
Electromagnetic Spectrum of Light

- Nucleic acids absorb UV-light maximum absorbance at wa nm (λ= 260nm)



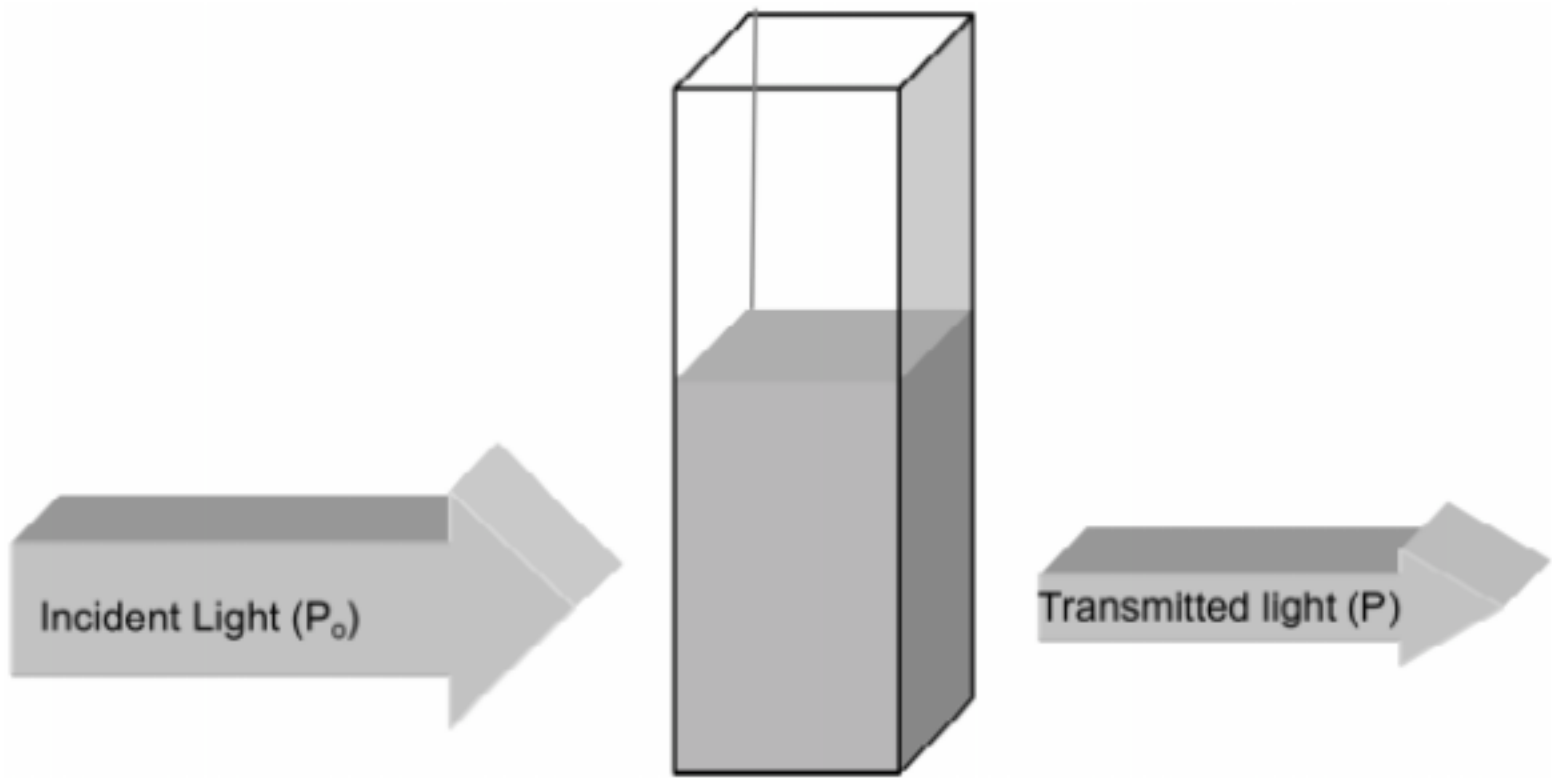
Typical RNA/DNA absorbance spectrum.

Assessment of Extracted Nucleic Acid



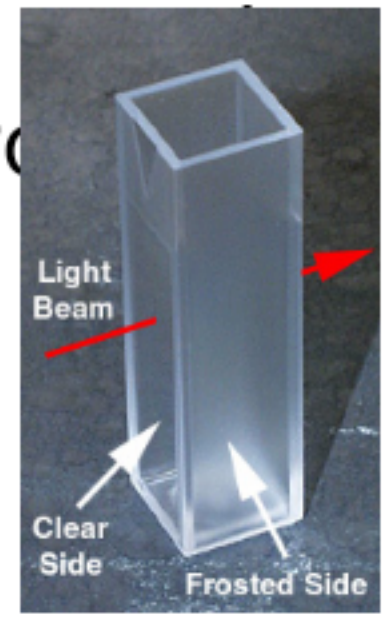
Assessment of Extracted Nucleic Acid

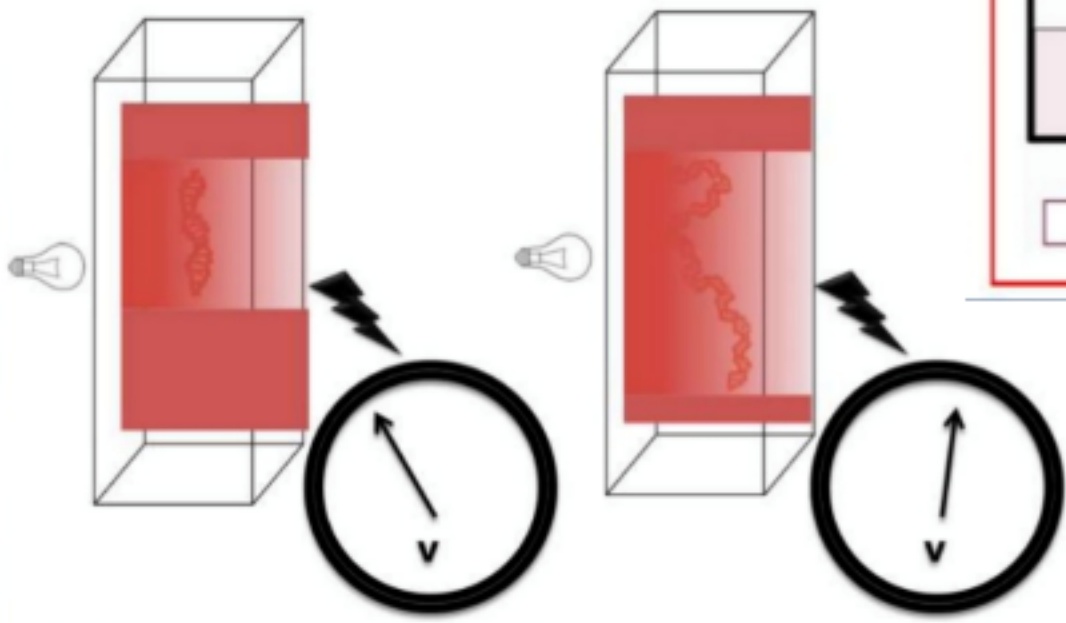
- Part of the incident light will be absorbed by the sample particles (depending on the concentration)
- The attenuation in the light that reaches the detector is measured in relation to the incident light and expressed as optical density (OD)

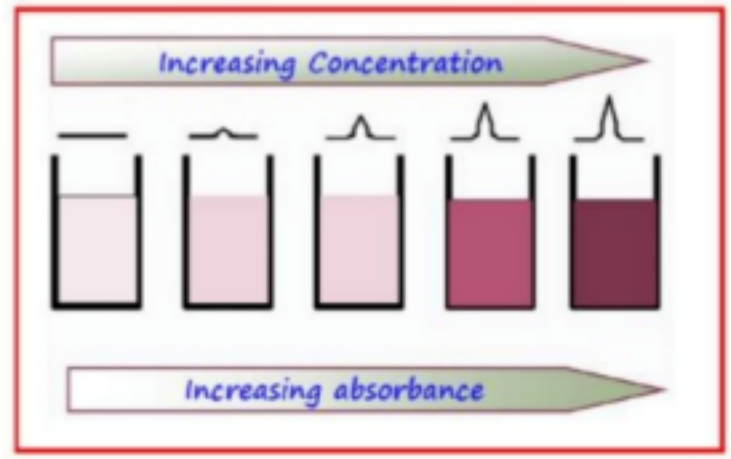


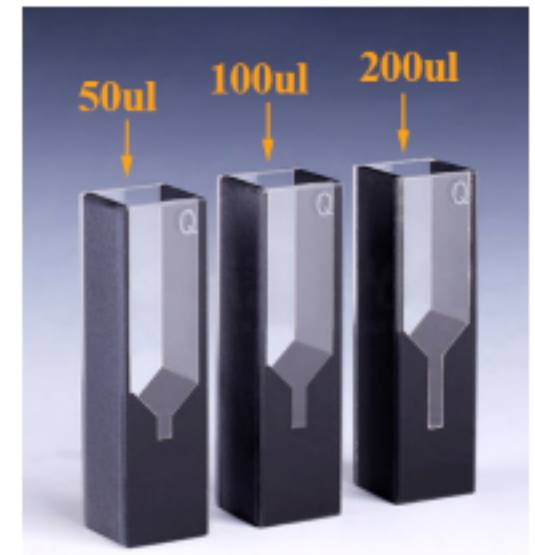
Assessment of Extracted Nucleic Acid

- Cuvette is made of plastic (disposable) or with two transparent and two opaque (foggy/fr sides
- Before sample measurement, a blank must measured (the buffer or solvent used to dissolve the sample)









Assessment of Extracted Nucleic Acid

- The concentration is estimated per one optical density unit (1OD) according to the type of nucleic acid (e.g. 1 OD unit = 50 µg/ml dsDNA)

Nucleic Acid	Concentration (µg/ml) or (ng/
ds DNA	50
ss DNA	33
ss RNA	40

- The concentration is calculated according to the following equation:

$$\text{DNA Concentration (µg/ml)} = OD_{260} \times \text{dilution factor} \times 50\ \mu g/ml$$

Assessment of Extracted Nucleic Acid

Example 1: OD_{260} = 0.6, dilution factor = 100, sample of genomic DNA

Answer:

DNA conc. = 0.6 x 100 x 50 ng/µl or µg/ml

= 3000 ng/µl or 3000 µg/ml or 3 µg/µl

Example 2: Calculate the dilution factor if 5µl of sample was added to 95µl of water?

Answer: dilution factor = total volume / sample volume

100 / 5 = 20 (dilution factor) 1:20

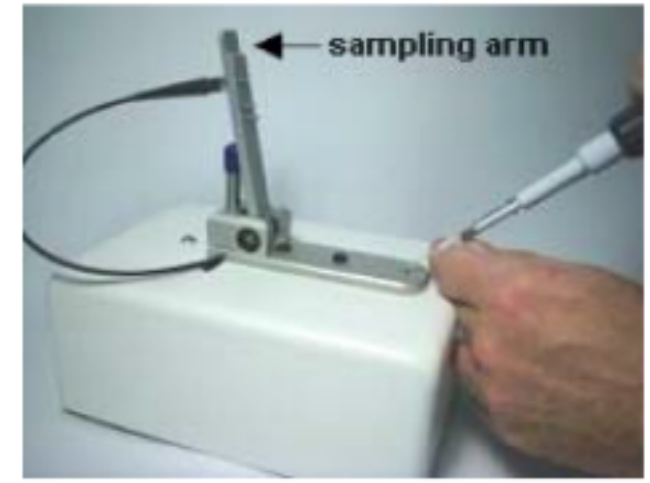
Example 3: Calculate the amount (yield) of DNA in the above example if total volume is 50 µl ?

Assessment of Extracted Nucleic Acid

NanoDrop is automatically
calculate the concentration in ng/µl

Assessment of Extracted Nucleic Acid

- NanoDrop spectrophotometer has similar principle to the standard cuvette spectrophotometer
- Load 1µl blank onto the lower pedestal, close the sampling arm and **click on blank** (wait 10-15 second)
- Wipe the pedestals and repeat the same steps using 1µl DNA or RNA sample and **click on measure**



Assessment of Extracted Nucleic Acid

DNA Quantitation

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Assessment of Extracted Nucleic Acid

Assessment of Extracted Nucleic Acid

2. Sample purity:

- The reading of absorbance at 260nm is divided by the reading at 280nm to estimate sample purity
- Aromatic amino acids of protein have maximum absorption at 280nm
- This ratio is most commonly used to determine the presence of protein in the isolated sample
- The acceptable range for this ratio:

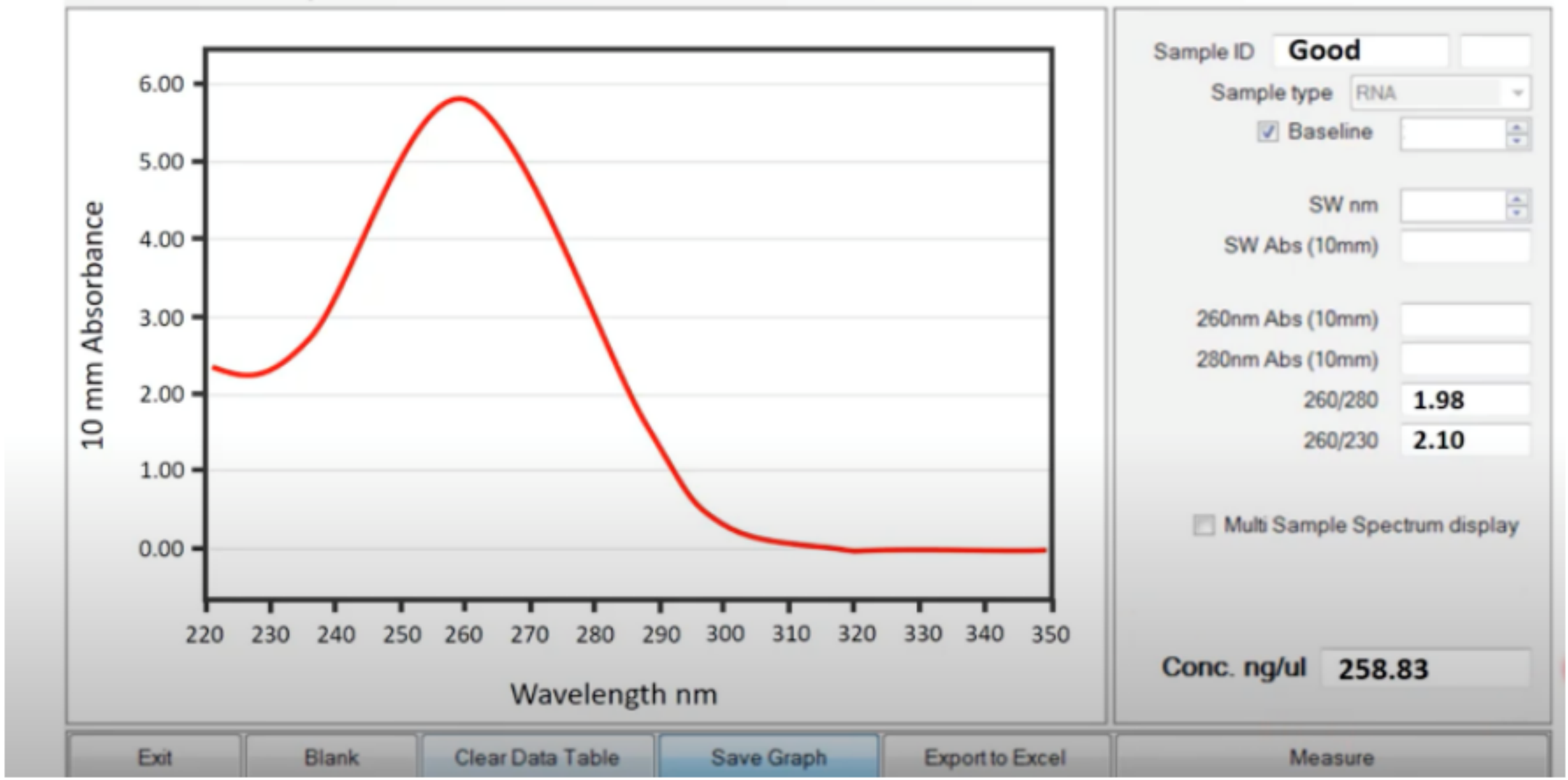
Sample type	Ideal	Accepted range
DNA	1.8	1.7-1.9
RNA	2.0	1.9-2.1

Assessment of Extracted Nucleic Acid

- The ratio of A260/A230 is used as secondary measure of nucleic acid purity (chaotropic salts, TRIzol and peptide bonds of protein absorb at 230nm)
- The expected value is 2.0-2.2 (should be greater than A260/A280 ratio)
- Low ratio indicate possible contaminants

Interpretation of Nanodrop Results

Sample 1



Interpretation of Nanodrop Results

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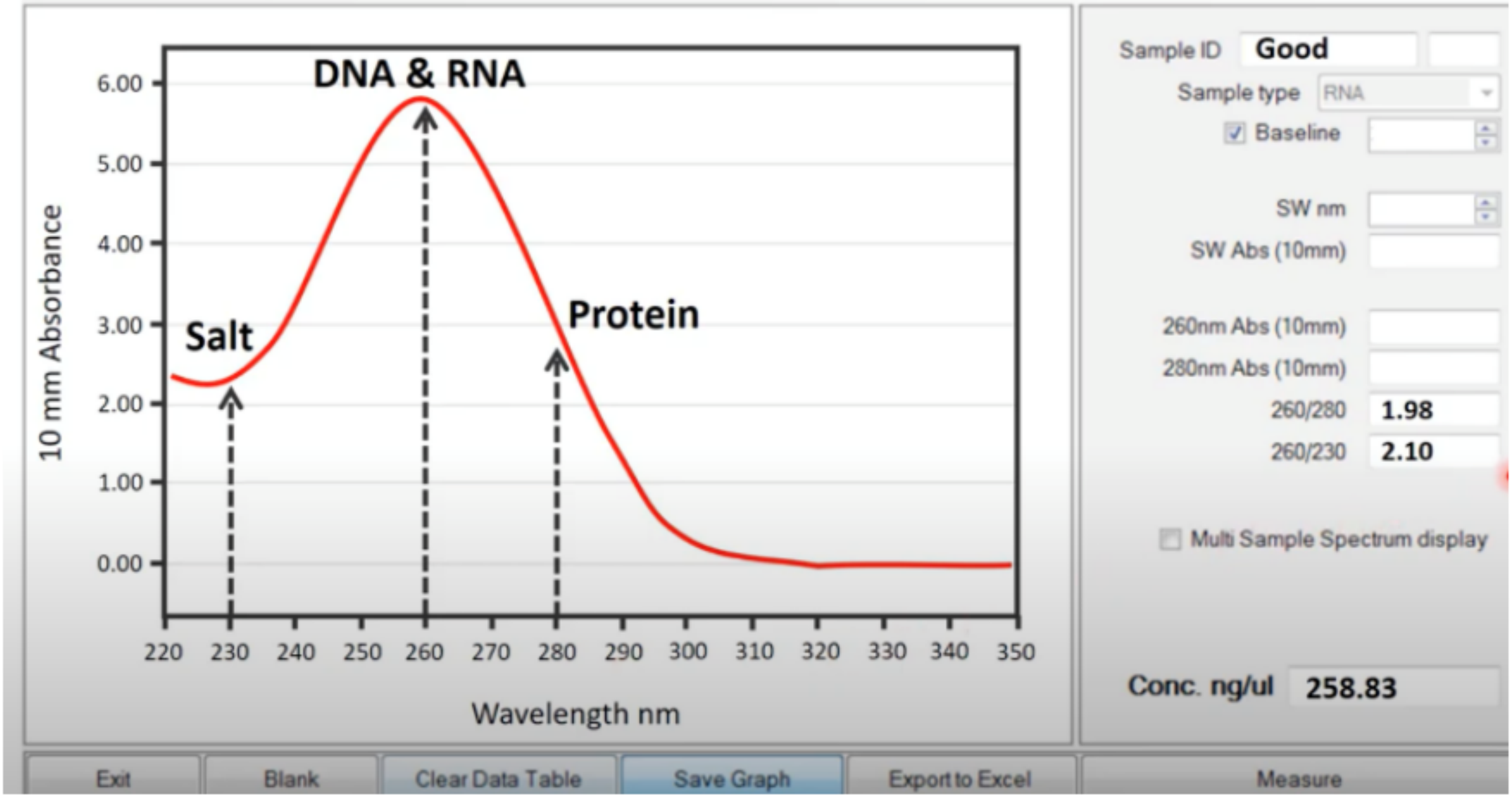
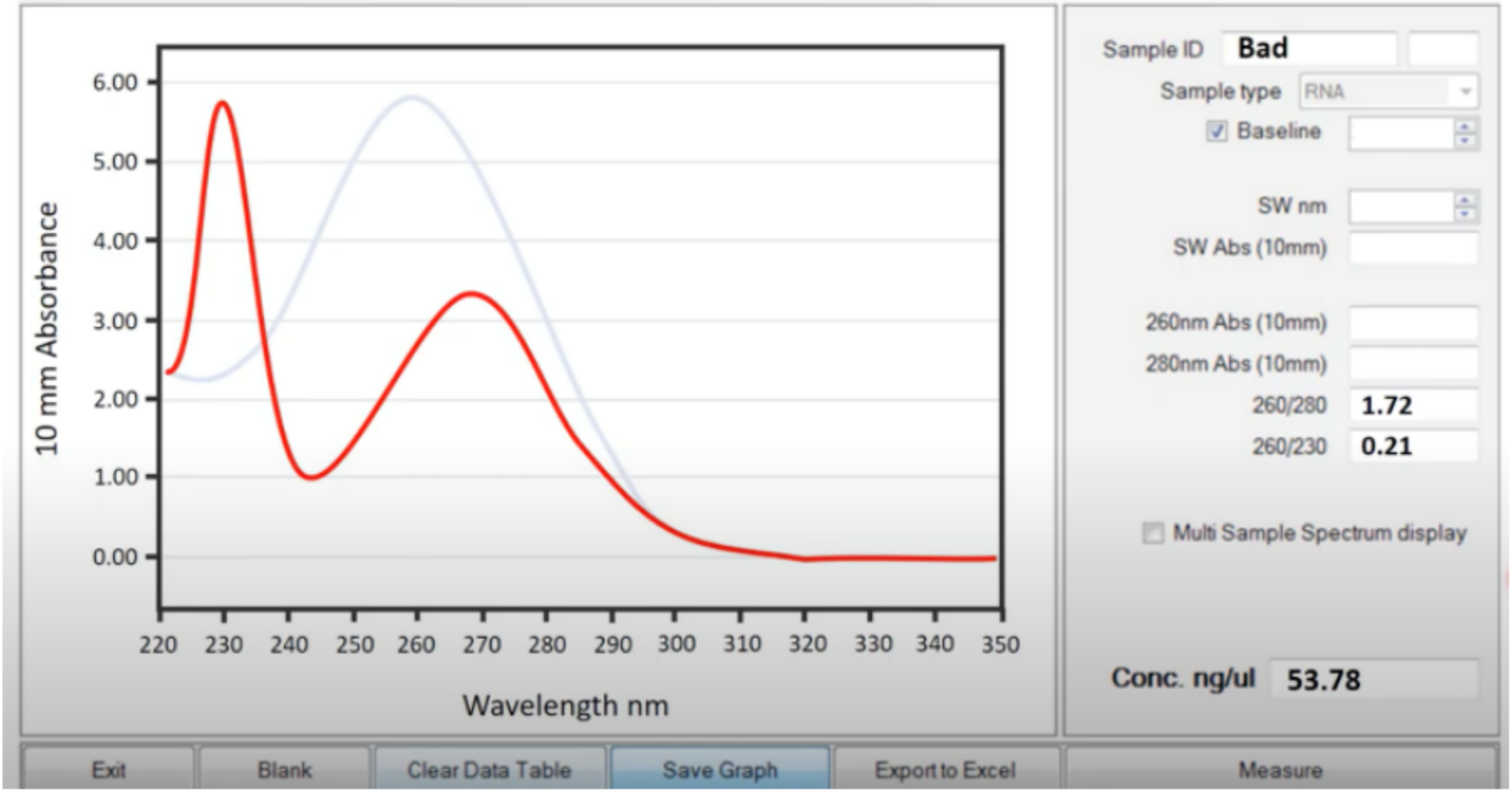


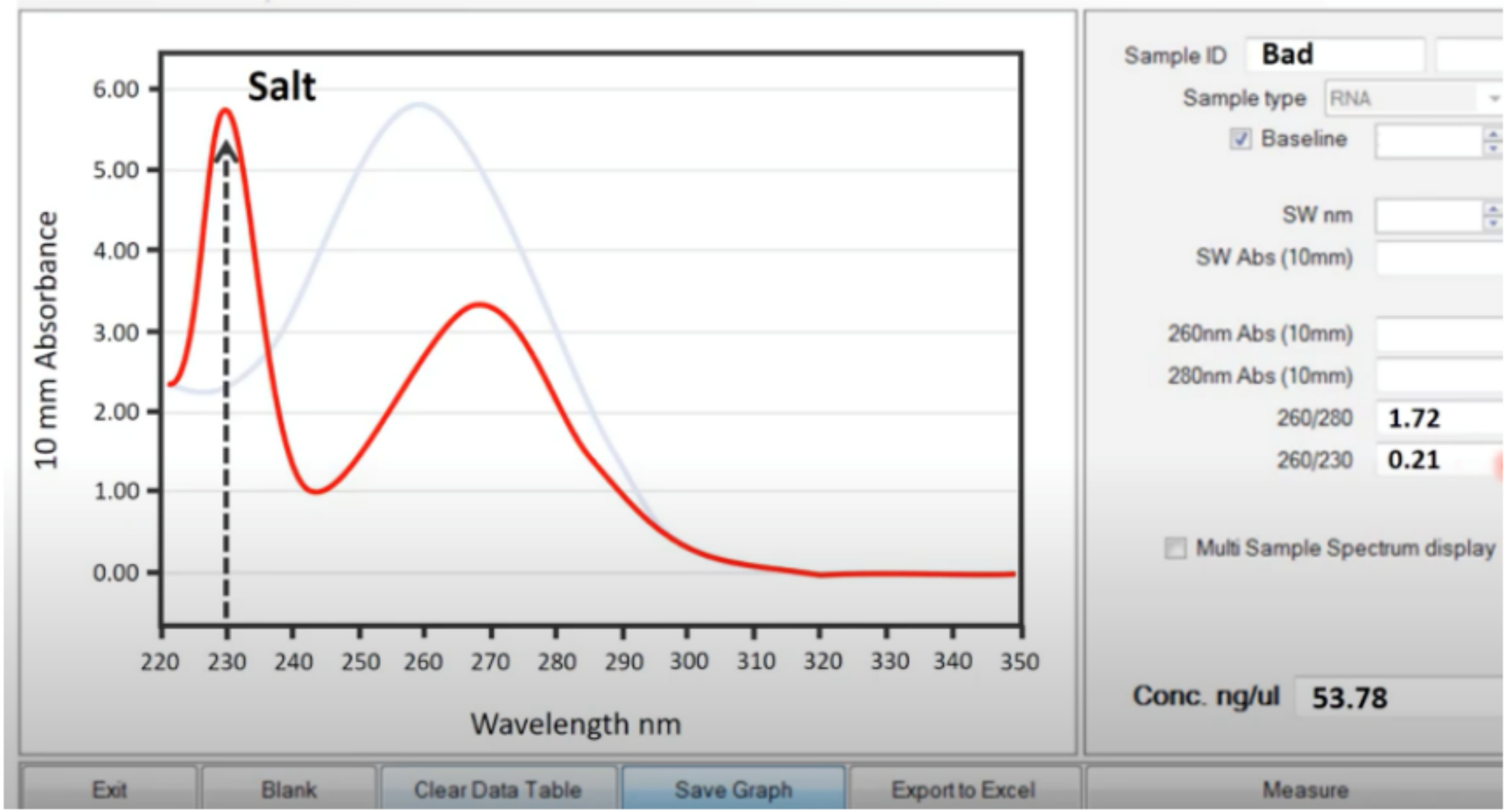
Interpretation of Nanodrop Results

Sample 2



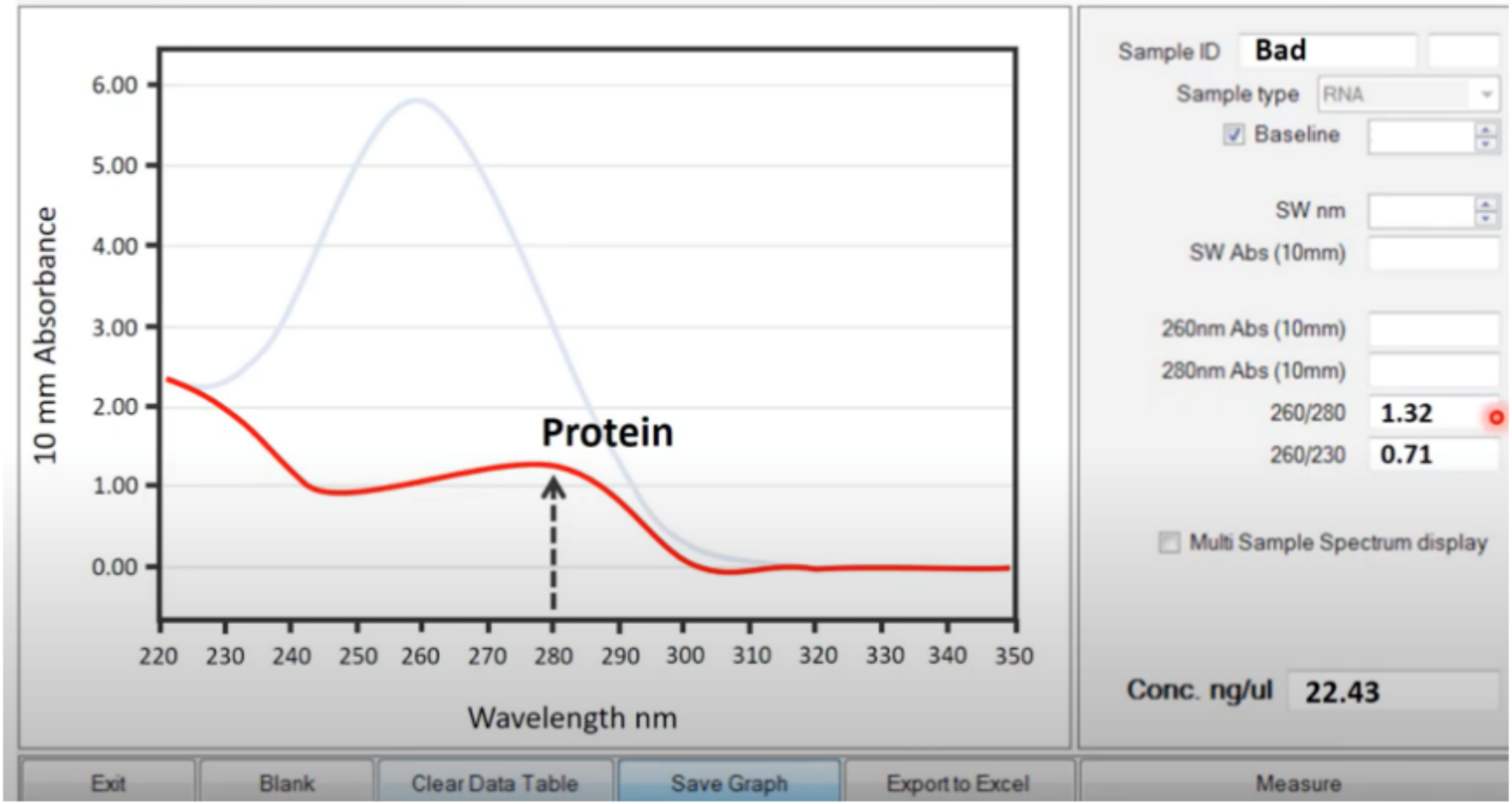
Interpretation of Nanodrop Results

Sample 2



Interpretation of Nanodrop Results

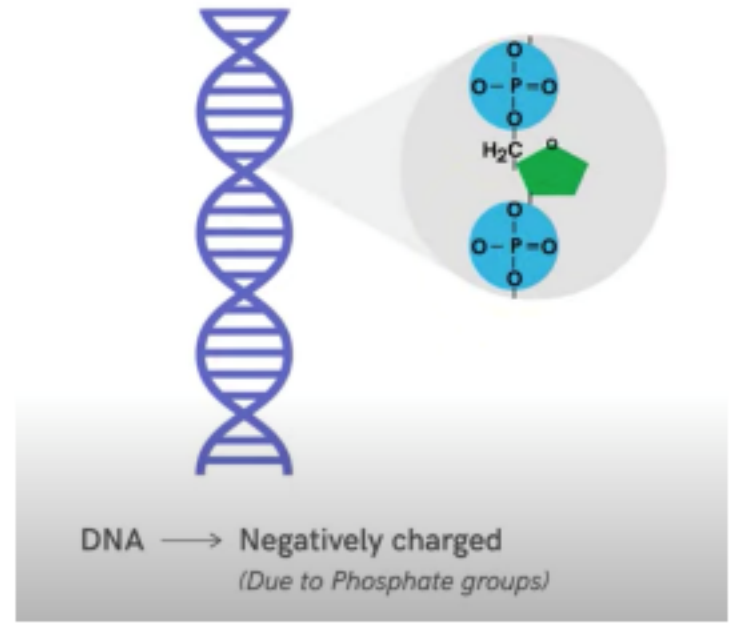
Sample 3



Assessment of Extracted Nucleic Acid

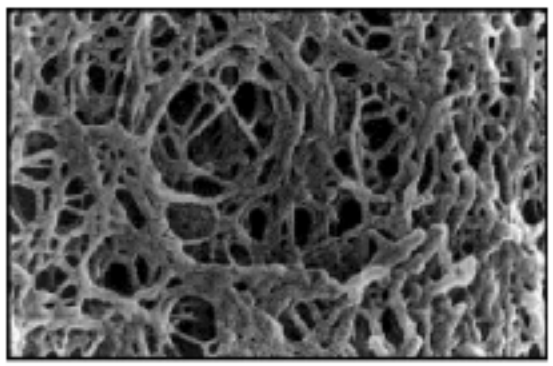
3. Gel electrophoresis:

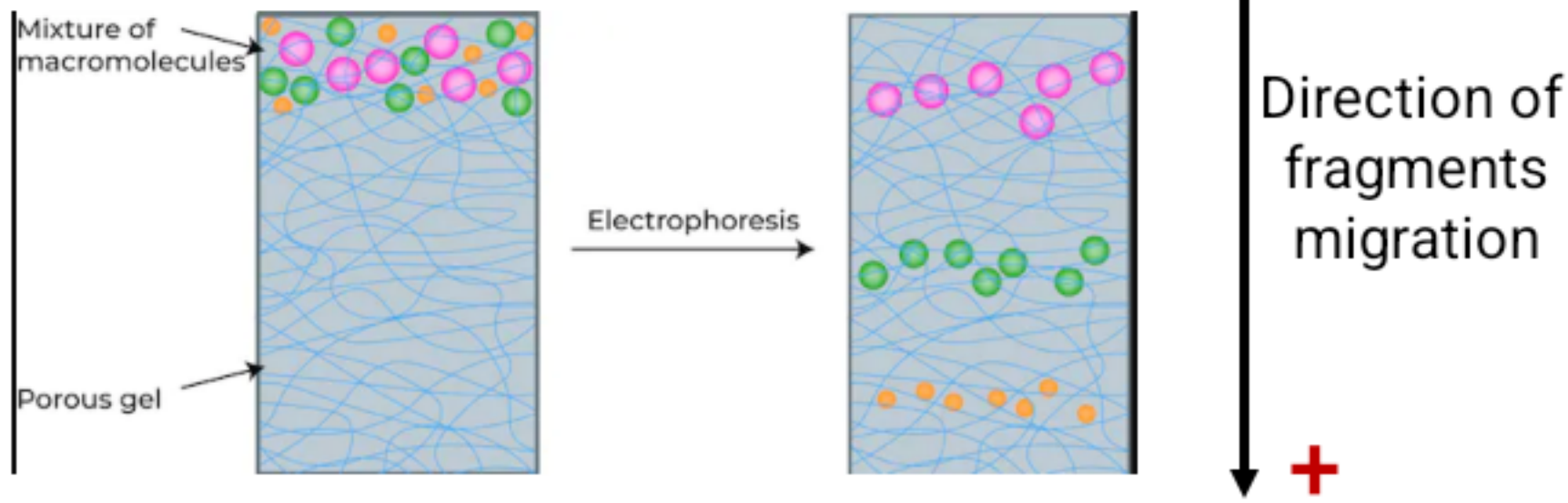
- Gel electrophoresis is a standard lab procedure for separation nucleic acids based on their sizes under the influence of electric field
- The concept: DNA and RNA are negatively charged molecules they move toward the positive electrode **(usually red)**



Assessment of Extracted Nucleic Acid

- Two types of gel can be used: polyacrylamide gel (suitable to separate small fragments up to 500bp) and agarose gel (suitable to separate larger fragments)
- Gel matrix acts as sieve or mesh (porous) and the smallest fragments migrate faster through the pores





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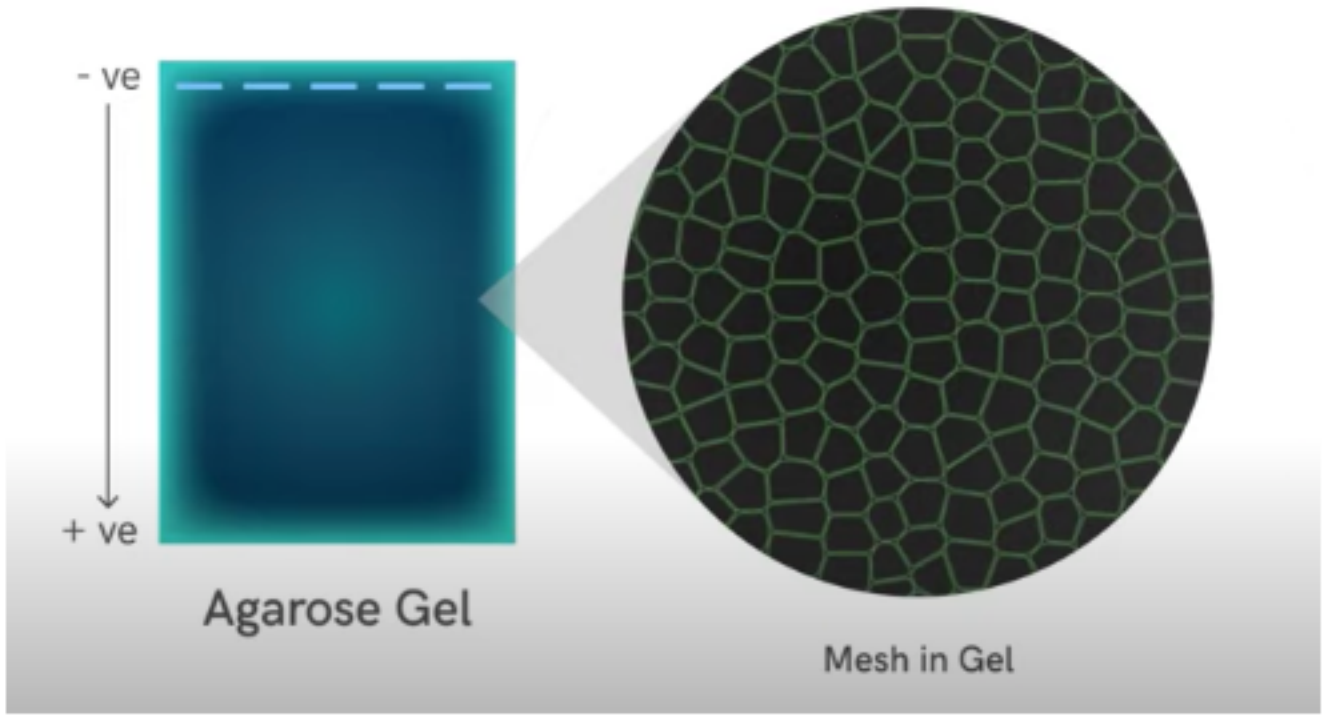

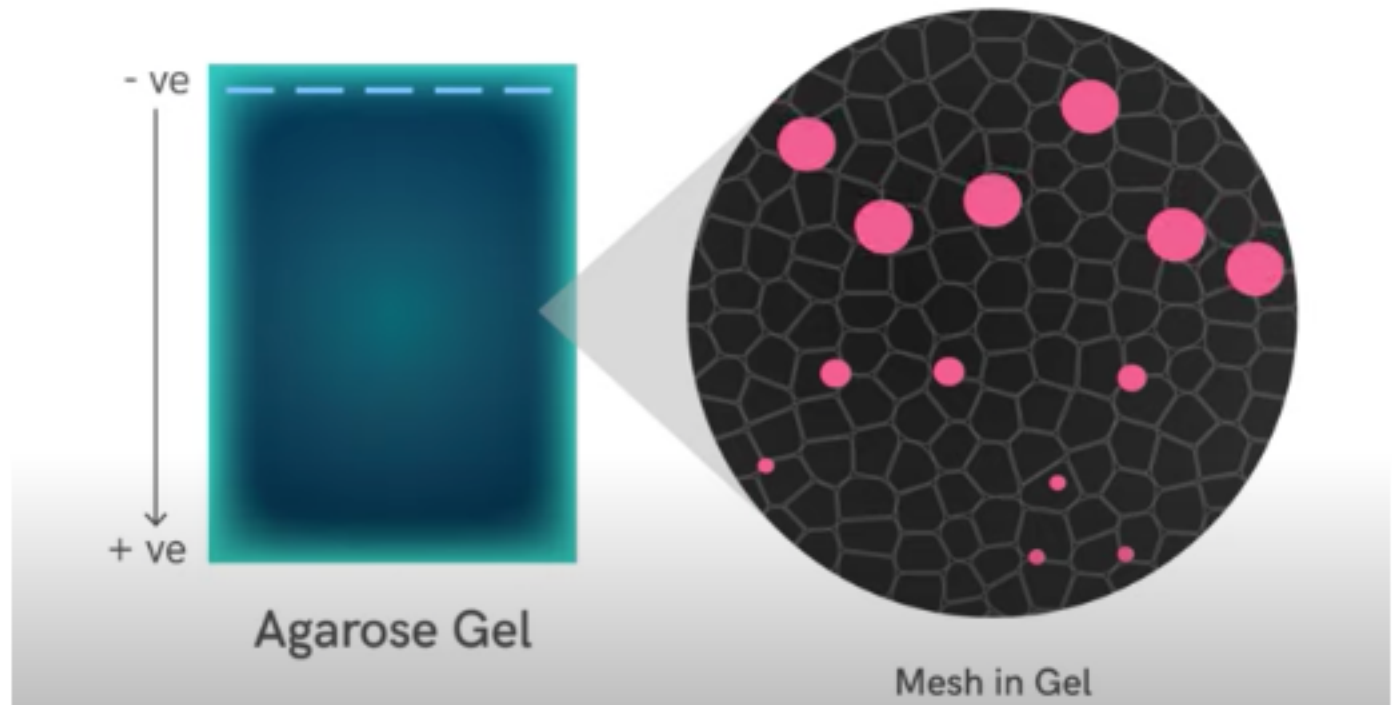

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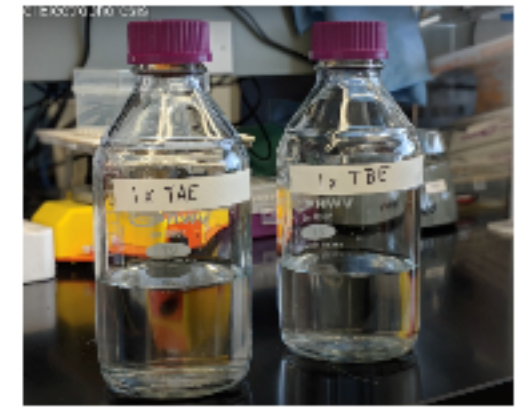


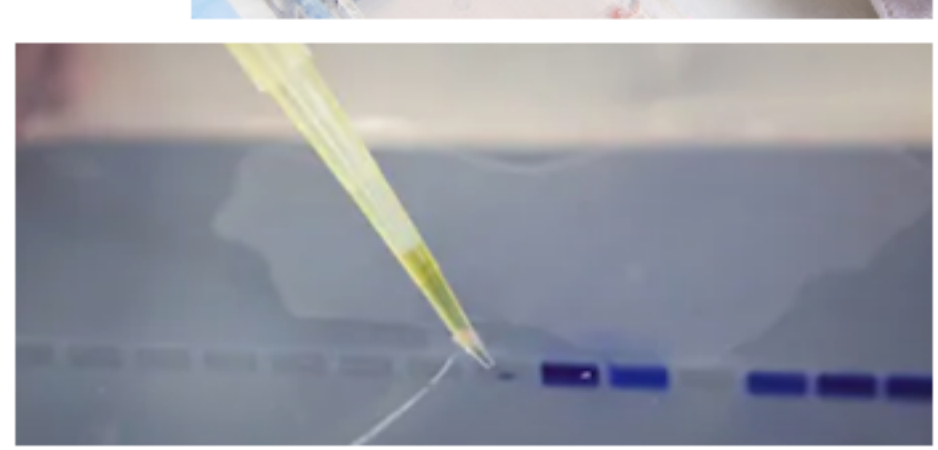
- Agarose gel (0.7% - 2%) is prepared by dissolving the powder in TAE buffer (Tris/Acetate/EDTA buffer) or TBE buffer (Tris/Borate/EDTA buffer)
- For example: to prepare 1% agarose gel (1g/ 100ml) dissolve 1g of agarose powder in100ml of 1X TAE
- add few drops of nucleic acid fluorescent dye to the dissolved gel solution then pour it into casting tray (don't forget to add the comb to make the wells)

Assessment of Extracted Nucleic Acid

- The nucleic acid fluorescent dye is used to visualize the nucleic acid under UV light (acts by intercalation): Ethidium Bromide (mutagen). GelRed (expensive but safe)
- The gel tray is placed in an electrophoresis chamber and filled with running buffer (1X TAE buffer) until it covers the gel piece. Buffer is used to provide ions that carry the current and to maintain pH

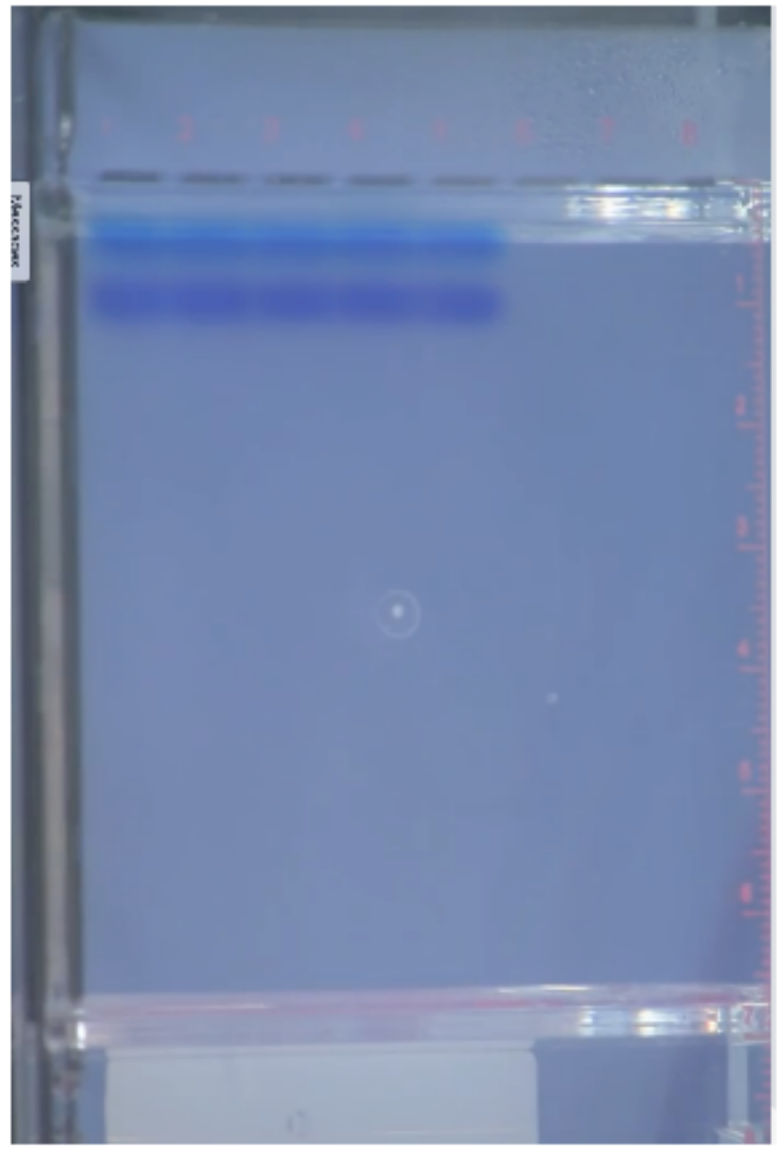
Assessment of Extracted Nucleic Acid

- Load the DNA or RNA sample into wells after mixing with loading dye (blue dye to increase viscosity of sample and prevents it from floating out of the wells and to track the migrated fragments)

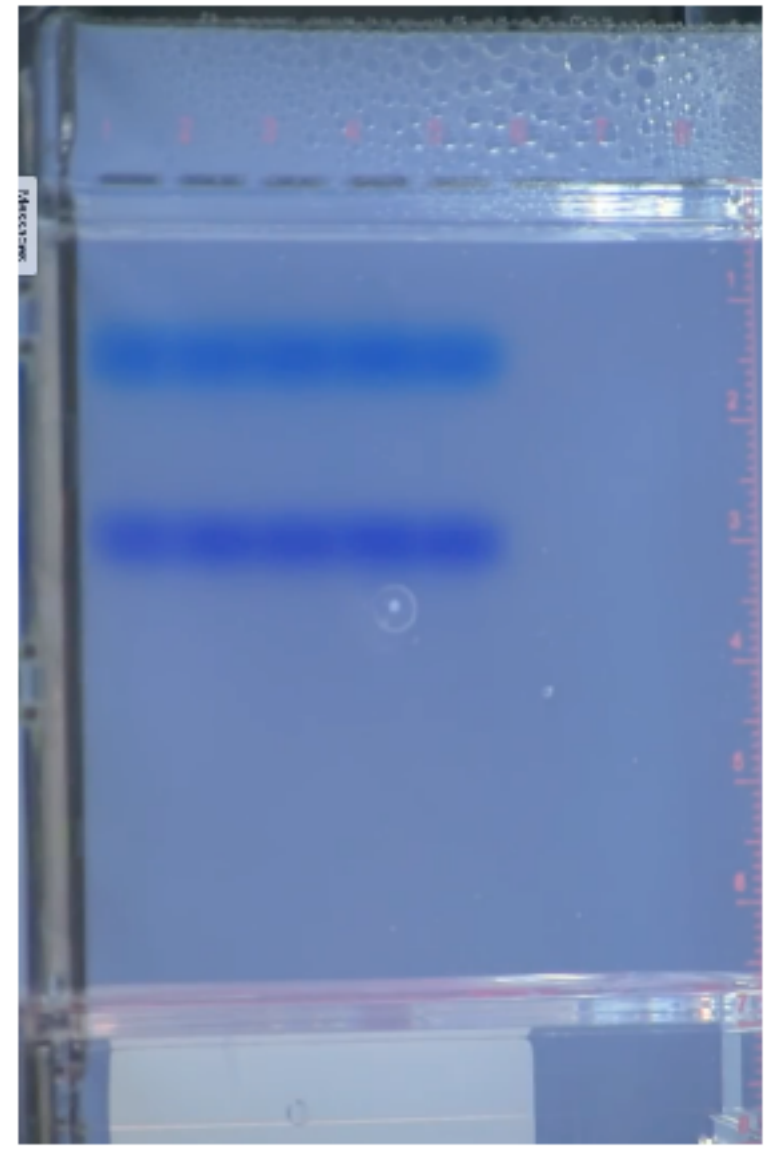


- The electrodes are attached to a power supply and an electrical current is applied

Assessment of Extracted Nucleic Acid



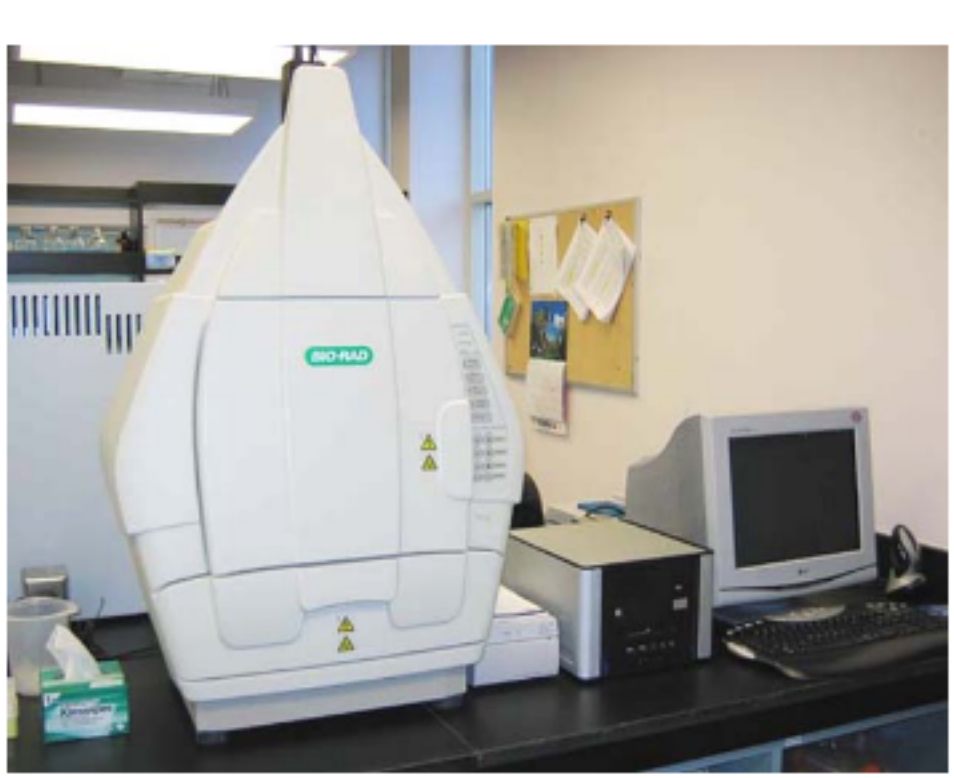
After 5 min



After 20 min

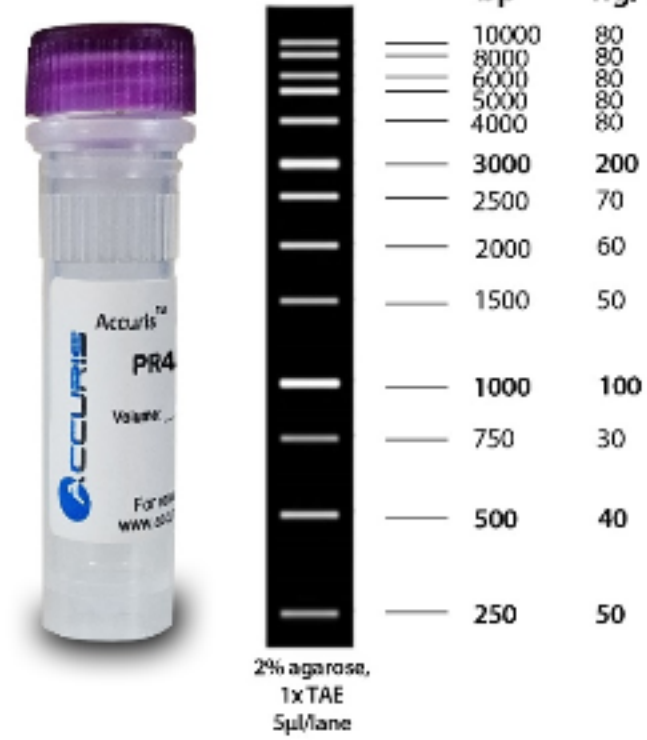
Assessment of Extracted Nucleic Acid

- Load DNA or RNA ladder (fragments with known sizes) into the first well (acts a ruler to compare and identify sizes of different bands)

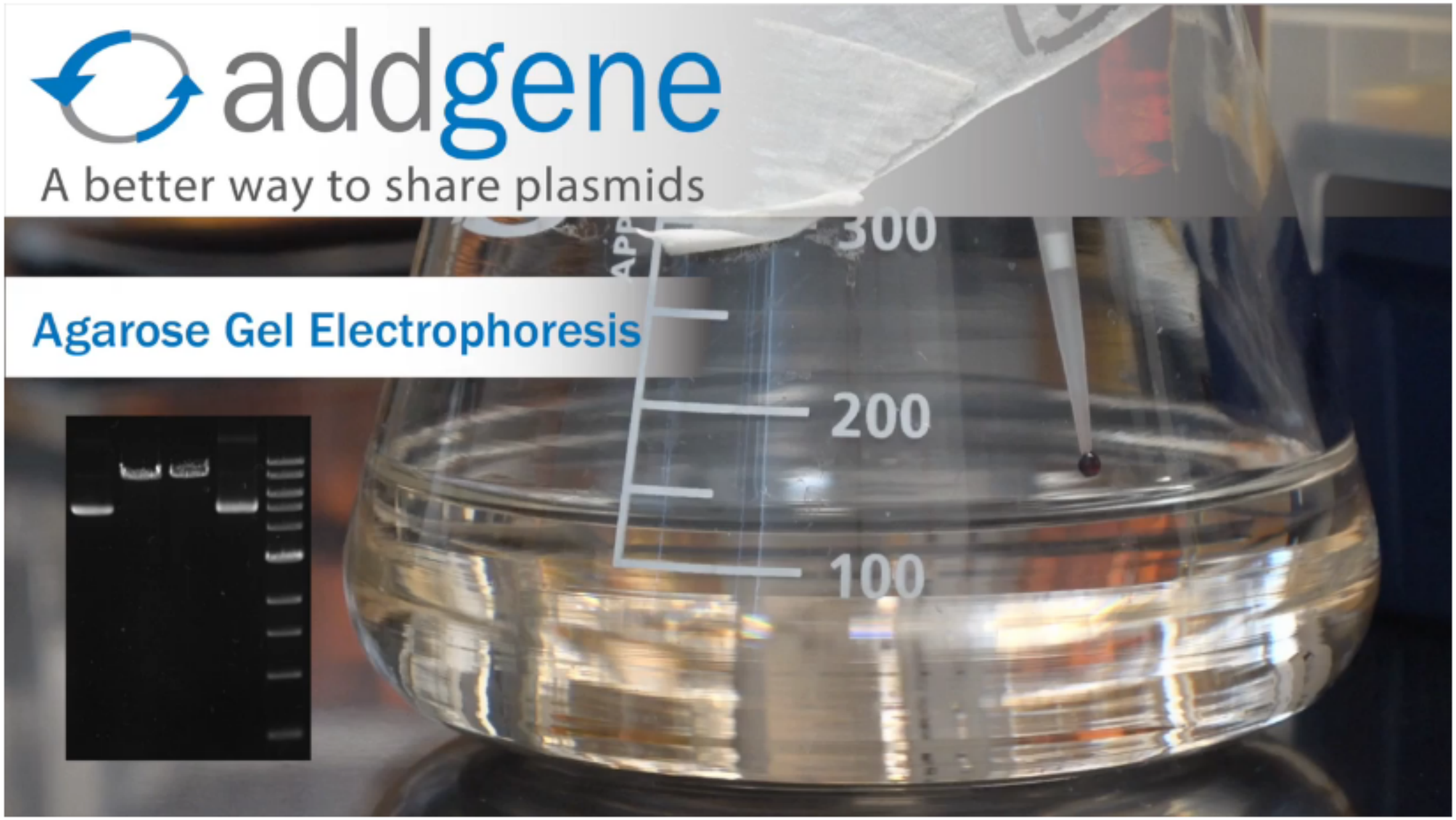


Gel documentation system

"Gel Doc System"



Agarose Gel Electrophoresis



Agarose Gel Electrophoresis

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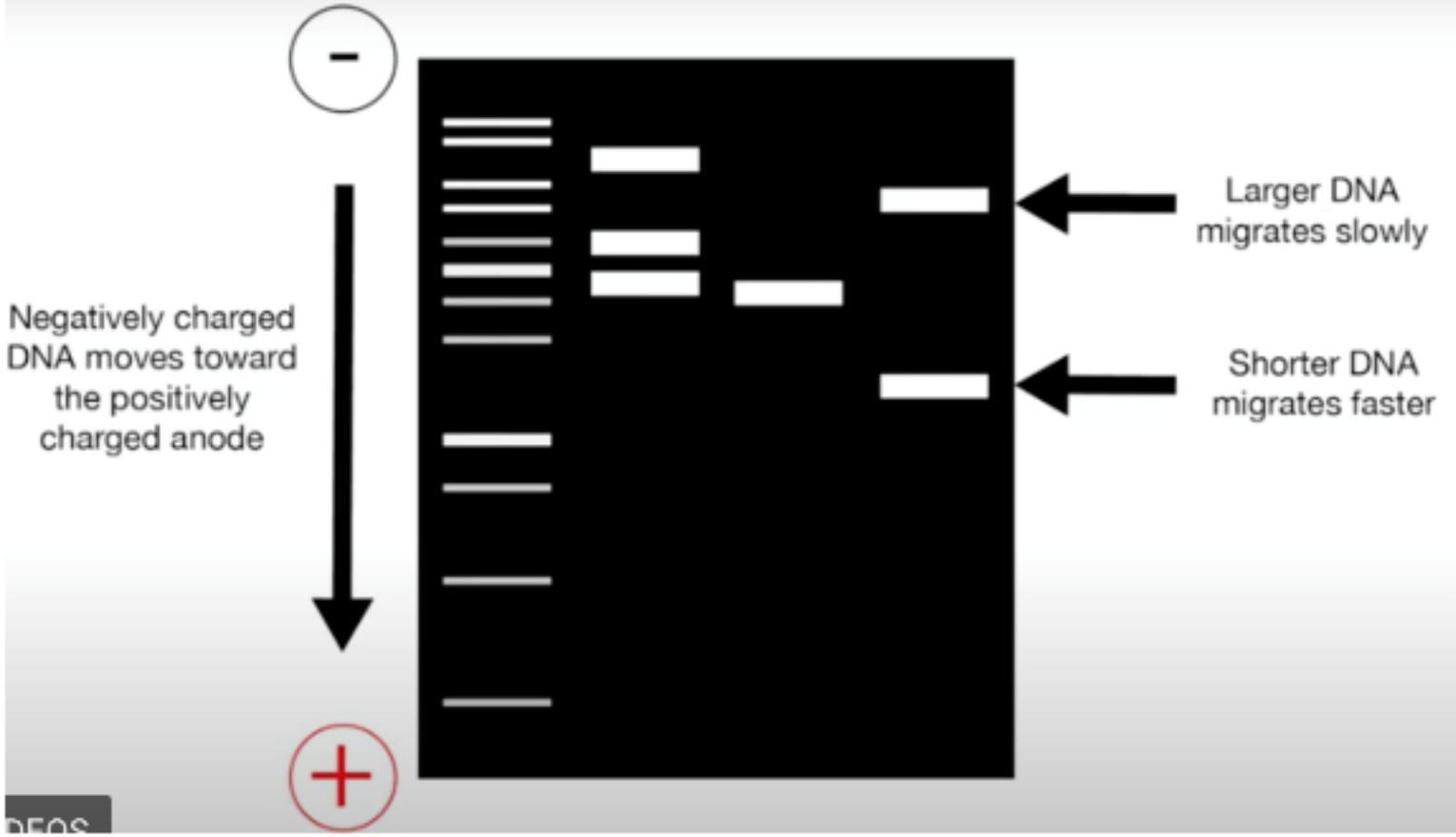
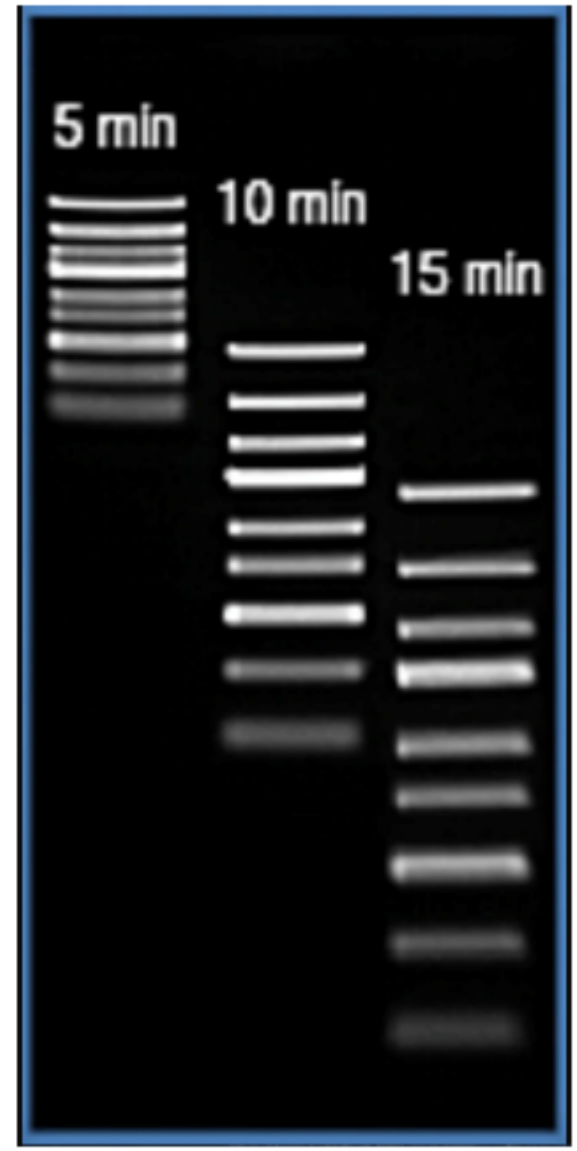
Assessment of Extracted Nucleic Acid





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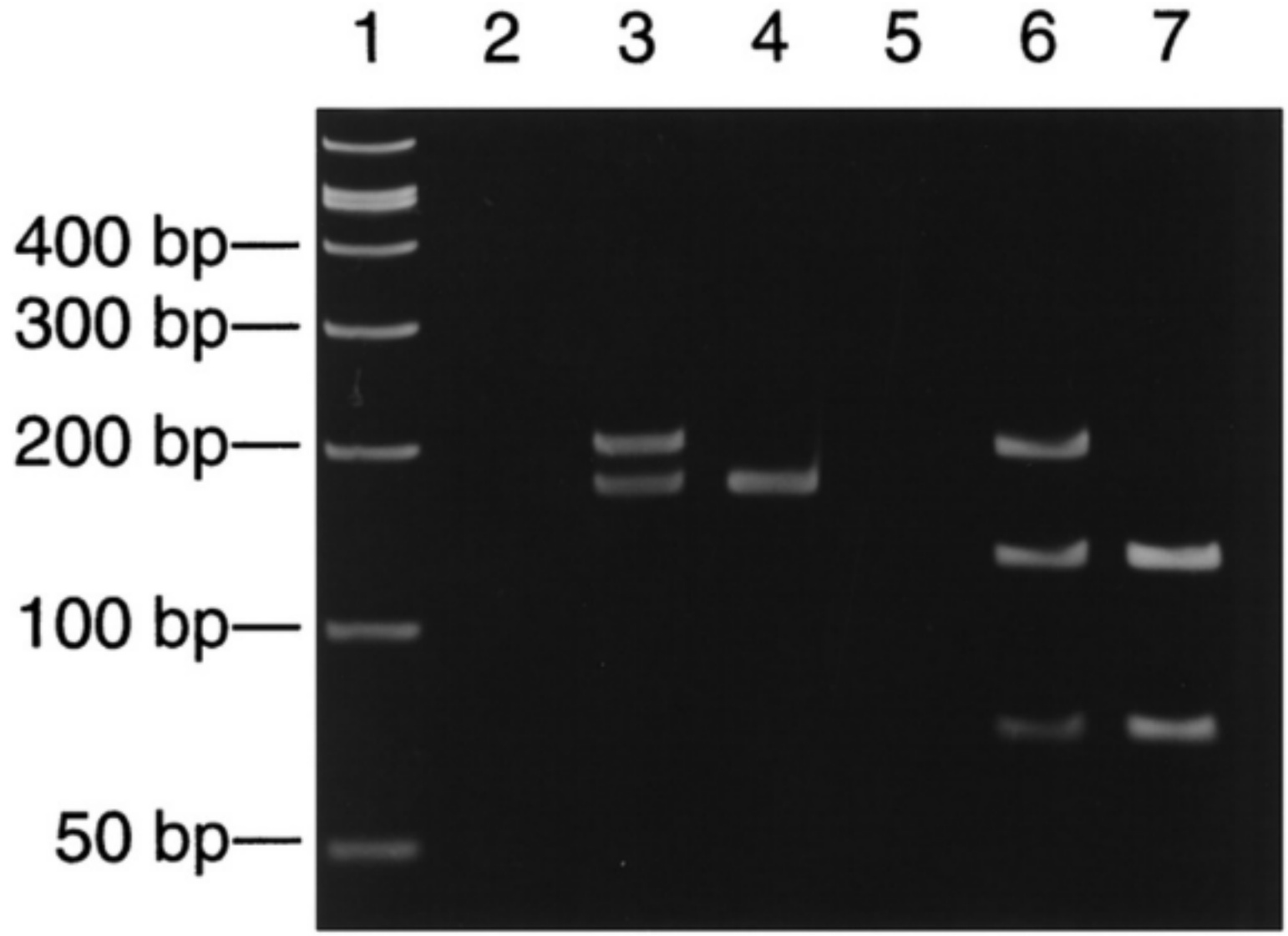

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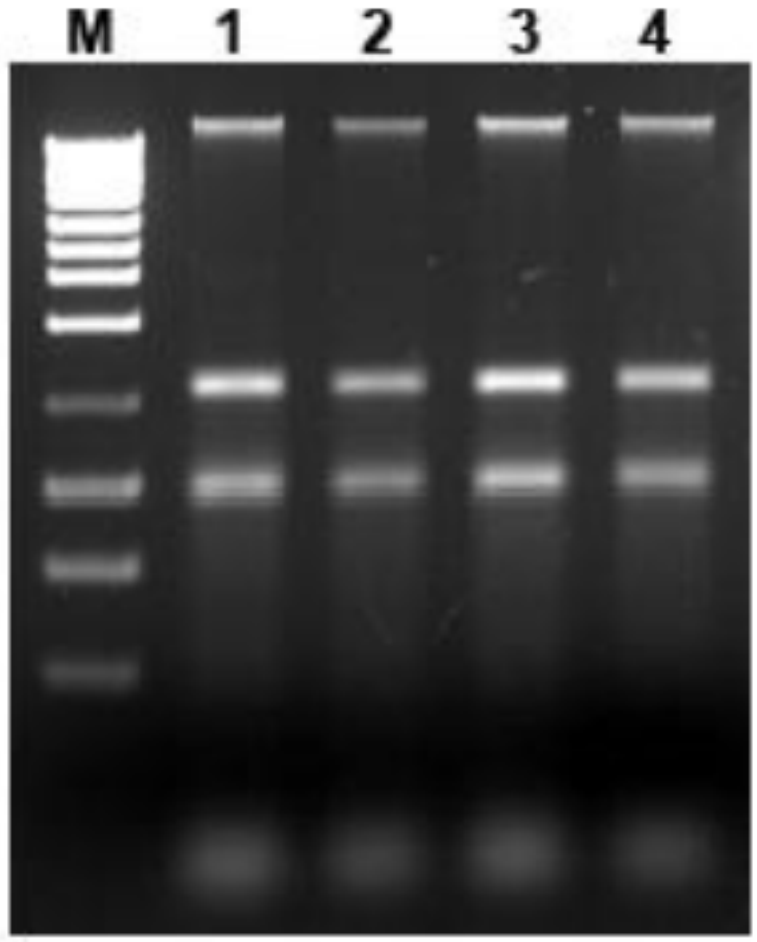
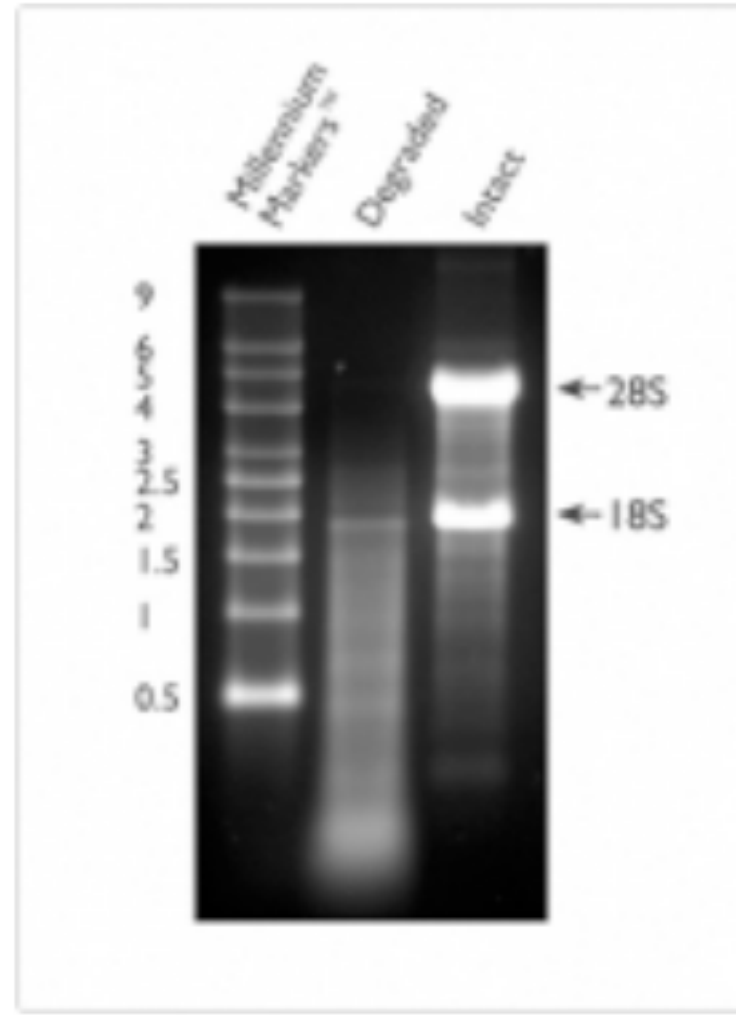


Total RNA



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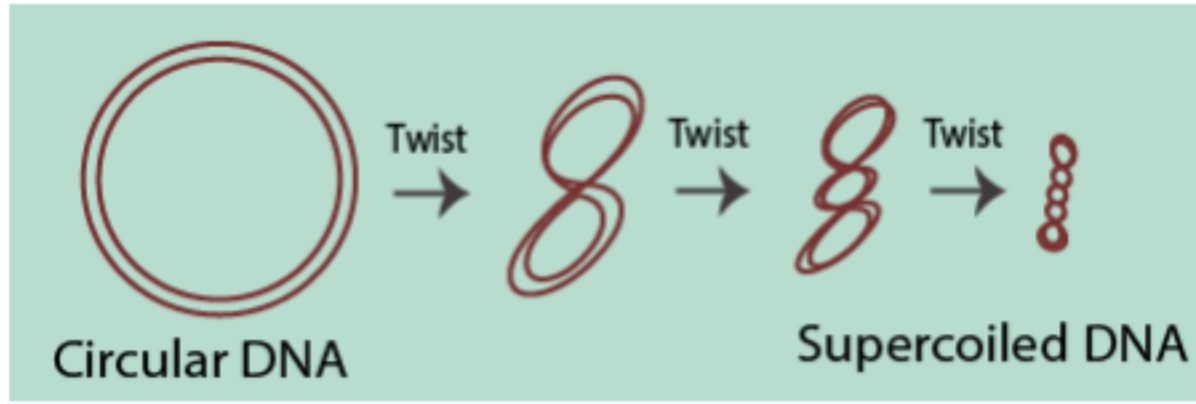

Relaxed circular form
(low mobility)

Linearized form
(moderate mobility)

Superhelical form
(high mobility)