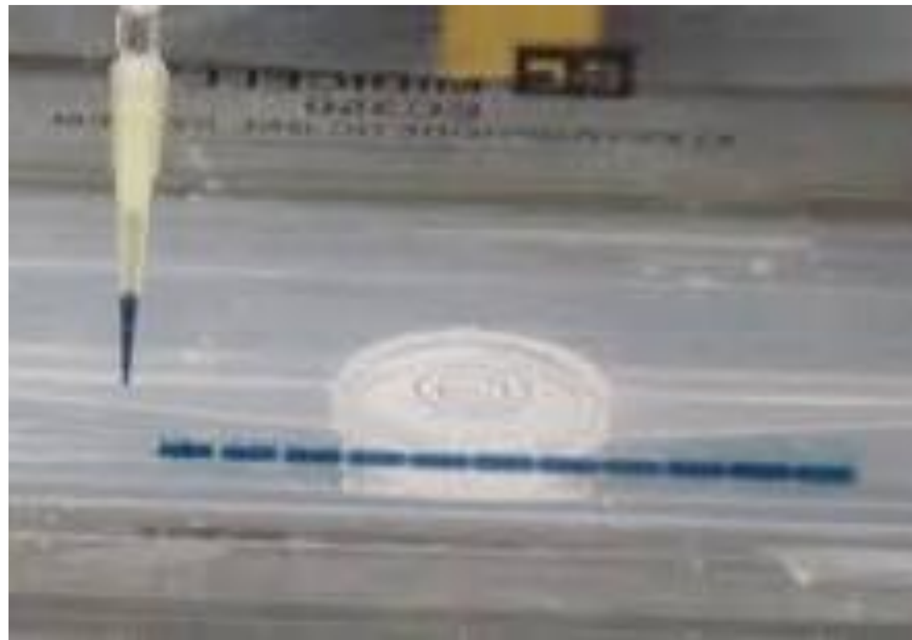
Agarose gel electrophoresis



Tissue

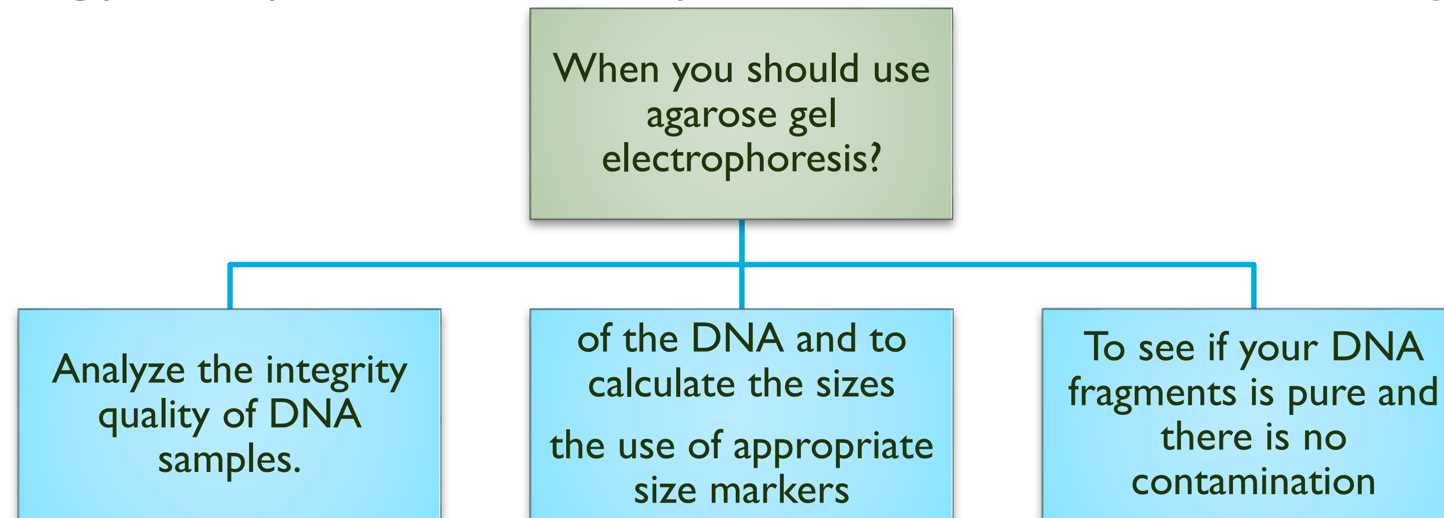
DNA Extraction

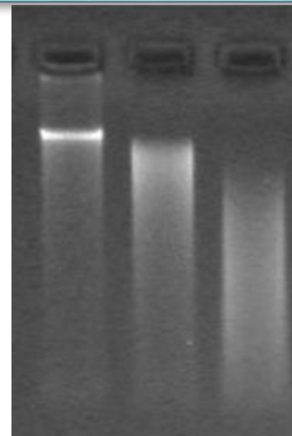
How to chick the integrity of DNA?

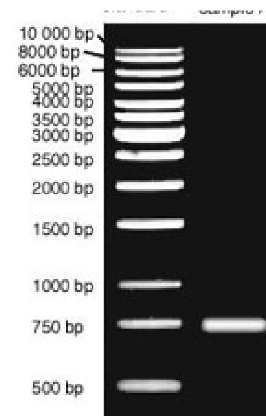
Agarose gel electrophoresis

Agarose gel electrophoresis

- Is a method of gel electrophoresis used in biochemistry and molecular biology to separate and analyze DNA or RNA molecules **by size**

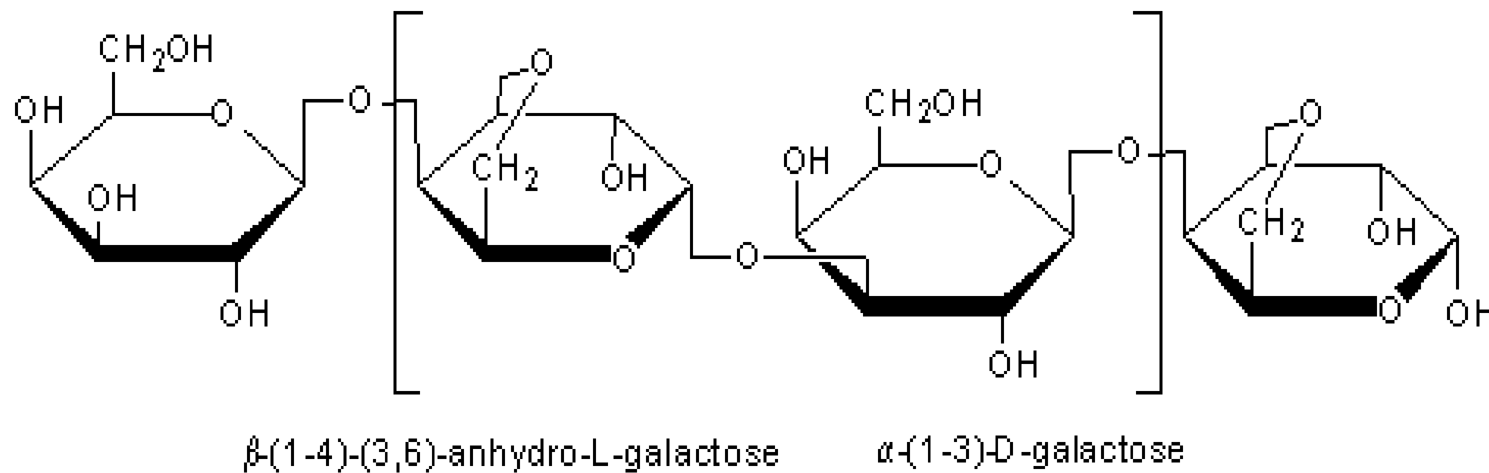




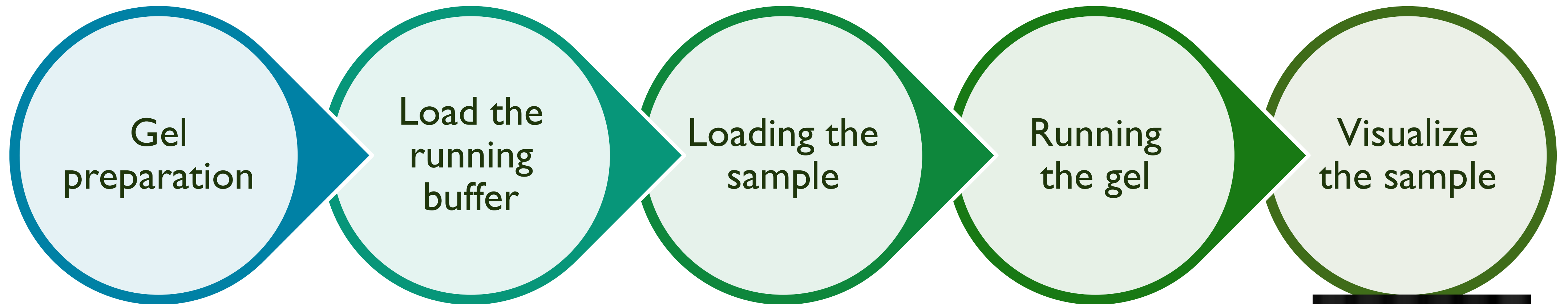


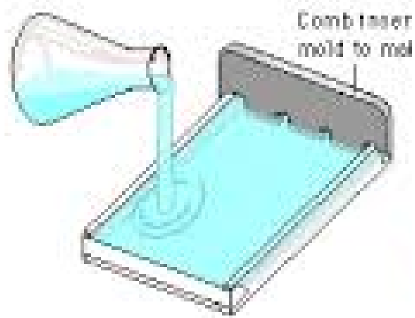
Agarose

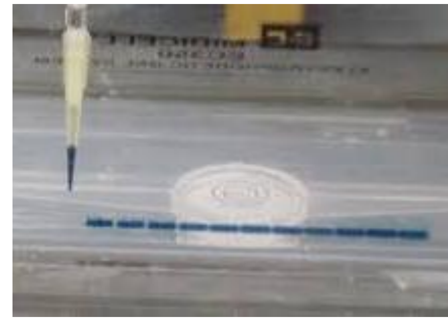
- Agarose is a linear polysaccharide made up of the basic repeat unit agarobiose, which comprises alternating units of galactose and 3,6-anhydrogalactose

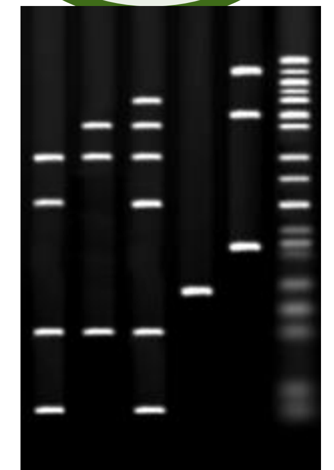


Agarose gel electrophoresis









1-Electrophoresis components

electrophoresis chamber

Gel casting trays

Combs

2-Prepare the gel

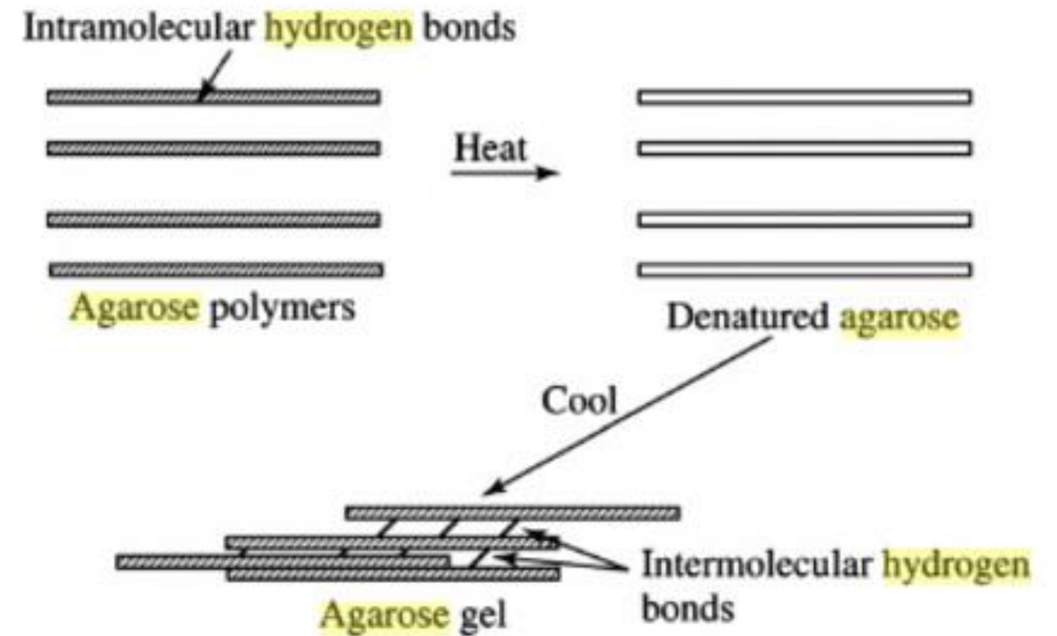
- Agarose gels are formed by suspending dry agarose in aqueous **buffer**, then boiling the mixture until a clear solution forms.

- This is poured and allowed to cool to room temperature to form a rigid gel (polymerized).

2-Preparation of gel

- Heating disrupts the intramolecular hydrogen bonding pattren of agarose while cooling allows the reformation of hydrogen bonding, some of these bonding formed intermolecular .

- The gelling properties are attributed to both inter- and intramolecular hydrogen bonding within and between the long agarose chains.



2-Preparation of gel

- The sample that you will use is a DNA extracted from blood, (genomic DNA)
- Assemble the gel tray and put the combs
- Prepare 50 ml of 1% agarose in TAE buffer. (why)
- Add Ethidium bromide
- Put the flask in the microwave oven and run until you see the bubbles.
- Be careful not to touch the flask with your hands
- Remove the flask from the microwave oven and wait until the temperature decreases slightly (don`t allow to solidify again).
- Load the gel into the plate until the half of the comb and wait until the gel is solidified.

2-Preparation of gel

- The concentration of the material in the gel determines the size of the pores.
- [high concentration of the gel → small pore size]

- Agarose gels have larger pore sizes compared to Dextran and polyacrylamide gels.

- This makes it useful for the analysis or separation of large globular proteins or long, linear molecules such as DNA.

3-Loading the sample and running the gel

- Add 5 µl DNA ladder into the 1st well
- Mix 5 µl DNA sample with 2 µl loading dye and add them into the 2nd well
- Loading dye consists of :
- Glycerol
- Tracking Dye (Orange Dye)

- Put the cover of the container (Insure that you have put it in the right way)
- Run at 90 volts and wait until the dye passes at least the half of the gel.

3-Running the sample

- **Polymerized agarose is porous, allowing for the movement of DNA or RNA**

- DNA or RNA are separated by applying an electric field, so these negatively charged molecules [-] will move through an agarose matrix towards the anode [+] , and the biomolecules are separated by size in the agarose gel matrix.

- The largest molecules will have the most difficulty passing through the gel pores,whereas the smallest molecules will move faster.
- The pore size in the gel is controlled by the initial concentration of agarose; large pore sizes are formed from low concentrations and smaller pore sizes are formed from the higher concentrations.

4-Gel staining and visualization

- The DNA in the gel needs to be stained and visualized, The reagent most widely used is the fluorescent dye ethidium bromide **"EtBr“**, that emits orange light after binding to DNA.

- Note: That the gel will be viewed under ultraviolet light.

- [Ethidium bromide is a cyclic planar molecule that binds between the stacked base-pairs of DNA.].

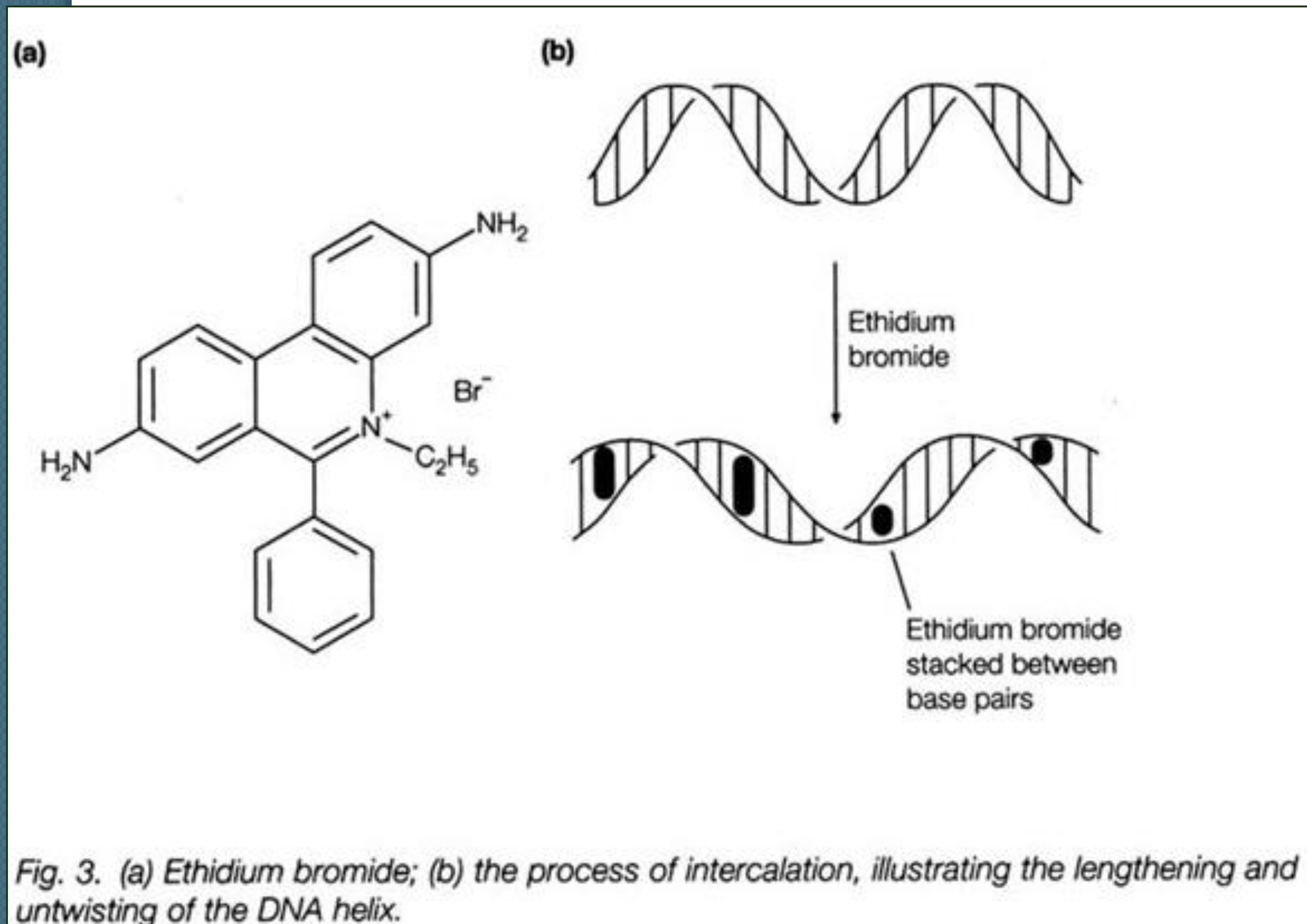
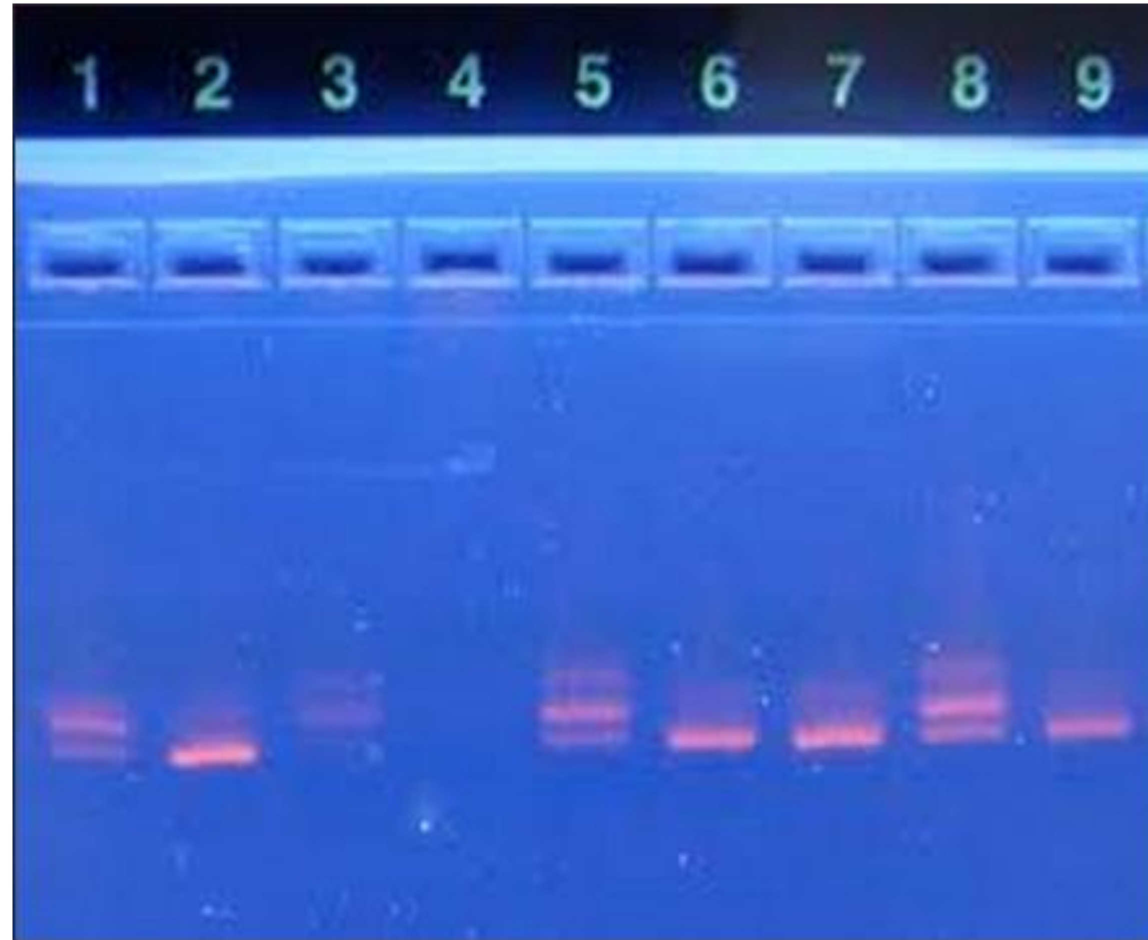


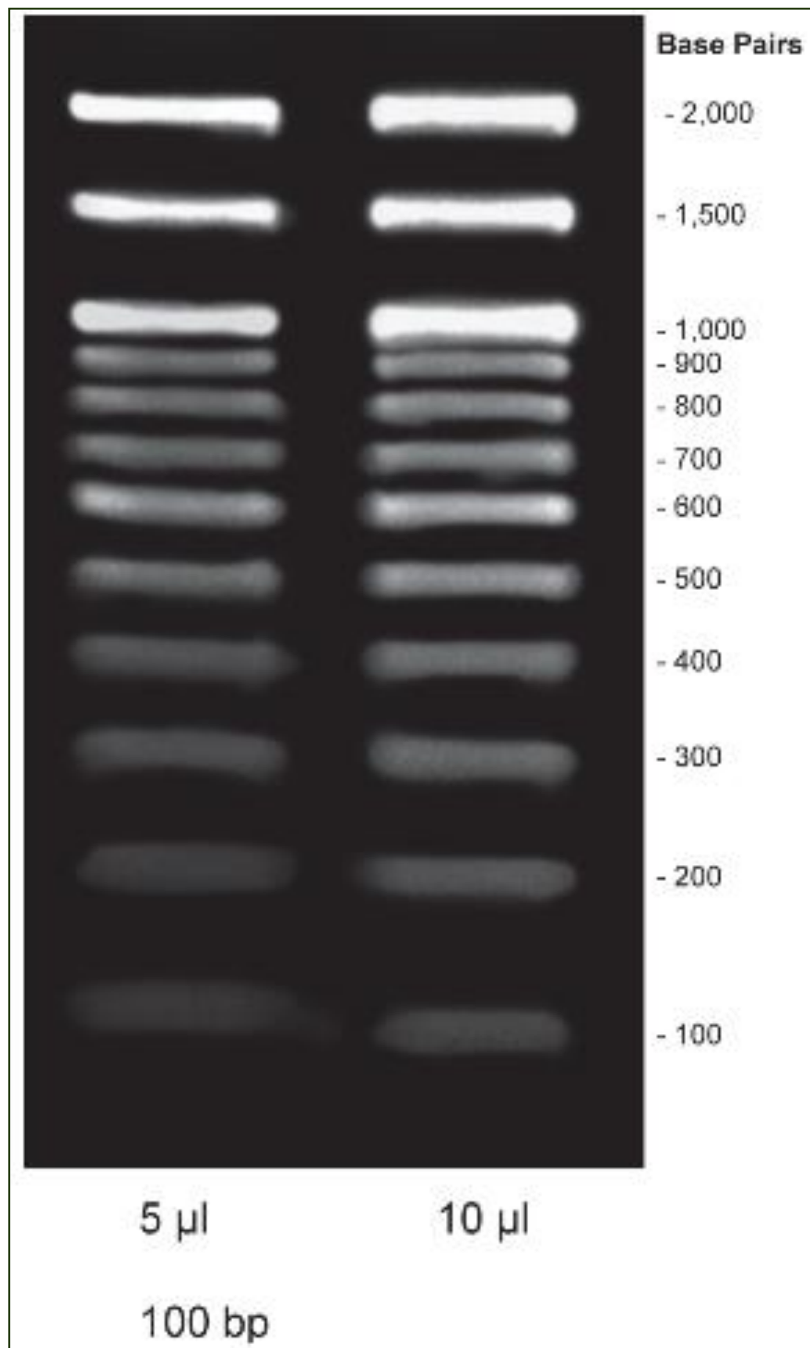
Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

Nucleic Acid-Specific Dyes

Intercalating agents intercalate, or stack, between the nitrogen bases in double-stranded nucleic acid. Ethidium bromide [3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide (EtBr)] is one of these agents and was the most widely used dye in early DNA and RNA analyses. It is carcinogenic and should be used with care, however. Under excitation with ultraviolet light at 300 nm, EtBr in DNA emits visible light at 590 nm. Therefore, DNA separated in agarose or acrylamide and exposed to EtBr will emit orange light when illuminated at 300 nm. After elec-



"EtBr“orange light after binding to DNA.



Determine the size of the DNA fragment:

-Since agarose gels separate DNA according to size, the size of a DNA fragment may be determined from its electrophoretic mobility by running a number of standard DNA markers of known sizes on the same gel.

- **Ladder can come in different ranges of fragments!!You must choose your ladder carefully!!!!!**

Figure: The 100 bp DNA Ladder is suitable for sizing double-stranded DNA fragments from 100-2000 bp.

Buffer used:

-helps is deliver the electric current through the gel.

Buffer used is either TBE or TAE.

- TBE buffer: is made with Tris/Boric acid/ EDTA.

- TAE buffer: is made with Tris/Acetic acid/ EDTA.

Tracking Dye:

A dye such as bromophenol blue [or orange dye] is also included in the sample;
it makes it easier to see the sample that is being loaded and also acts as a marker of the electrophoresis front.

Note that:

-The higher the voltage → the more quickly the gel runs.

[↑ Voltage → ↑ rate of migration].

-Gel concentrations must be chosen to suit the size range of the molecules to be separated.

Animation

- http://learn.genetics.utah.edu/content/labs/gel/