Plasmids & Transposable Elements

Lecture 4

BY

Dr. Wiaam Ahmed Al-Amili

Methods of Molecular Weight Evaluation for Plasmids

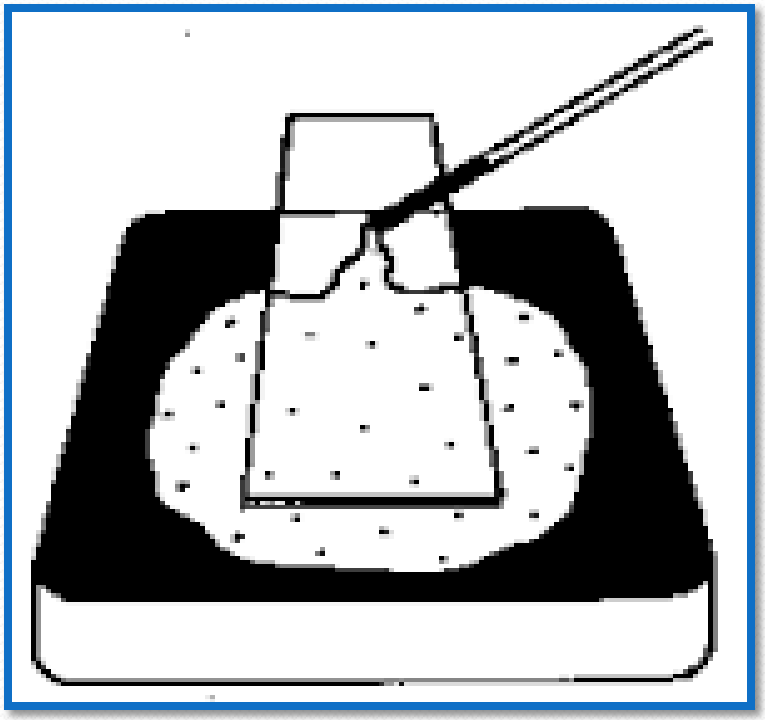
1. Using Electron Microscope

- An electron microscope is a microscope that uses a **beam of accelerated electrons** as a source of illumination .
- As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons , electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects.

- electron microscopes use electromagnetic lenses to control the electron beam and focus it to form an image. These electron optical lenses are analogous to the glass lenses of an optical light microscope.
- electron microscope can achieve better magnifications of up to about 10,000,000 x whereas most light microscopes are limited by useful magnifications below 2000 x.

- The transmission electron microscope (TEM) consists of a metal column from which air is evacuated and through which a linear beam of electrons is accelerated and focused by electromagnetic lenses.
- The biomolecules are adsorbed onto a support film,
- **Support Film** : A thin film of plastic or carbon that is attached to an EM grid and provides a substrate onto which biomolecules can be adsorbed.

- 1. Mix nucleic acid , cytochrome c , and buffer (Spreading solution).
- 2. Pour hypophase solution (usually, water or ammonium acetate is used).
- 3. Insert a glass slide in the trough with one end resting on the rim.
- 4. Apply spreading solution on the glass slide.
- 5. Wait until solution has run down the ramp and spread out.



- 6. Touch the film side of a grid (Normally, carbon- or parlodion-coated grids are used) to the surface of the hypophase.
- 7. Stain the grid (Ethanolic uranyl acetate is widely used).
- 8. Dehydrate the specimen.
- 9.Rotary shadow for contrast enhancement (Usually, platinum/palladium (80 : 20) is used for rotary shadowing.

- **Heavy-metal Shadowing** : Evaporation of a film of heavy metal (e.g. platinum–palladium or tungsten–tantalum) onto a dehydrated preparation.
- The deposit of metal particles around the biomolecules improves contrast and gives a shadowed, three-dimensional appearance.

- Whereas some of the electrons collide with atoms in the specimen , lose energy, and are scattered, the remaining electrons pass through the preparation and are focused to form an image on a phosphorescent screen (for direct viewing) or on a photographic plate (for later examination).

- Using standard DNA with known molecular weight
- Compare the length of sample DNA with stander DNA .

The reason of the bad preparation may be

- contaminating agents in the DNA solution(detergents
- bad Cyt c (stock solution too old)
- contamination of buffer.

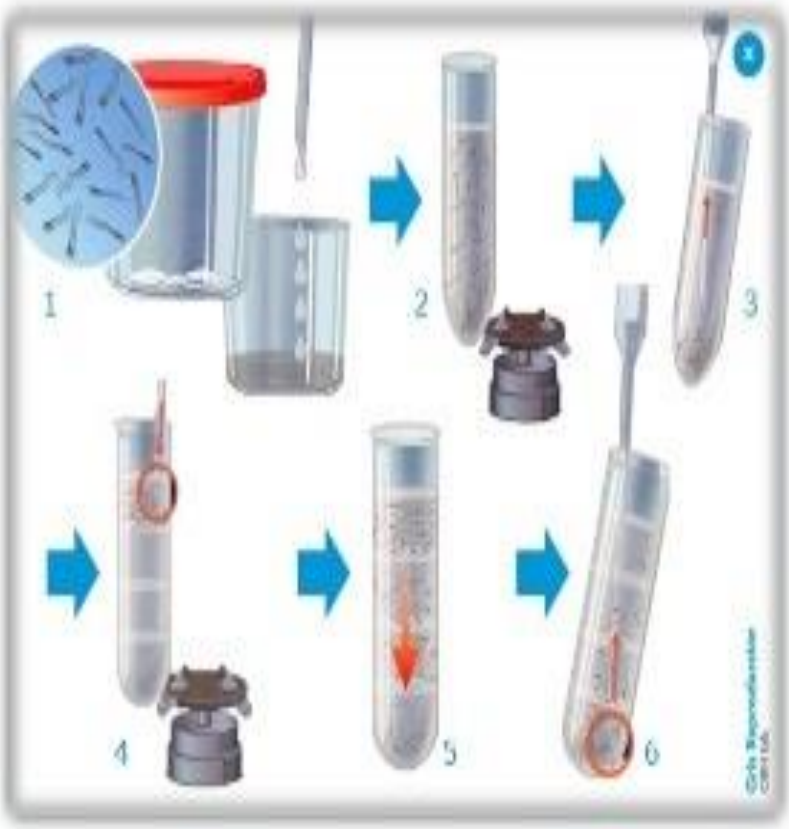
- - Is not supported method

2-Sucrose Gradient

- Whole lysates containing labeled DNA may be sedimented directly in alkaline sucrose gradients to determine the number and size of resident plasmid species within a bacterial strain.
- Moreover, it is often necessary to include a differentially labeled standard plasmid DNA of known molecular weight in sucrose gradients to calculate the size of any unknown plasmid species.

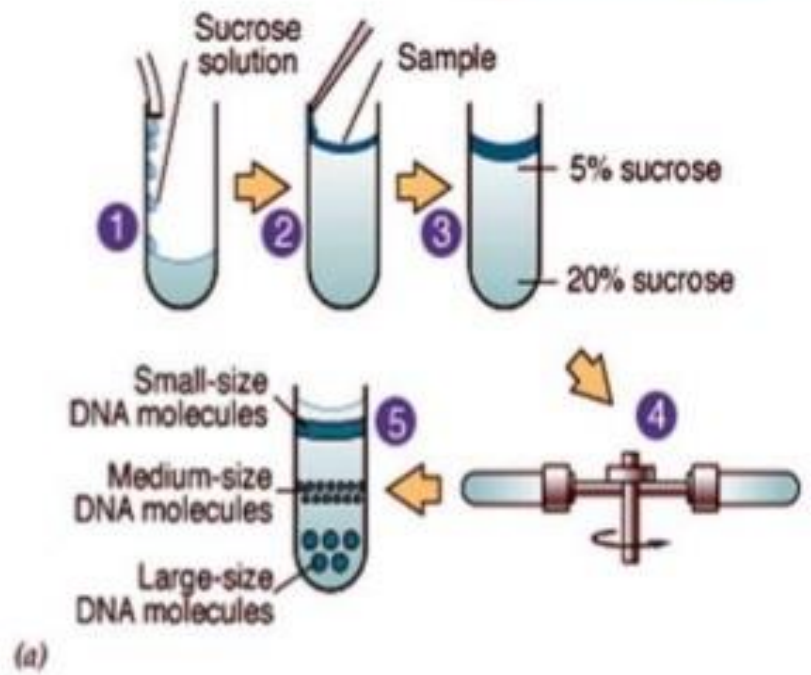
Density gradient separation.

- A density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in a centrifuge tube, from 5% to about 20% sucrose.
- Gradient mixers can be used to form a gradient.
- The sample is placed on top of the gradient and centrifuged at forces in excess of 150,000 x g.
- The particles travel through the gradient until they reach their isopycnic point, i.e. the point in the gradient at which their density matches that of the surrounding sucrose.



SUCROSE GRADIENTCENTRIFUGATION

- A homogenate is placed on top of a special medium e.g. sucrose solution
- that progressively increases in concentration density also
- when this sucrose gradient is centrifuged at high speed each particle in the homogenate will move down in the tube and will come to rest at the point in the gradient where a density equals that of the sucrose solution.



Agarose Gel Electrophoresis

- Consequently , we have been searching for an inexpensive screening method for the detection of plasmids and the determination of their size that could be applied to a wide variety of bacterial species.
- Recently, agarose gel electrophoresis has been widely employed.

- Analysis of bacterial plasmid profiles has been shown to be very important in epidemiological studies, especially those involving outbreaks of nosocomial infections.
- The method has proved to be a useful tool for survey work , as well as an important complement to the genetic analysis of plasmids.

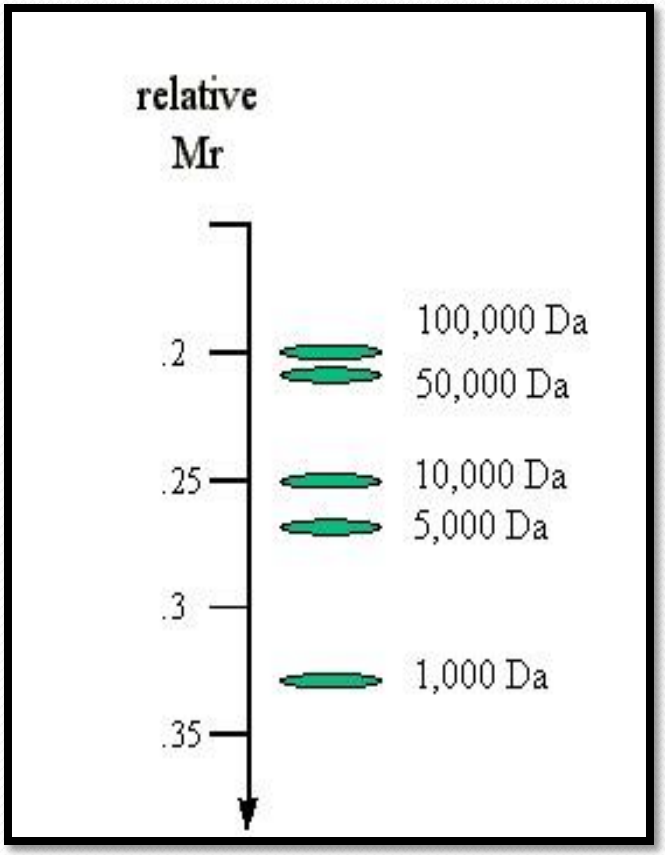
- may be employed effectively
- The method is sensitive and does not require radioisotopes or ultracentrifugation.
- The molecular weight size of unknown plasmids is determined by comparing their band pattern obtained in agarose gel electrophoresis with those obtained with DNA that have been used as molecular weight or size standards.

- Gel electrophoresis is used to characterize one of the most basic properties - **molecular mass** - of DNA.

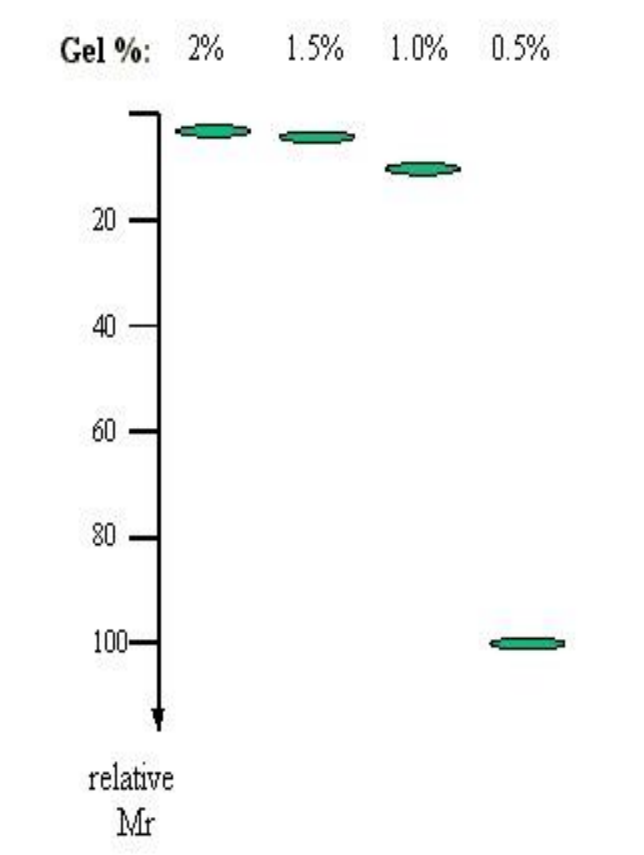
DNA agarose gels

- The electrophoretic migration rate of DNA through agarose gels is dependent upon ***four main parameters***:

- 1. The **molecular size** of the DNA. Molecules of linear duplex DNA travel through agarose gels at a rate which is inversely proportional to the log of their molecular weight.



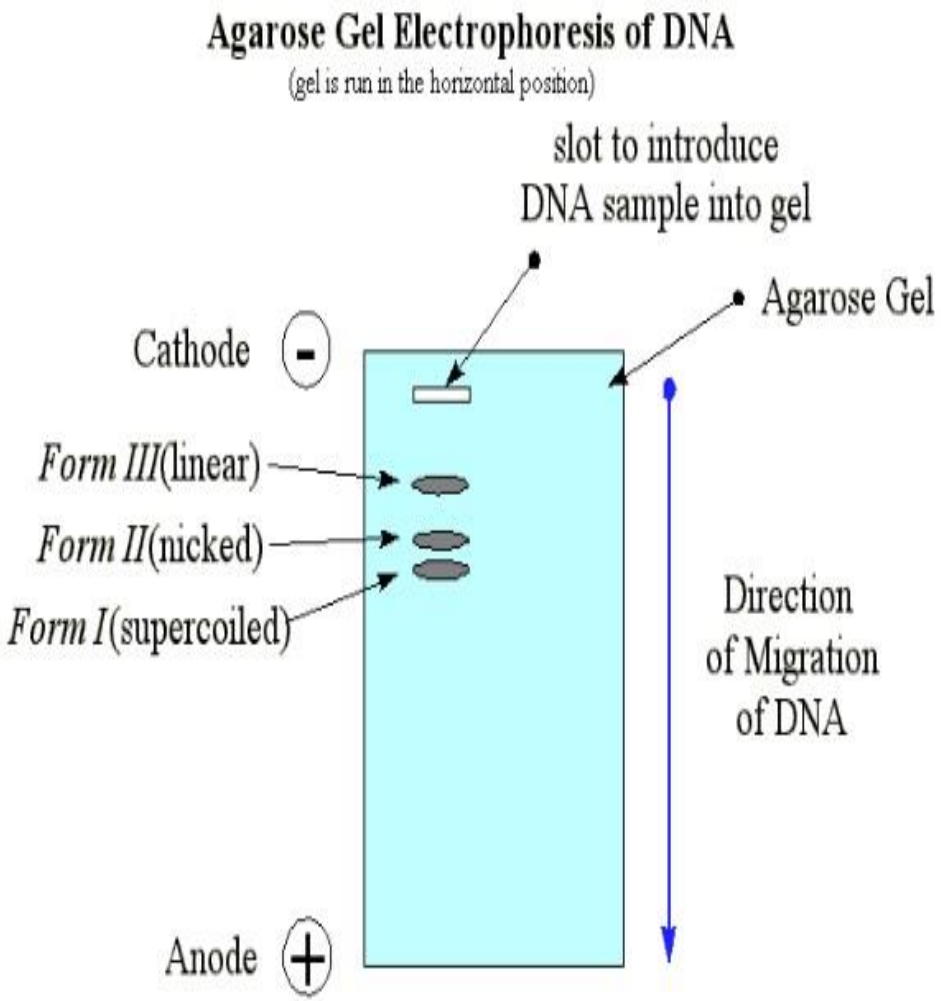
- 2. The **agarose concentration**. There is an inverse linear relationship between the logarithm of the electrophoretic mobility and gel concentration.

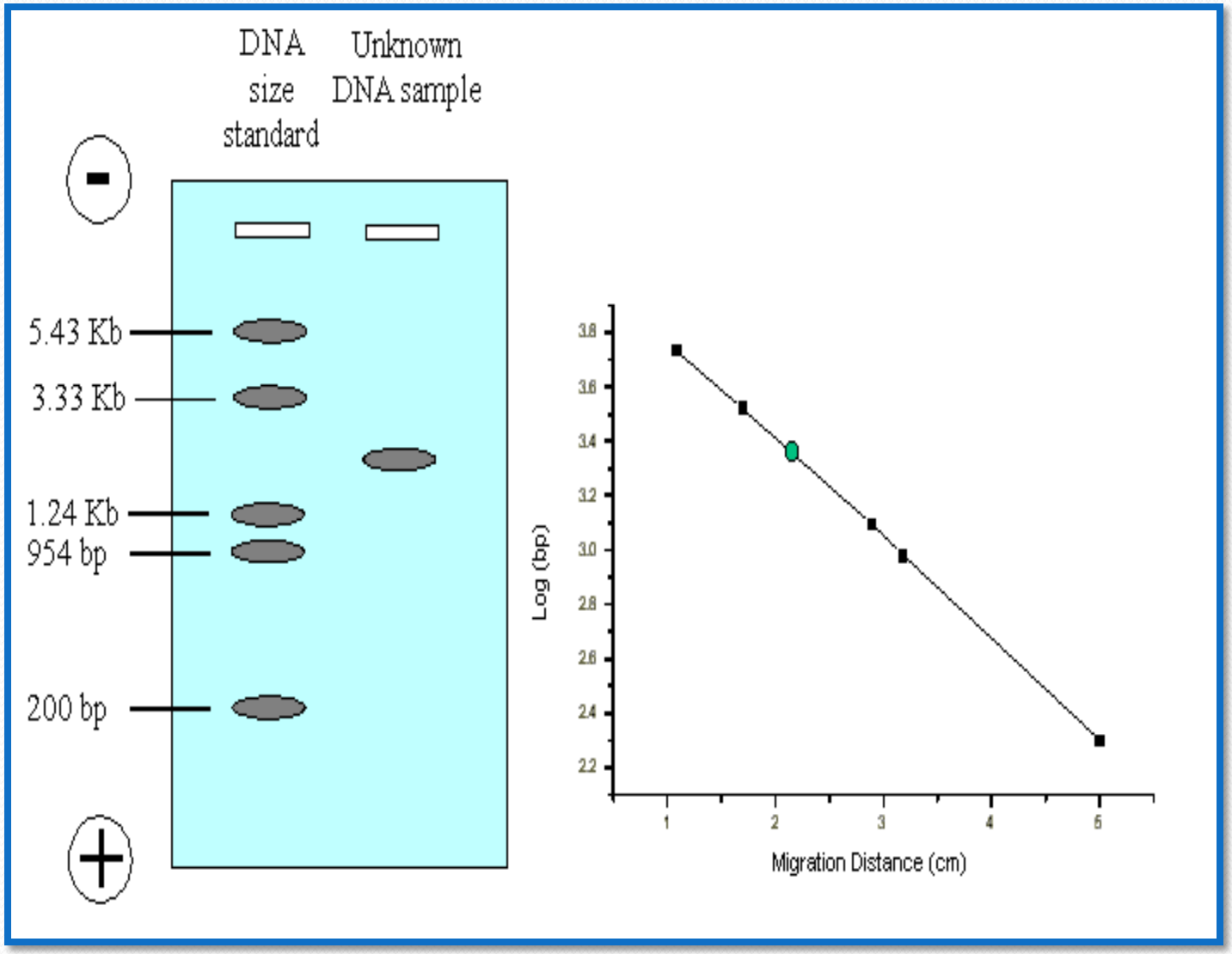


- 3. The conformation of the DNA.
- closed circular DNA (***form-I***) - typically supercoiled
- nicked circular (***form-II***)
- linear DNA (***form-III***)
- These different forms of the *same DNA* migrate at different rates through an agarose gel.
- Almost always the **linear form** (form-III) migrates at the **slowest** rate of the three forms
- Supercoiled DNA (form-I) usually migrates the **fastest**

- 4. The applied **voltage**.
- Typical value for running an agarose gel is **5 volts per cm** (length of gel).
- Agarose gels are usually poured and run horizontally

Finally, the DNA being an acidic molecule, migrates towards the positively charged electrode (cathode).




DNA size standard
Unknown DNA sample
5.43 Kb
3.33 Kb
1.24 Kb
954 bp
200 bp
Log (bp)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
1
2
3
4
5
Migration Distance (cm)


Good
Luck!