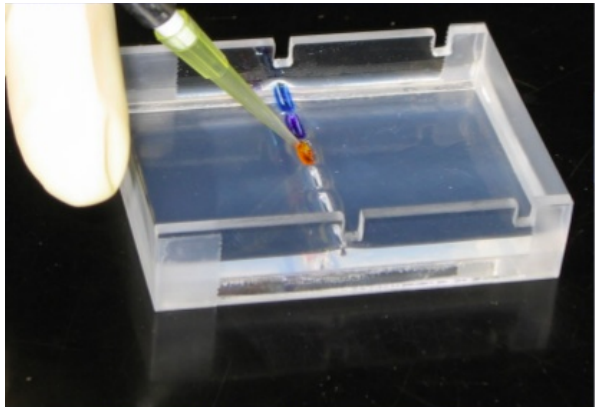
Assessment of the Extracted Nucleic Acid



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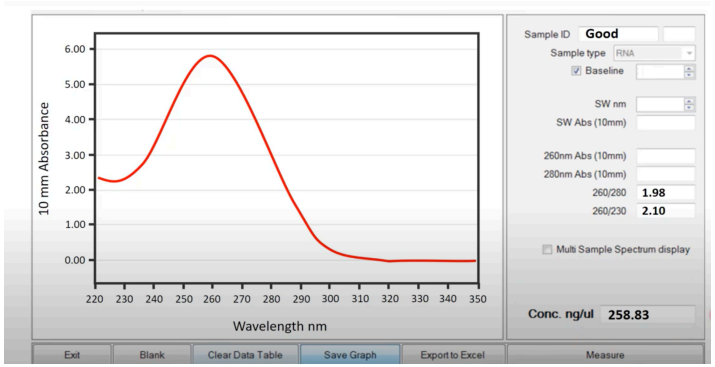
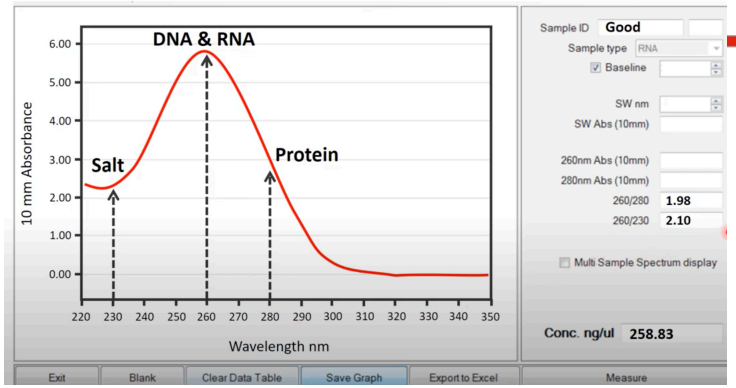
Assessment of Extracted Nucleic Acid

- 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
- **Sample purity chaotropic salts=>Some times it continous bind to DNA Although it washed**

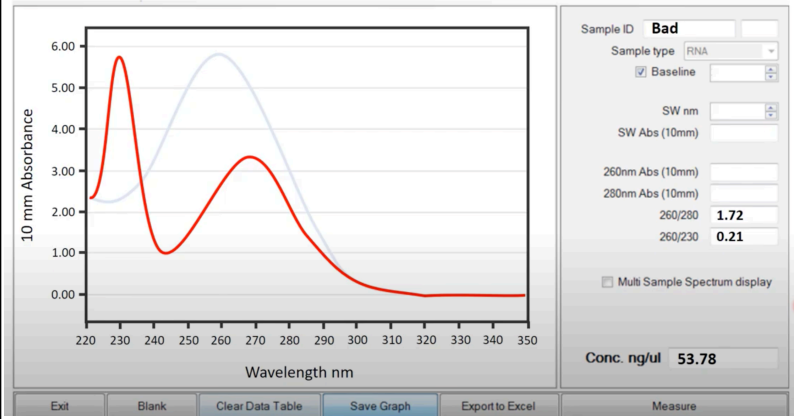
Interpretation of Nanodrop Results

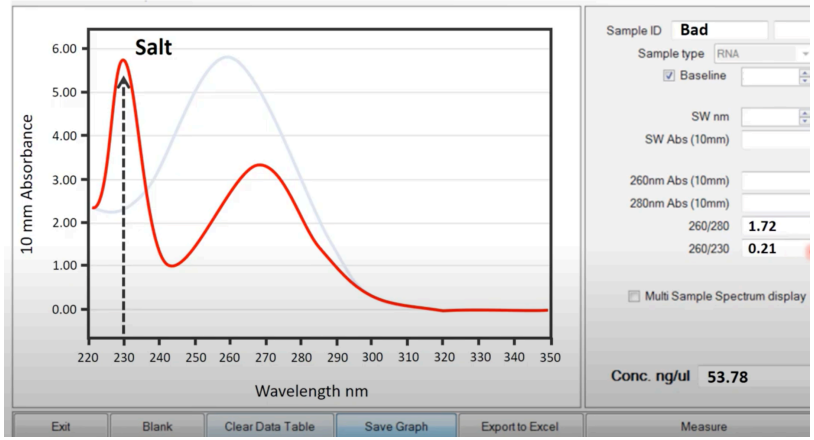




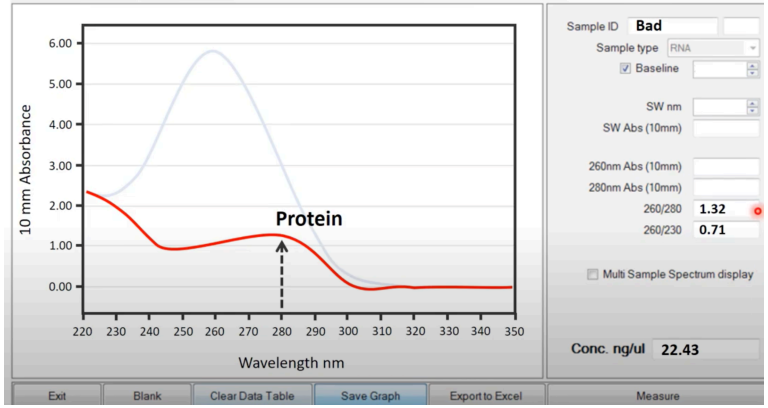
- In this picture =>Amount =258.83 X 50(the sample is correct and suitable.)
- DNA , RNA 250 high amount

Interpretation of Nanodrop Results





In this picture the sampleis false./Bad RNA because 1.72 is less, 0.21 , Also less. Not pure The Sample have avery large amount of quanidium hydrachloric,protein, salt .



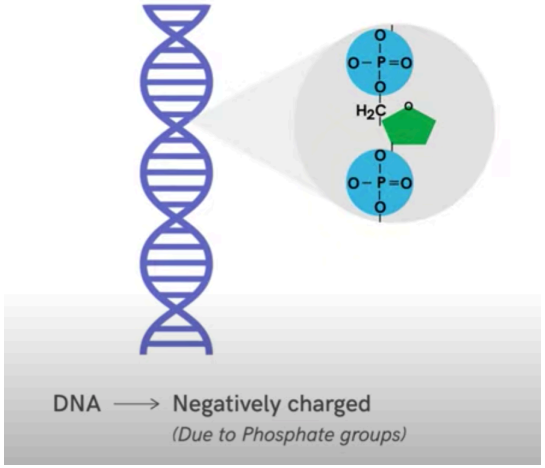
This sample have a very amount of protein

Assessment of Extracted Nucleic Acid

3. Gel electrophoresis:

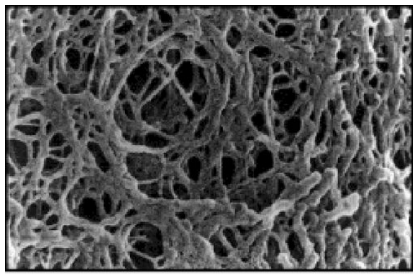
- Gel electrophoresis is a standard lab procedure for separation nucleic acids based on their sizes under the influence of electric field
- The concept: DNA and RNA are negatively charged molecules they move toward the positive electrode **(usually red)**

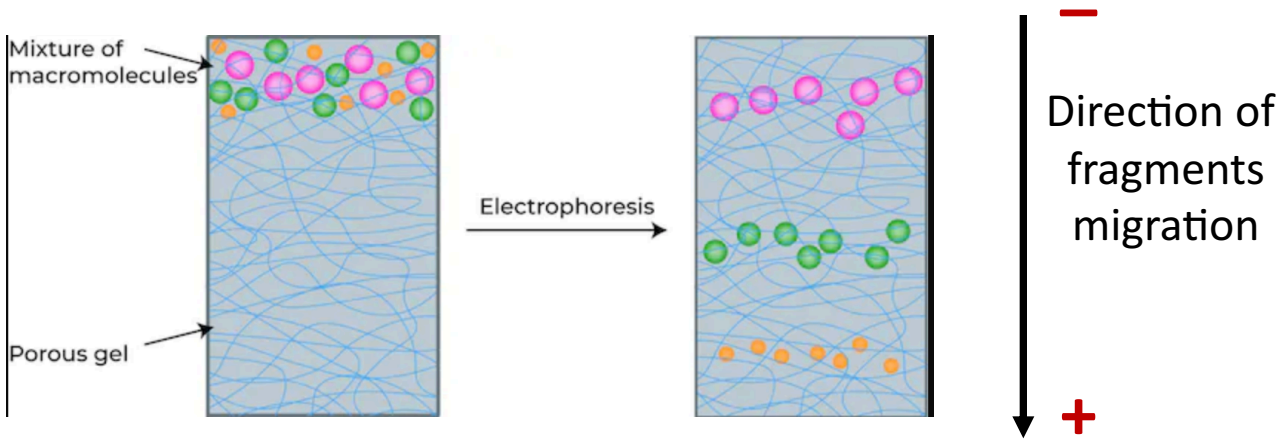
Gel electrophoresis ⬆️ size ⬇️ Volcity or speed of molecule



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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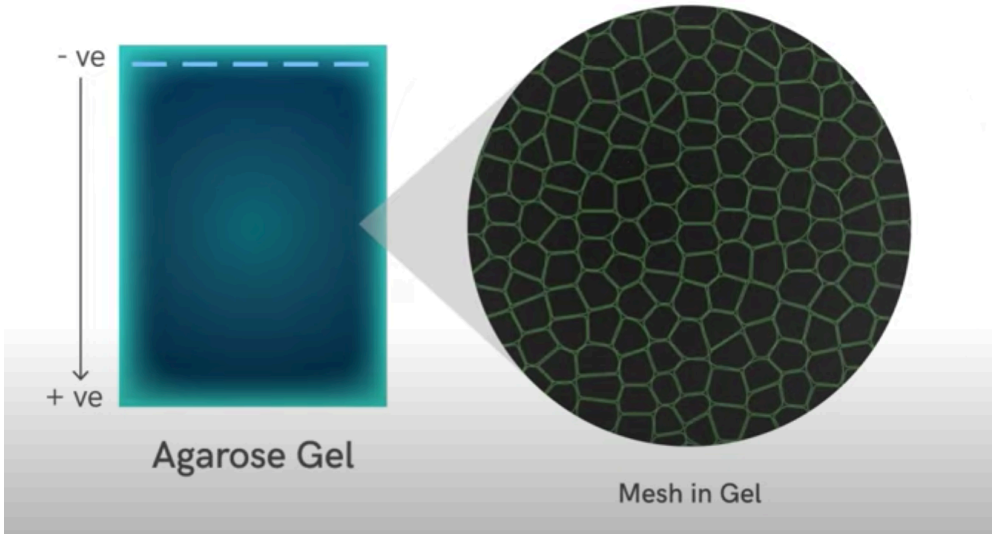
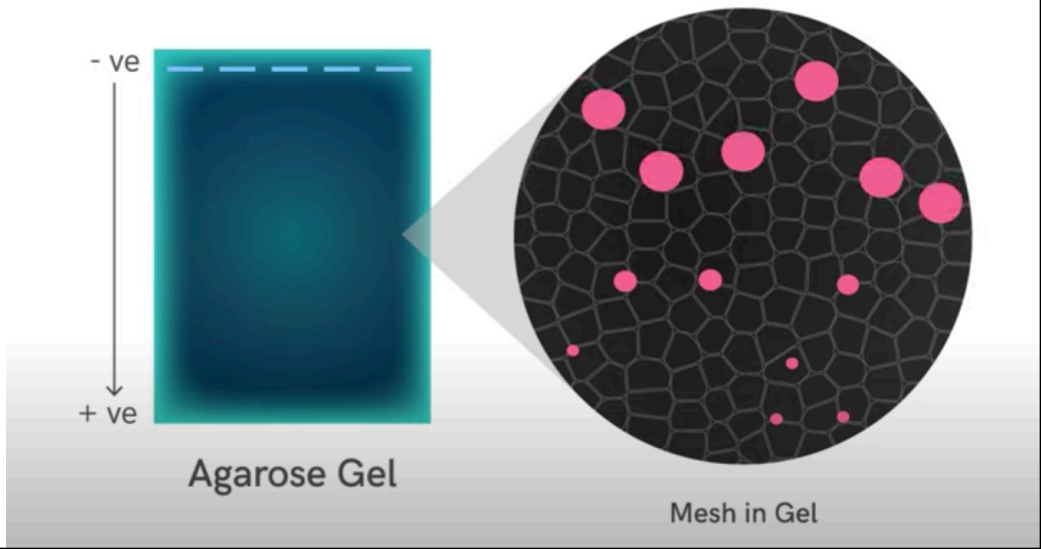
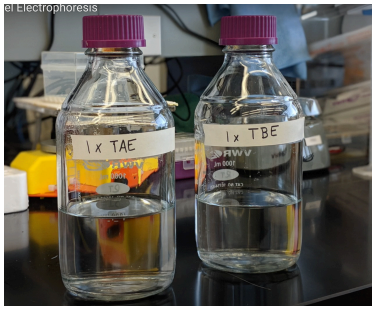


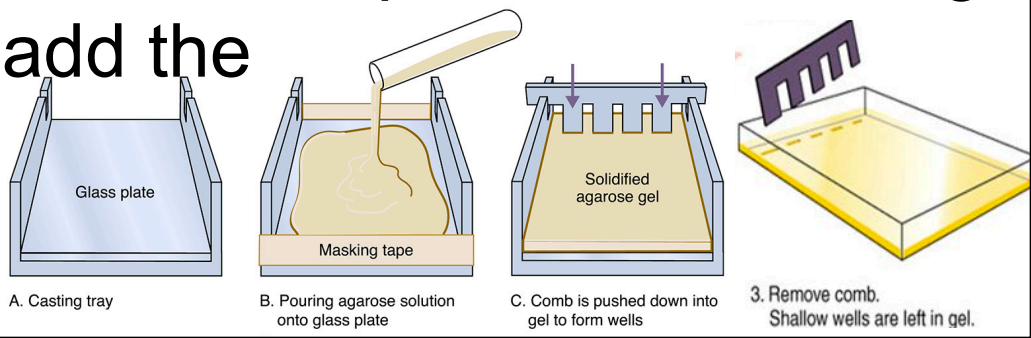


Assessment of Extracted Nucleic Acid

- 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)

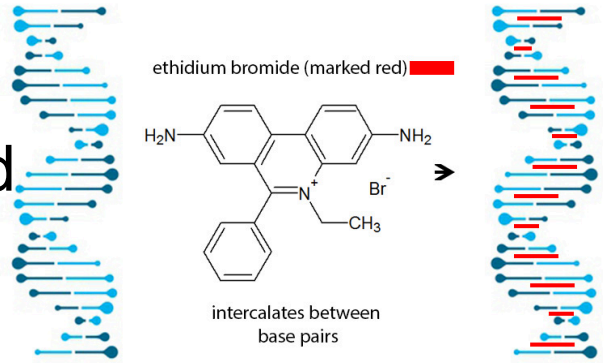


- For example: to prepare 1% agarose gel (1g/100ml) dissolve 1g of agarose powder in100ml of 1X TAE
- add few drops of nucleic acid fluorescent dye to the dissolved gel solution then pour it into casting tray (don't forget to add the comb to make the wells)

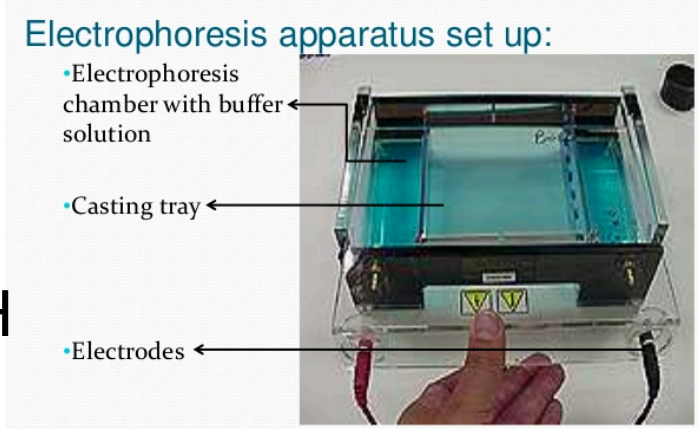


Assessment of Extracted Nucleic Acid

- The nucleic acid fluorescent dye is used to visualize the nucleic acid under UV light (acts by intercalation): Ethidium Bromide (mutagen). GelRed and SYBR green I (expensive but safe)



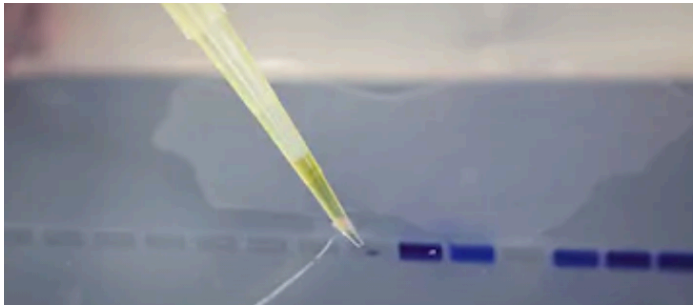
- The gel tray is placed in an electrophoresis chamber and filled with running buffer (1X TAE buffer) until it covers the gel piece. Buffer is used to provide ions that carry the current and to maintain pH



- **intercalation=> enter the DNA and bind to it.mean1x TAE=> How many time dilution of buffer When weuse the buffer, should be used in anormal Concentration (1 x)In lap use (50 x)-> should dilute it , even become1 X**

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)



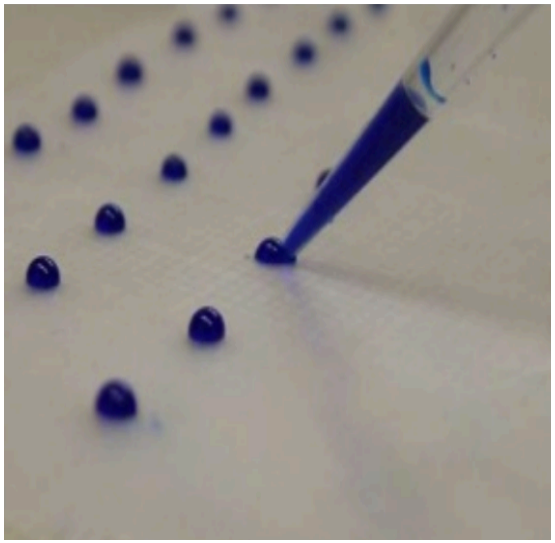
- The electrodes are attached to a power supply and an electrical current is applied

What the meaning of track=> follow the sample.

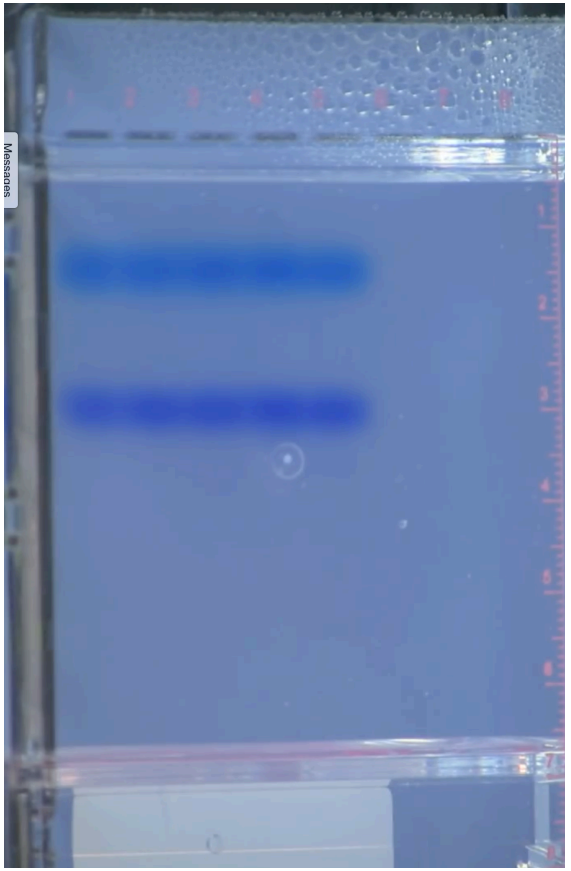
Assessment of Extracted Nucleic Acid

- سؤال بالامتحان الفرق بين fluorescent, loading dye
- loading dye=> to track migrated fragment, prevent it from Floating In experiment, it faster than the migrated fragmentFluorescent dye=> to visuale nucleic acid bands under uv light.Gel documentation system =>connect to the printer=> to print the gel in paper

Assessment of Extracted Nucleic Acid



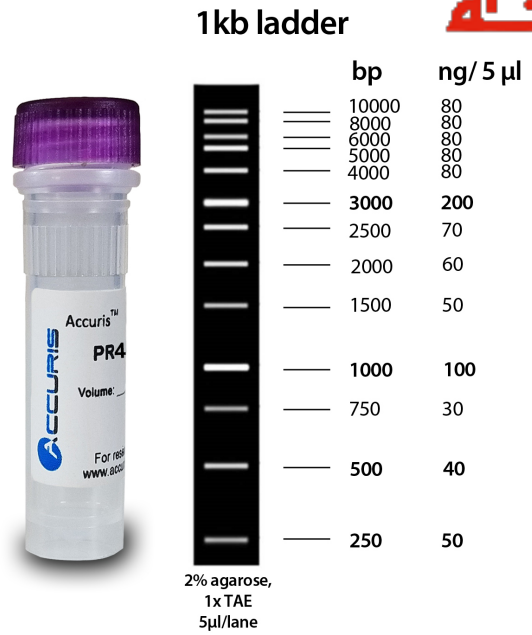
After 5 min

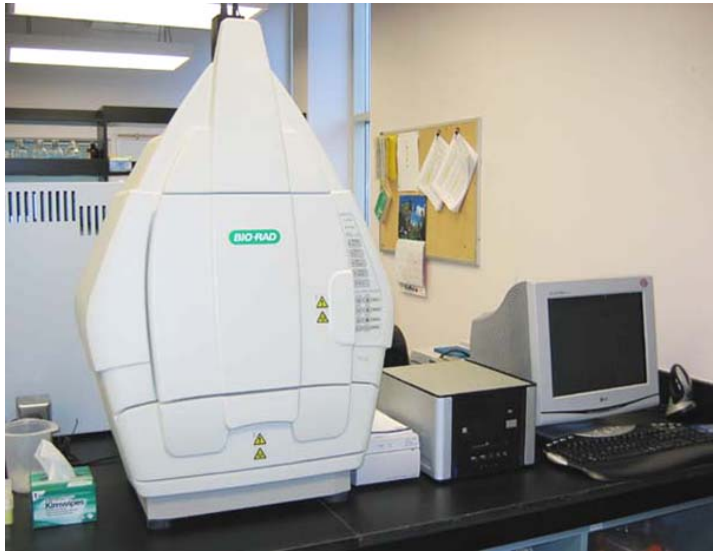


After 20 min

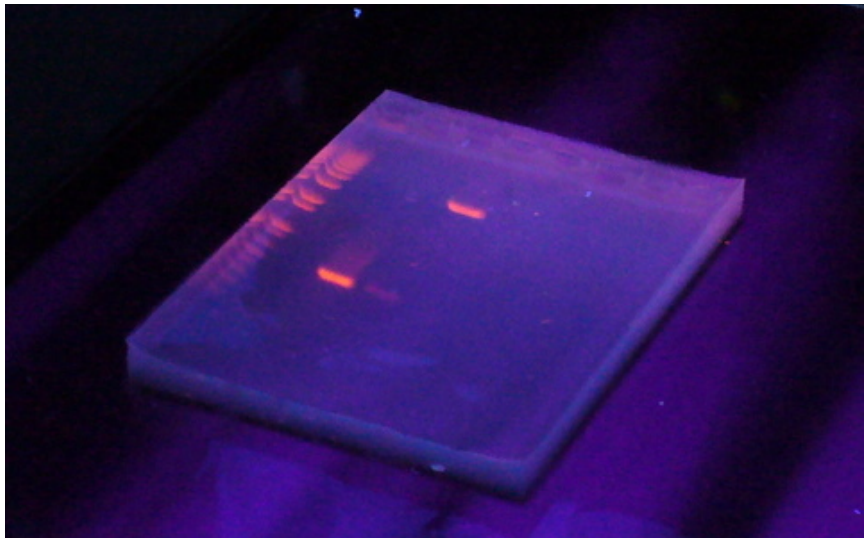
Assessment of Extracted Nucleic Acid

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

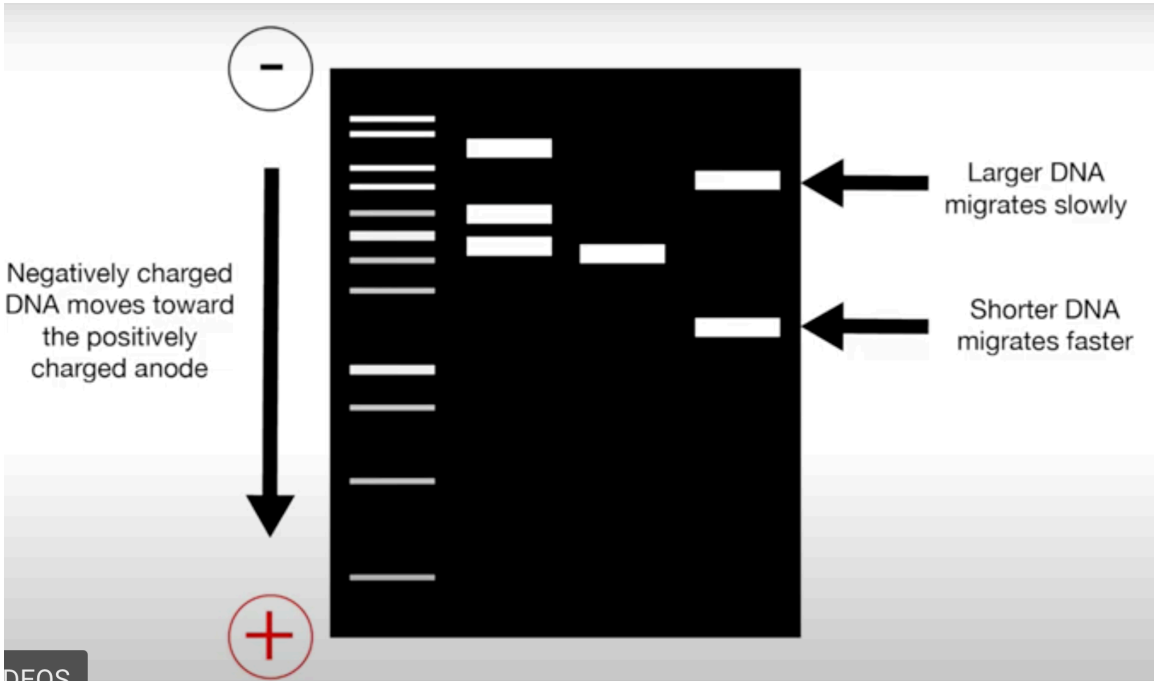


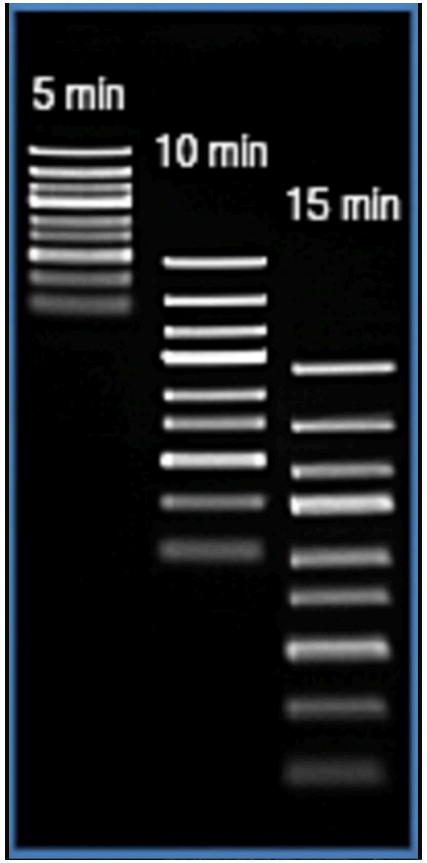


Gel documentation system "Gel Doc System"



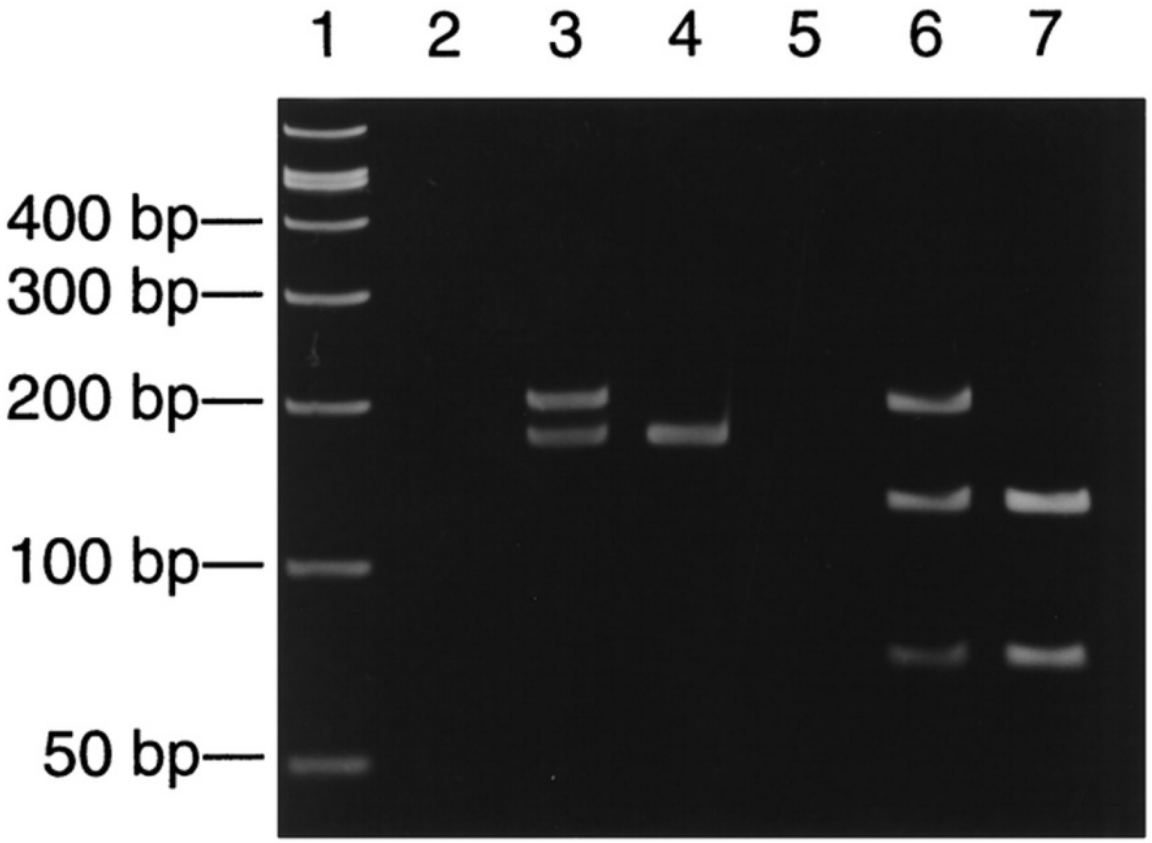
Assessment of Extracted Nucleic Acid





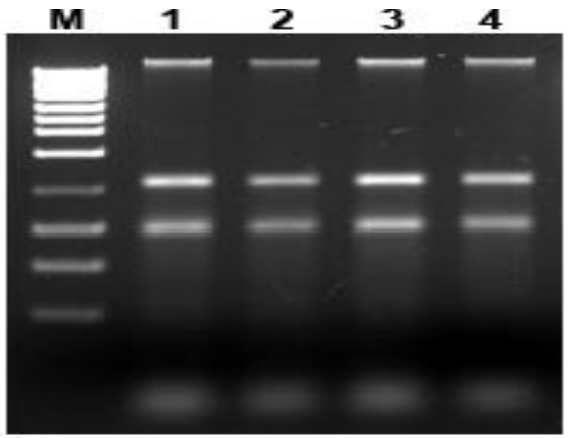
comparingthe size => increasing time will increase the space between fragment

Assessment of Extracted Nucleic Acid

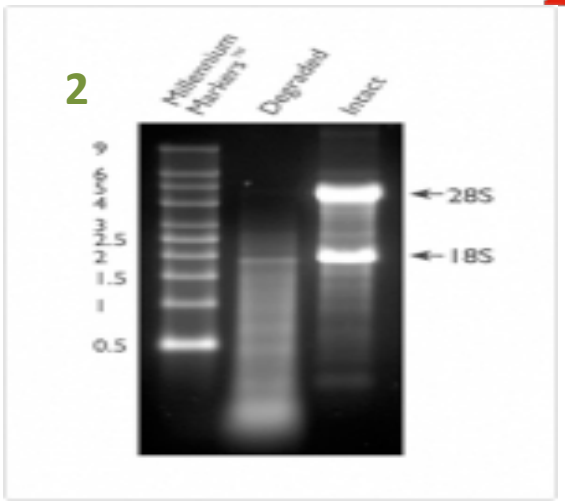


compare between your hypothesis or expectationand result from expirement.1 and 5 => false work / bad

Assessment of Extracted Nucleic Acid



Total RNA



…mRNA is not seen by gel electrophoresis as it is large in size.

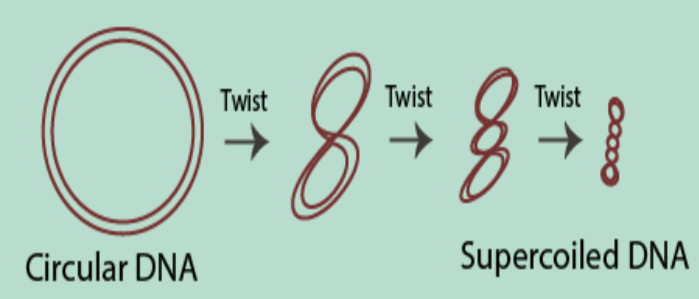
Pic 2 indicates degradation of RNA as it appears like a smear , if … rRNA is intact, this means that all RNA type, are atintact, too.

Electrophoresis is applied and seperate RNA as two bands. So you can used these sample to complete your work ,If it smear you can't used these sample to complete your work

In case you mix between two sample of DNA and RNA , How do I distinguish between samples?

In RNA sample, mRNA cantbe seen, but rRNA that have two Part example 28s (faster) / 18 S Can be seen.why can't see the mRNA=> becouse it large in size

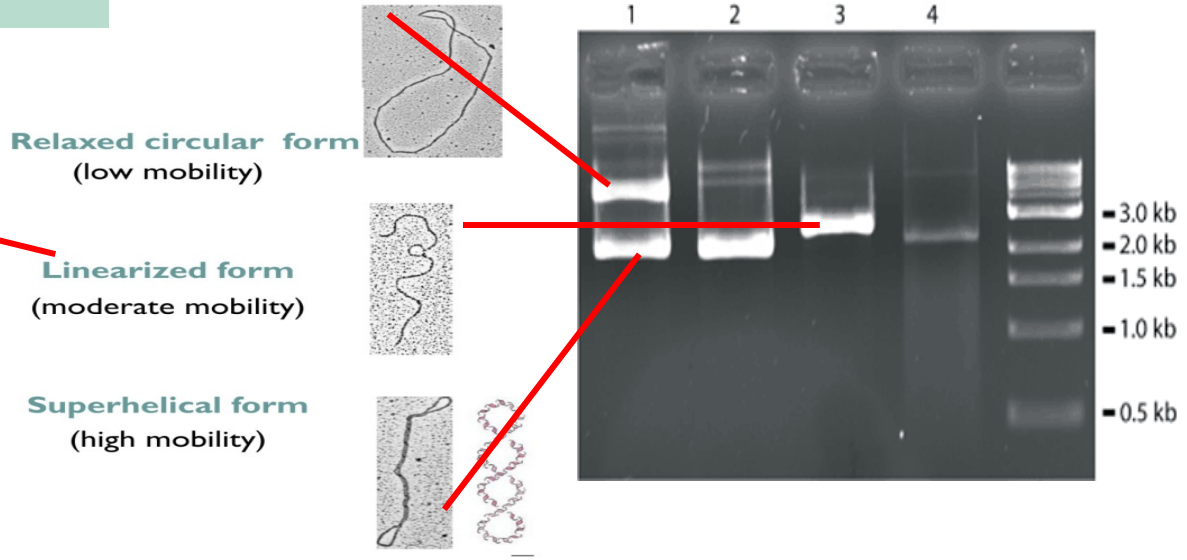
Assessment of Extracted Nucleic Acid



Plasmid→
…identify Presence of Plasmid
…contain gent of interest

we use a special restriction enzyme to cut Plasmid molecule in the site of gene of interests so the fragrant becomes linear

Sample *1* Plasmid RNA ,it seems like attached waves
Sample *4* linearized form in low Concentration



((plasmid),(Vector)=> the Same method of preparation RNA & DNA
How can I determine if a plasmid contains the required gene (gene interest) ?
We use restriction enzyme before we add loading dye