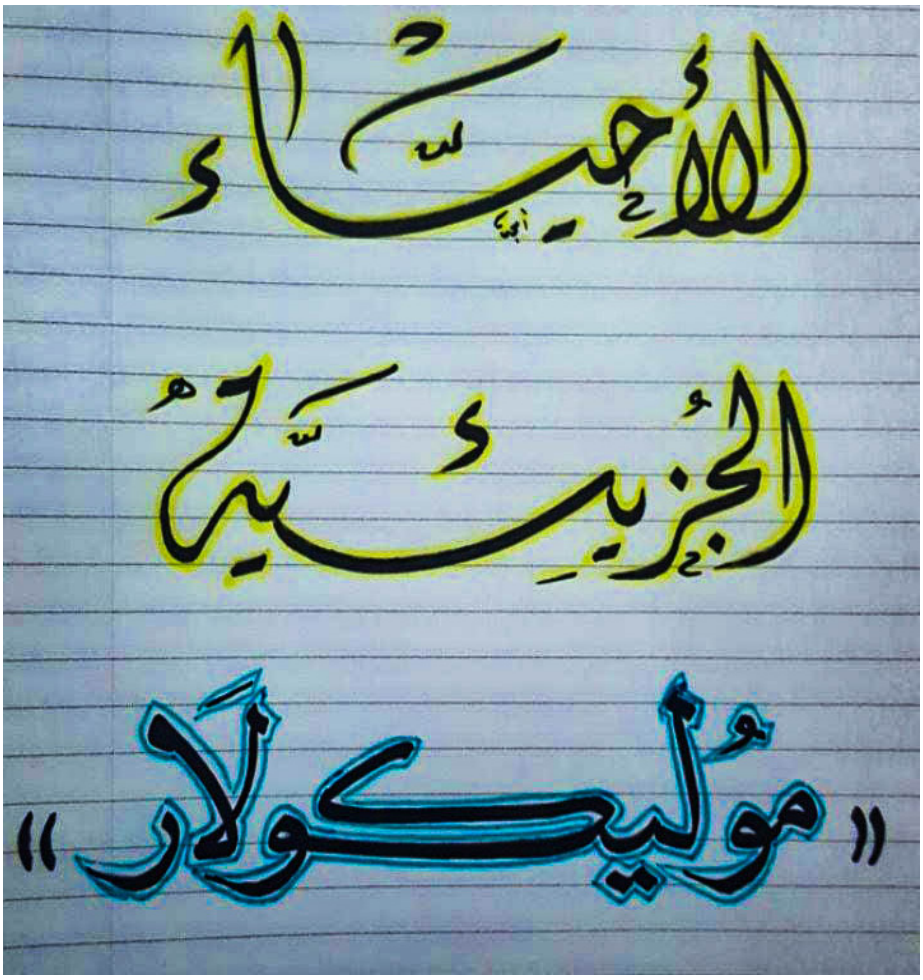
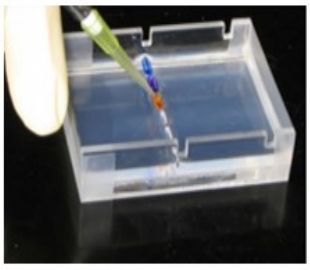


Assessment of the Extracted Nucleic Acid



Dr. Nesrin Mwafi
Biochemistry & Molecular Biology Department
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Slide (2): Assessment of Extracted Nucleic Acid

- (DNA) or (RNA) is extracted from the sample or cell...
The Process of extraction ends up with 50 microliter of the extracted DNA/RNA so it looks transparent, appearing in the form of drops inside a small tube called ep-pendorf which is named after the manufacturer company, and it's capacity is 2 ML.

-Three routine tests can be performed to check for quantity (concentration/amount) and quality (purity and integrity) of the extracted product.

-Take an aliquot of the sample (5µl out of 50µl)

-Integrity--> Intact, Aliquot--> Apportion of larger sample.

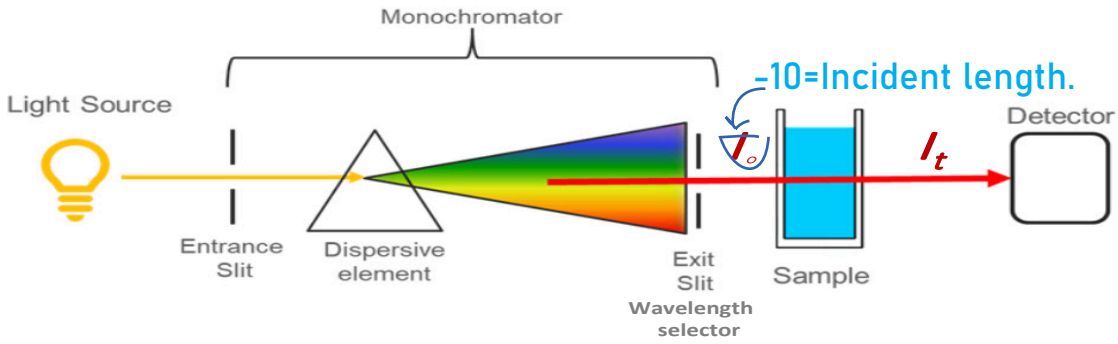
-The The work is done in the laboratory on the aliquot in order to protect our original sample from contamination.

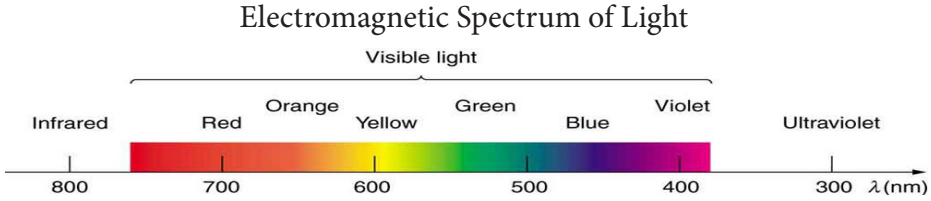
1-Measurement of concentration:

-10=Incident length.

Slide (3): 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) .



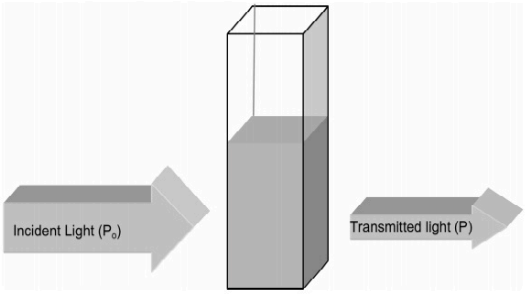


Continue Slide (3):

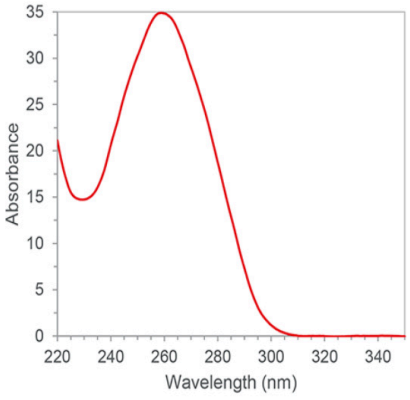
-Ultraviolet light--> 200-400 λ
-Visible Light--> 400-800 λ
-Glass prism does sperate the light which passes through it into the 7 colors of spectrum "Rainbow"
-Exit slit contains wave length selector.
-Uv-Vis Spectrophatometer is used to measure Protein concentration, usage of this device is usually done by fixing lambda (λ) at the maximum absorbance.
-The maximum absorbance of nucleic acid is 260 λ.
-Detector--> Measures the intensity of the incident light

Slide (4): 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 absorbance value (optical density).



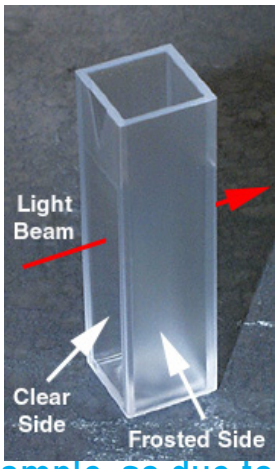
-Nucleic acids absorb UV-light with maximum absorbance at wavelength of 260 nm (λ= 260nm).



Typical RNA/DNA absorbance spectrum.

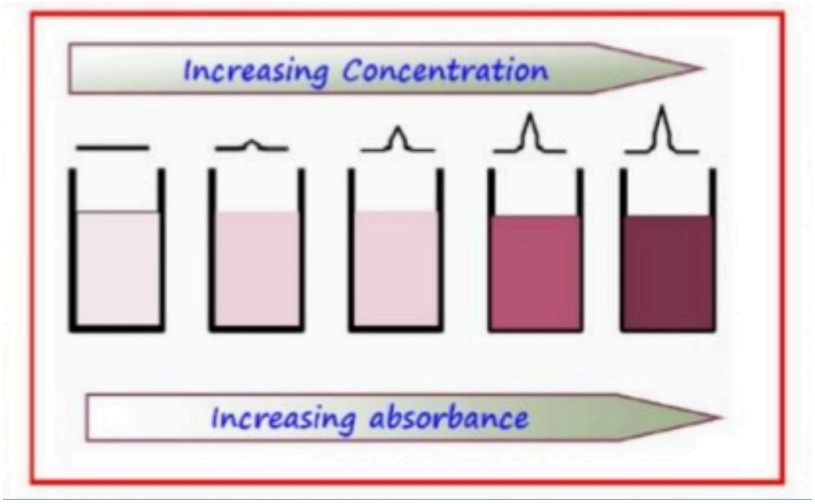
-The higher the concentration of the sample "Particles" is, the higher the light absorbance rate, which leads to a higher result on the detector.

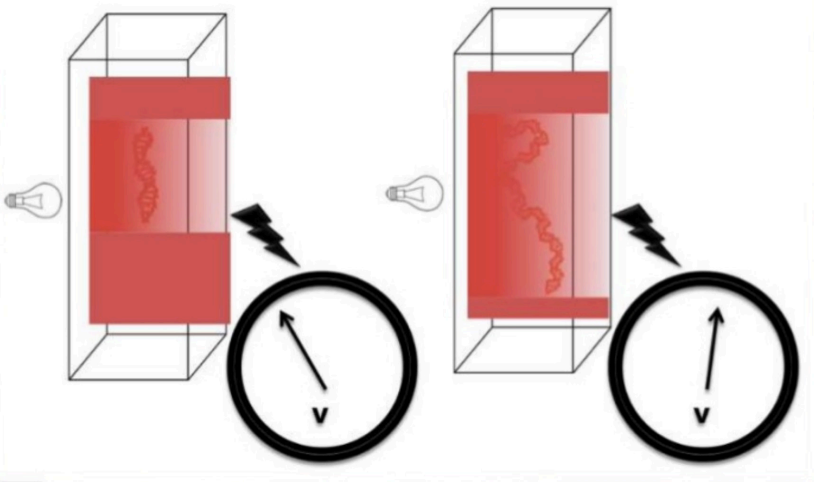
Slide (5): Assessment of Extracted Nucleic Acid



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

-Cuvette has a sign indicates the 100 microliter capacity, so we make delution to the sample until we reach the sign.
-We only take 1 or 2 from the aliquot which is about 5 microliter the Indication “result” which is done by the device is the deluted sample, so due to that we multiply the result with the delution factor to calculate the original sample result (stock).
-Opaque: nontransparent frosted, and tarped.
-Cuvette-->nondisposable and it’s made of quartz and it’s better than the plastic one which is disposable, also the quartz is more expensive.
-The light should be aimed to the transparent side.
-The sample stick to the Opaque.
-We dissolve the sample with a solvent.
-The solvent= Water (Milli-Q water and Ultraviolet water)..





Slide (6): 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 OD260 unit = 50 µg/ml dsDNA)

Nucleic Acid	Concentration (µg/ml) or (ng/µl) per A260 Unit
ds DNA	50
ss DNA	33
ss RNA	40

- (1) OD = (1) Optical density.
-When we study a sample of (1) microliter we dilute it using (99) microliter untill we get a sample of 100 microliter "reach the sign".

-Dilution Factor= Total volume/ Volume sample.

-The concentration is calculated according to the following equation:

DNA Concentration (µg/ml) = OD260 × dilution factor × 50 µg/ml

Slide (7): Assessment of Extracted Nucleic Acid

Example 1: OD260 = 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 ?

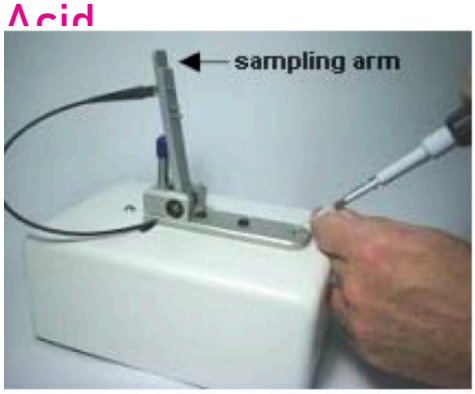
Answer: Amount = concentration X total volume of the extracted sample
= 3000 ng/µl X 50 µl
= 150000 ng or 150 µg

Note: 1g = 1000mg 1L = 1000 ml
1mg = 1000 µg 1ml = 1000 µl
1 µg = 1000 ng

Slide (8): Assessment of Extracted Nucleic Acid

NanoDrop is automatically
calculate the concentration in ng/µl

Slide (9): Assessment of Extracted Nucleic Acid

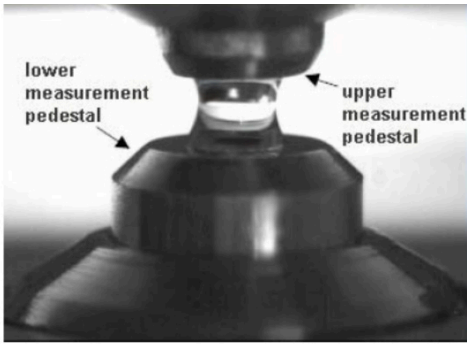


-NanoDrop spectrophotometer has similar principle to the standard cuvette spectrophotometer.

-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.

-The winping is done to get rid of any plankton that may have contaminant project resulting from the previous sample



Slide (10): 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:

-Lower range than the accepted range means contaminant with protein or phenols.

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

Slide (11): 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).

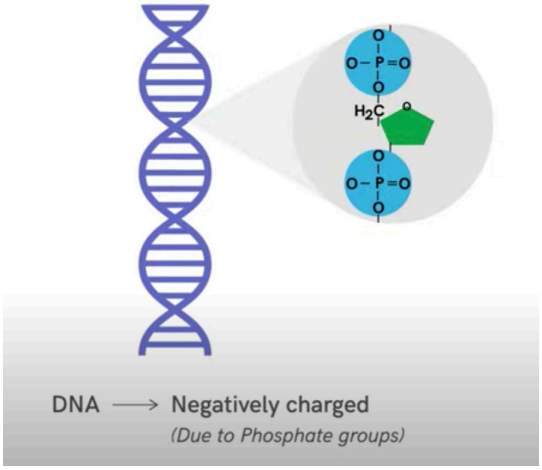
-Low ratio indicate possible contaminants .

-The higher the reading at 280 means more protein we have, so the denominator will increase and the ratio will decrease.
-Peptide bone, Chaotropic and trizol =230 λ

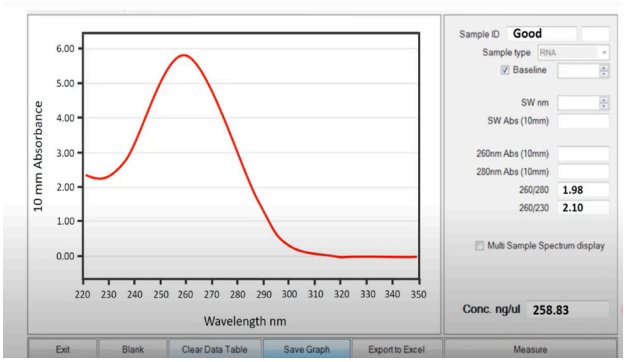
Slide (13): Assessment of Extracted Nucleic Acid

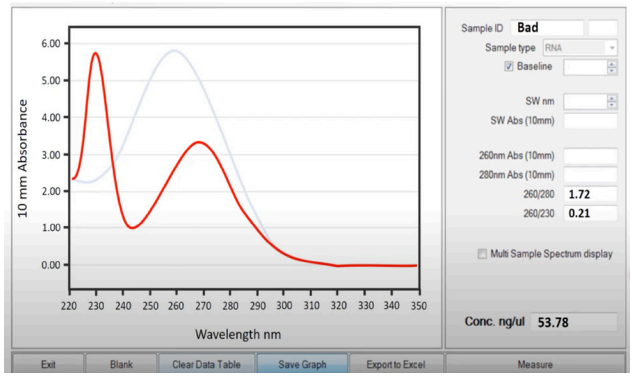
3-Gel electrophoresis:

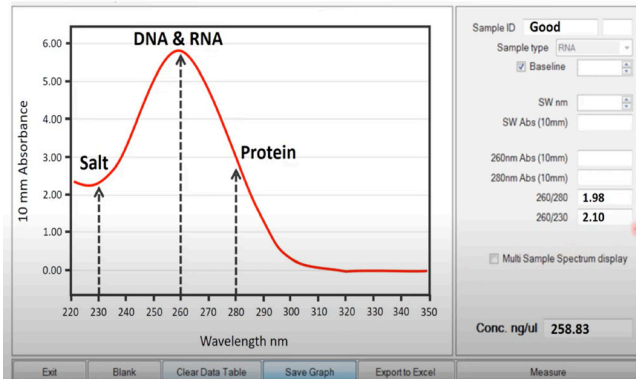
-It's done to be sure about the inegrity of the DNA.
-This method is used to distinguish the sample if it's a DNA or RNA or Plasmid.
-By this method we can know the size of the fragment
-Gel electrophoresis is a standard lab procedure for separation nucleic acids based on their sizes under the influence of electric field.
-As the sample is smaller,it moves rapidly on the gel under the influence of the electric field.
-The concept: DNA and RNA are negatively (due to the negative charge of the phosphate group) charged molecules they move toward the positive electrode (usually red).
-The negative elctrode is black.

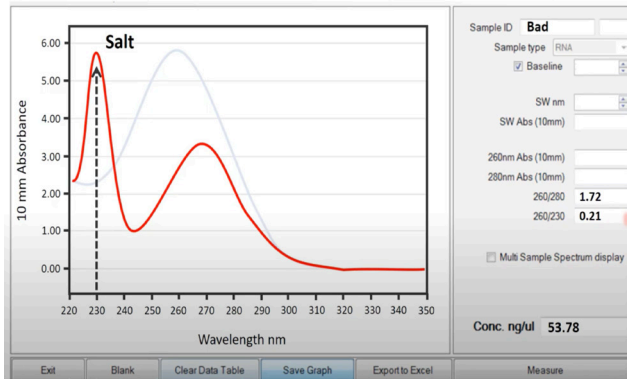


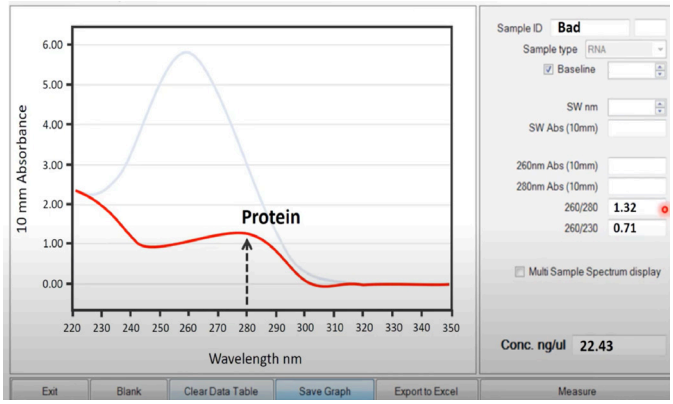
Slide (12): Assessment of Extracted Nucleic Acid





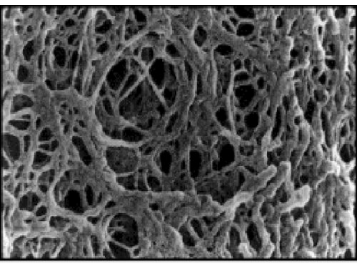


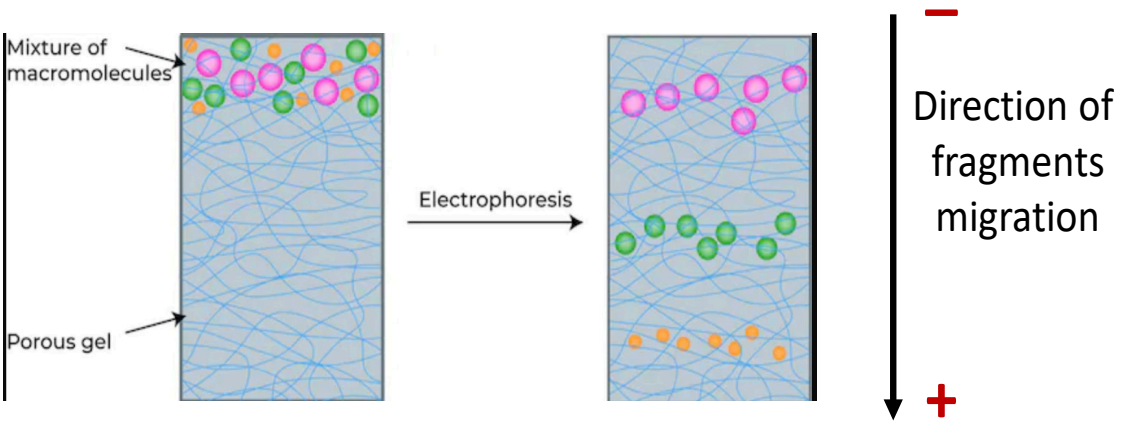




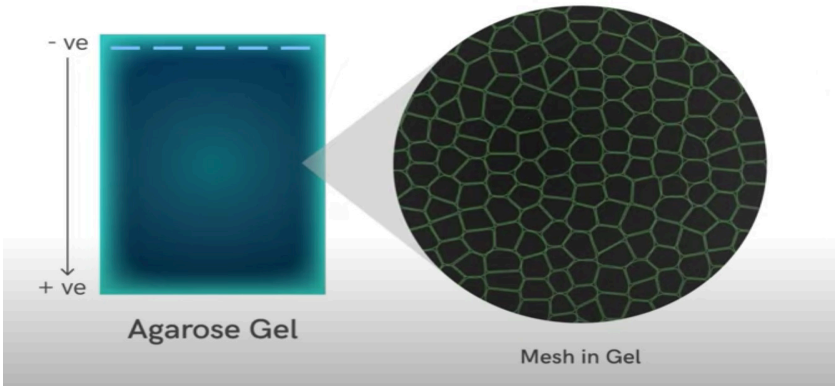
Slide (14): Assessment of Extracted Nucleic Acid

-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 "500-2000").
-Gel matrix acts as sieve or mesh (porous) and the smallest fragments migrate faster through the pores.
-The size of the pores in the end of the matrix is determined based on the kind and the amount of the gel..
-The size of the pores controls the sepration of the fragments.

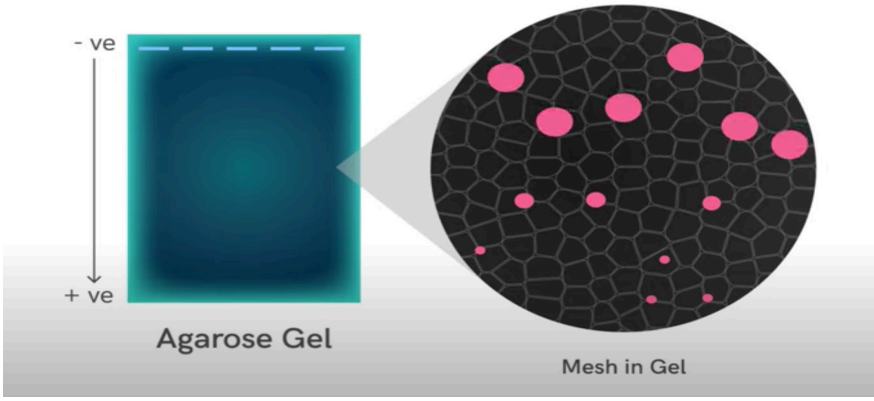




Slide (15): Assessment of Extracted Nucleic Acid



-It take 20-30 minutes to complete the sepration



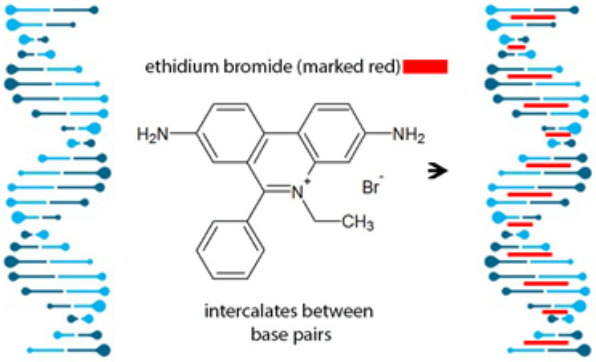
Slide (16): Assessment of Extracted Nucleic Acid

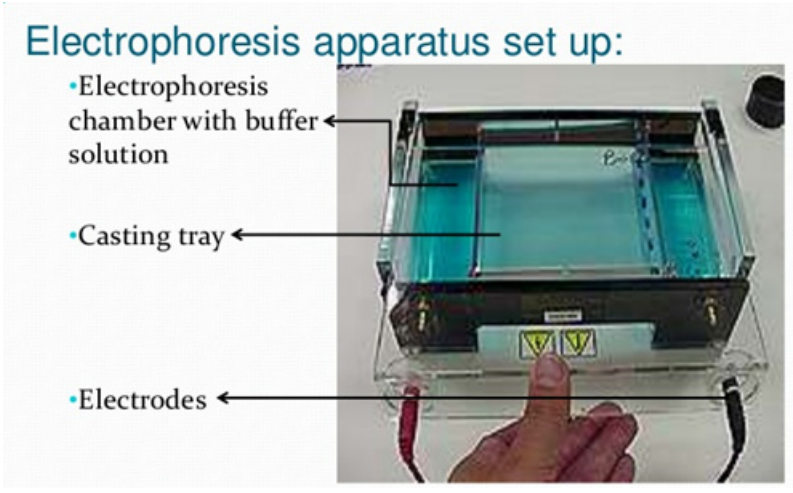
-Agarose gel (0.7% - 2%) is prepared by dissolving the powder in TAE buffer (Tris/Acetate/EDTA buffer) or TBE buffer (Tris/Borate/EDTA buffer).
-2%--> dissolve 2g of agarose powder in 100ml of TAE.
-The buffer doesn't dissolve in water.
-1x means that the buffer is filuted (not concentrated).
For example: 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).
#After pouring the gel into the casting tray, the gel will solidify after (20-30) minutes at room tempreature. So, the comb helps in making the wells to put the samples in it.
-The DNA is translucent, so there are two types of dyes: 1.Dye specific to visualise the nucleic acid (DNA,RNA)--> it usually a fluroscent dye--> shows the fragments with orange color in UV--> the dye is either put with the gel our put with the sample in the wells.
2.Loading Dye.

Slide (17): Assessment of Extracted Nucleic Acid

-The nucleic acid fluorescent dye is used to visualize the nucleic acid under UV light (acts by intercalation: In DNA, enter between the two strands. In RNA, enter between the base pairs.) : Ethidium Bromide (mutagen "Eithidium Bromide is mutagenic (cause mutation) and cancerogenic (cause cancer)"). GelRed and SYBR green I (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.





Slide (18): Assessment of Extracted Nucleic Acid

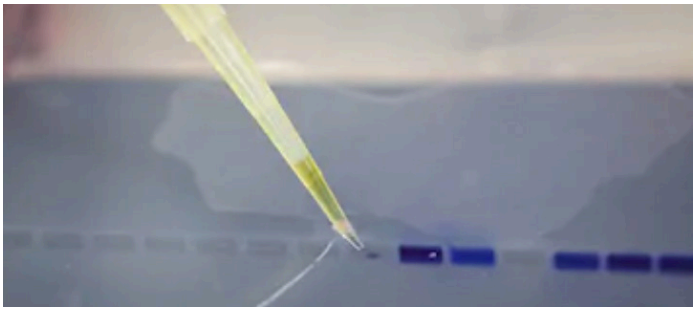
-Load the DNA or RNA sample into wells after mixing with loading dye (blue dye to increase viscosity " make the sample heavier, it will move to the bottom of he well" of sample and prevents it from floating out of the wells and to track the migrated fragments).

-There is a loading dye that specific to DNA and another ne that is specific to RNA.

-If we put the sample directly with the buffer it will not appear because it's trans-parent and it's size is small, so it's easy to leak out of the gel.

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

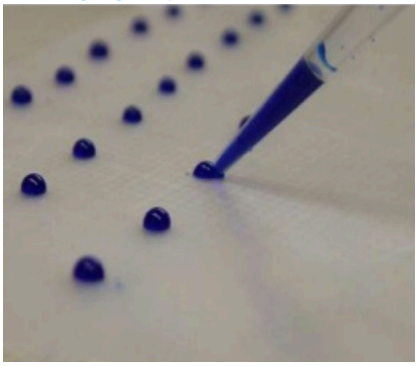
-Then it starts to separate the sample.



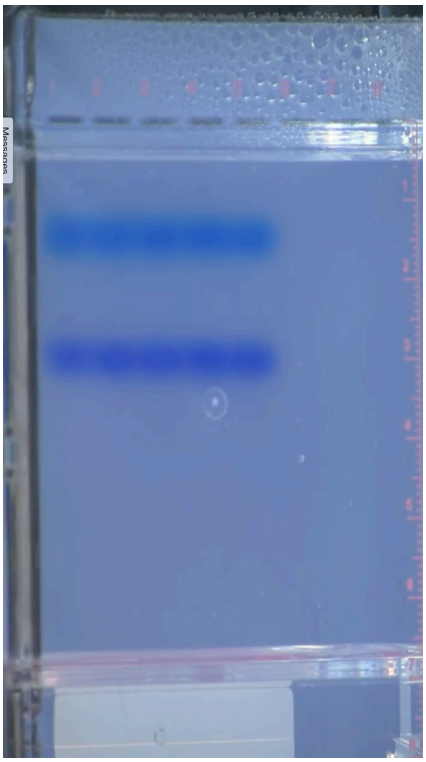
Slide (19): Assessment of Extracted Nucleic Acid

-Always when I do loading I don't put any sample in the first well, but I put 1 microliter from DNA ladder.

-Para film



After 5 min

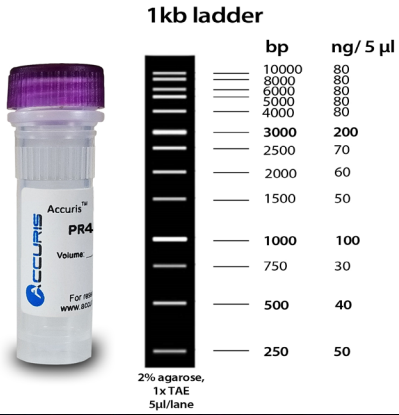


After 20 min

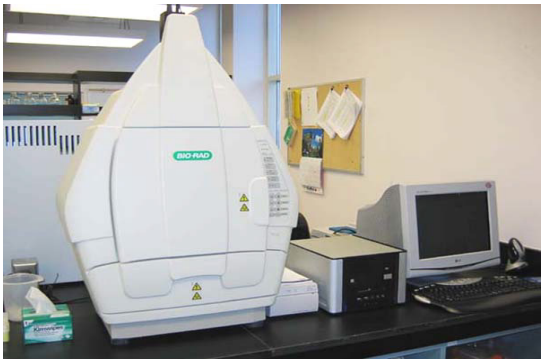
-Loading in well

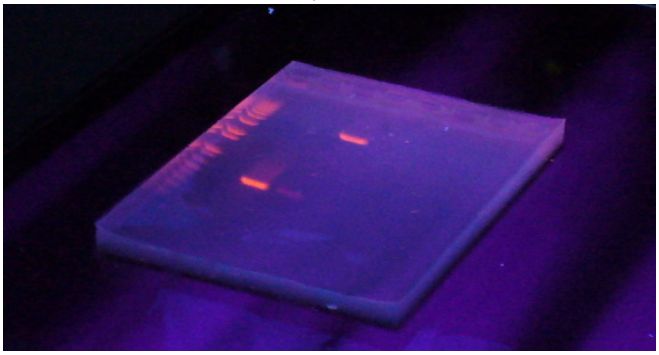
Slide (20): Assessment of Extracted Nucleic Acid

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



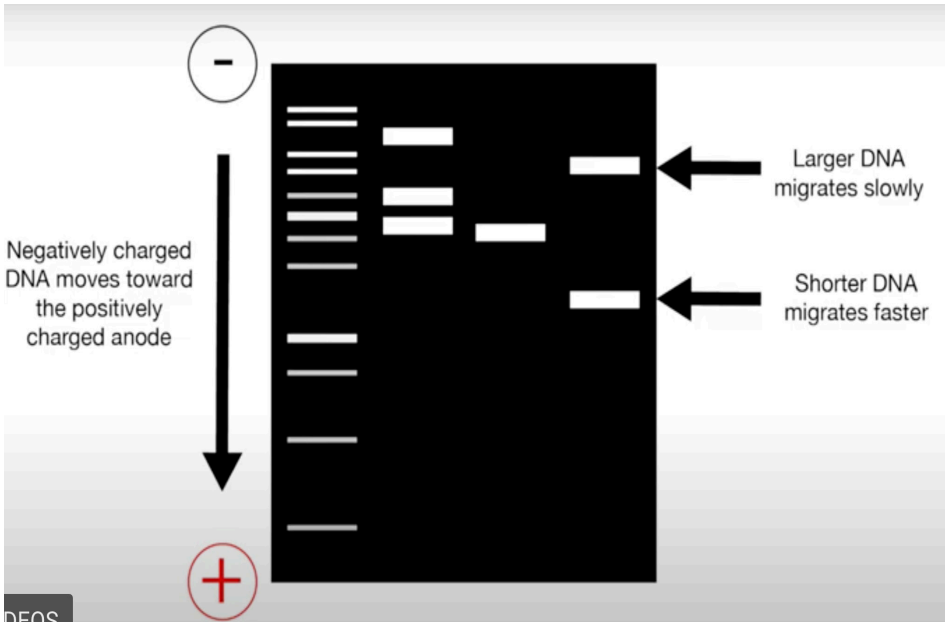
-The ladder is different according to my work.

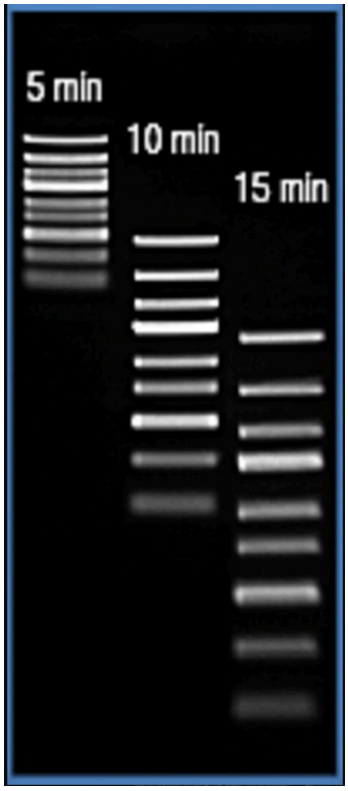




-The shape which is seen in the computer

Slide (21): Assessment of Extracted Nucleic Acid

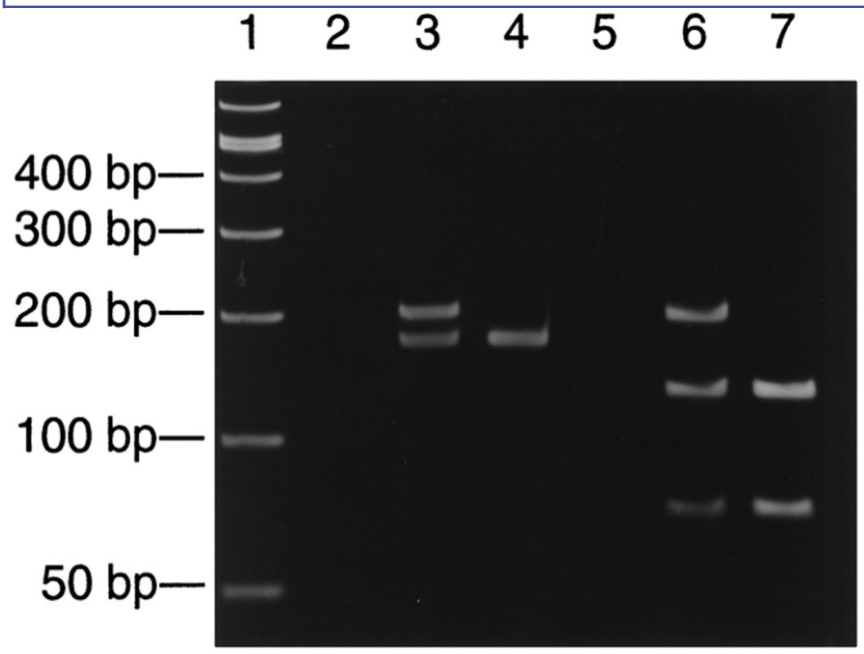




-When the loading dye reaches to the gel we must stop power supply.

-The separation will be more clear when we increase the separation.

Slide (22): Assessment of Extracted Nucleic Acid

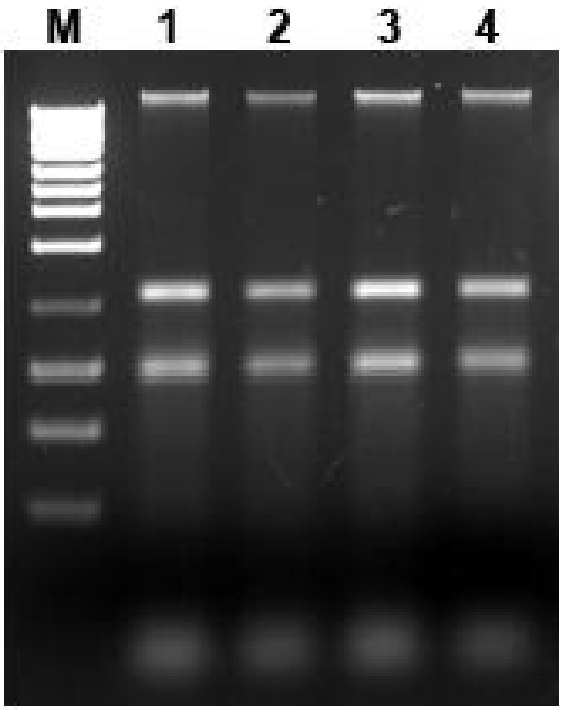


-The gel is used to make a separation of the sample according to the size and I can determine their size from making a comparison with ladder.v

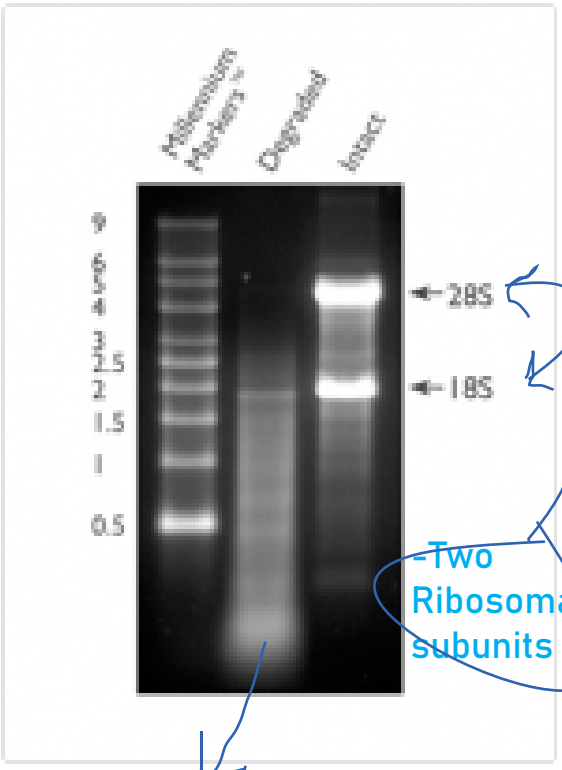
Slide (23): Assessment of Extracted Nucleic Acid

-The slow band will be deeper than the fast one.

-The apperance of two ribosomal sub-units clearly indicates that the sample is intact.



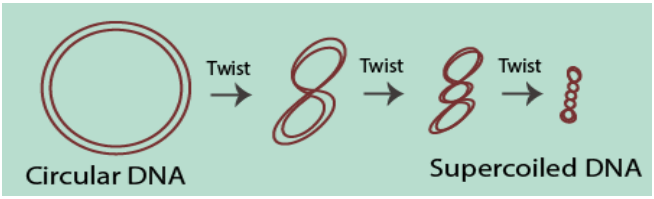
Total RNA

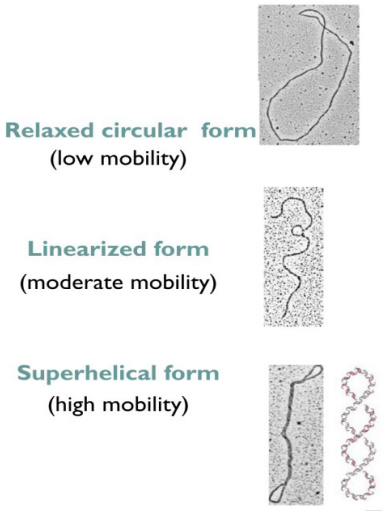


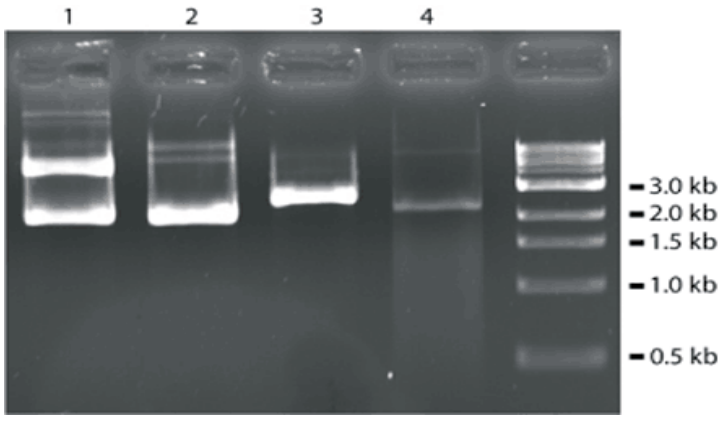
-Two Ribosomal subunits

-Degradated

Slide (24): Assessment of Extracted Nucleic Acid







-One of the properties of the plasmid that it's circular.
-I will make a loading for a sample which I made for it a direct purification to appear 2 bands.
-I will do my linearisation for another sample under the condition that I define the area of cut it contains gene of interest.
-If I have sample number 1 before linearisation and sample number 3 after it, means that the work is excellent, but if appear sample number 1 after linearisation means that the gene of interest is not found.