Técnicas de estudio de la interacción de compuestos metálicos con ADN:

IV. Electroforesis en gel

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Gel electrophoresis

1. Basics of electrophoresis
2. Nucleic acids gel electrophoresis
3. Agarose gel electrophoresis
4. Examples

Electrophoresis

Transport of particles through a solvent by an electric field

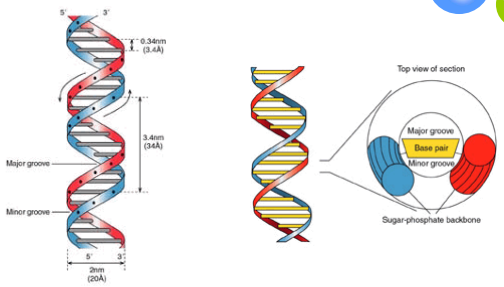
most biological polymers are charged and will move in an electric field

characteriza-tion of a molecule by its rate of movement in the electric field

determine protein MW

distinguish molecules by their net charge or shape

separate molecular species quantitatively



Electrophoresis

molecule of charge *q* submitted to an electric field *E*

electrical force

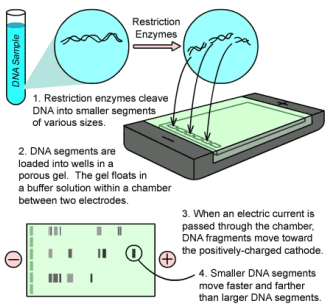
$$Eq = fv$$

$$\mu = v/E = q/f$$

viscous drag

f frictional coefficient
μ mobility

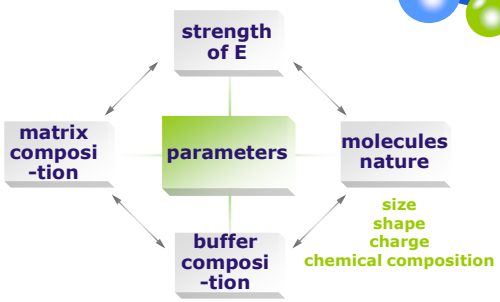
Gel electrophoresis



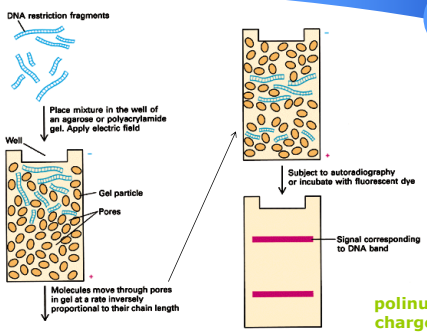
separation of charged molecules through a matrix

matrix: paper, starch, agarose, polyacrylamide

Gel electrophoresis: parameters

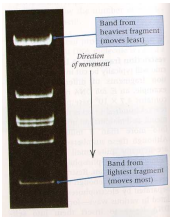


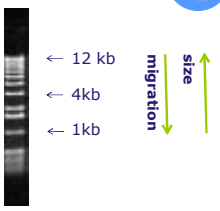
Nucleic acids gel electrophoresis



polinucleotides: charge/mass ≅ k

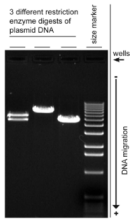
Nucleic acids gel electrophoresis





Migration of nucleic acids is inversely proportional to size.

Nucleic acids gel electrophoresis



agarose gel with a 1 kb ladder, right lane

A DNA ladder is a solution of DNA molecules of different lengths used as a reference to estimate the size of unknown DNA molecules.

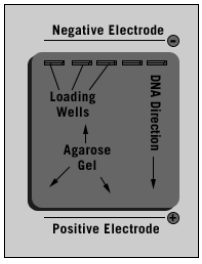
Nucleic acids gel electrophoresis

Nucleic acids are separated on a gel or matrix of agarose or acrylamide.

The gel is prepared in a mold with wells to put the sample.

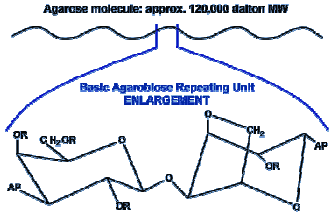
Each end of the gel is in contact with the electrophoresis buffer or the whole gel is immersed in it.

Buffer ions transport the charge and mantain the pH value approximately constant.



Electrophoresis on agarose gels: agarose

Agarose is a polysaccharide obtained from marine algae.



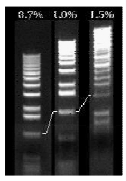
Low melting point (65ºC) agarose beds are used in Molecular Biology experiments for the analysis and separation of DNA fragments and shapes. It allows bands isolation and quantification.

Electrophoresis on agarose: % of agarose

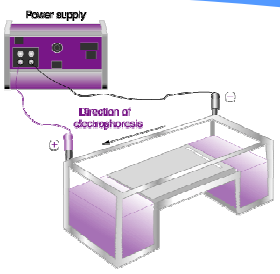
% agarose 0.7-2.0 in buffer (TBE or TAE)

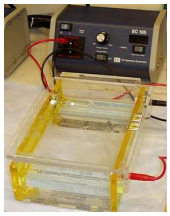
resolution varies with % agarose

% agarose	size range
0.7	5-10kb
2.0	0.2-1kb
3.0	very small fragments



Electrophoresis on agarose gels: technique





Gel electrophoresis apparatus - An agarose gel is placed in this buffer-filled box and electrical field is applied via the power supply to the rear. The negative terminal is at the far end (black wire), so DNA migrates toward the camera.

Electrophoresis on agarose gels: sample

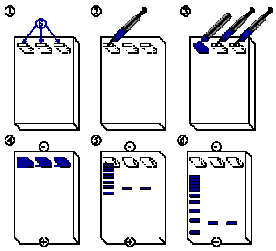
volume: 10-20 µL

buffer

dye*

MW marker

well size, number

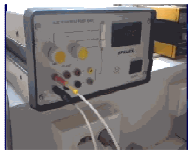


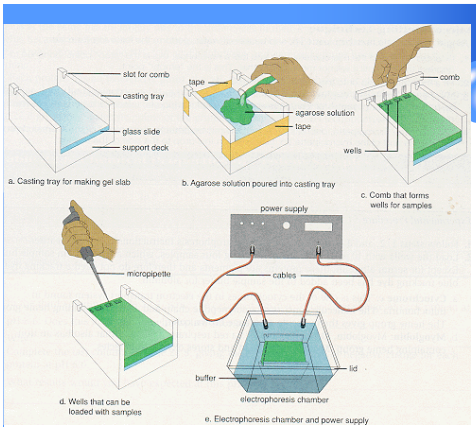
* bromophenol blue (300bp), xylencianol (4kb)

Electrophoresis on agarose: intensity

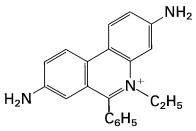
generally: 50-100 V
for a couple of hours

High voltages
diminish resolution.





Electrophoresis on agarose: NA visualization



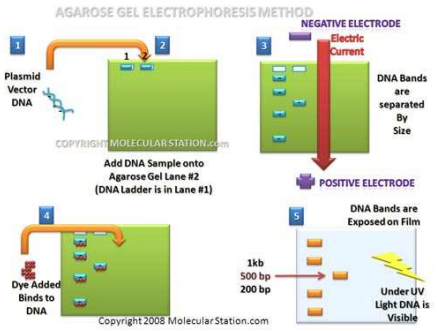
stain:
ethidium bromide

fluorescent intercalator: absorbs light and emits in the visible region (orange); its quantum yield is enhanced when bound to DNA

included in the gel before run, added to the sample in the buffer or after electrophoretic run

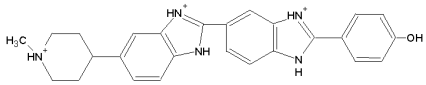
a transiluminator is needed

Electrophoresis on agarose: NA visualization



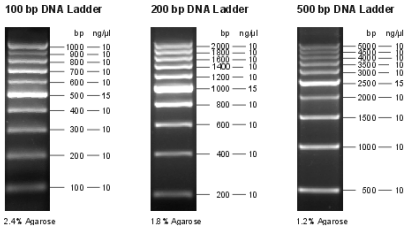
Electrophoresis on agarose: NA visualization

ethidium bromide: carcinogenic, mutagenic

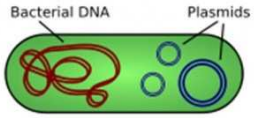


Hoechst 33258

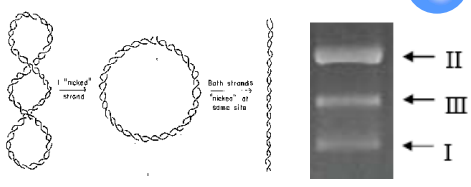
Electrophoresis on agarose: NA quantification



Electrophoresis on agarose: plasmid DNA



Electrophoresis on agarose: plasmid DNA

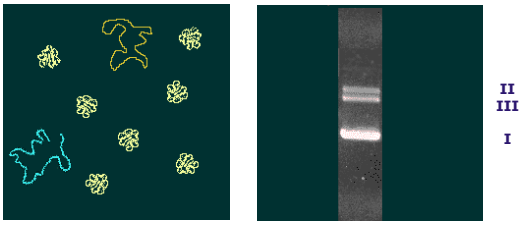


form I supercoiled

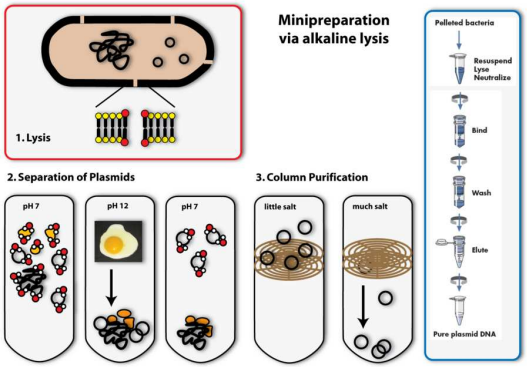
form II relaxed circular

form III linear

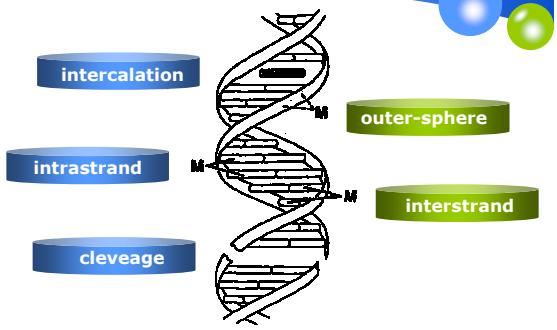
Electrophoresis on agarose: plasmid DNA



Electrophoresis on agarose: plasmid DNA



Electrophoresis on agarose: interaction

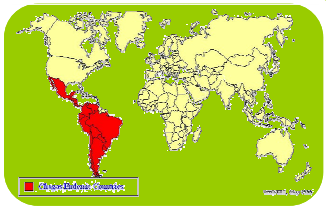


Neglected parasitic diseases

- Malaria
- Schistosomiasis
- Leishmaniasis
- Trypanosomiasis

Chagas disease: American Trypanosomiasis

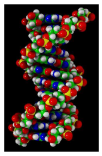
parasite:
Trypanosoma cruzi

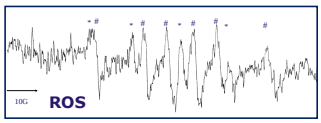


vector:*Triatoma infestans*

Bioactive metal complexes: Mechanism of action

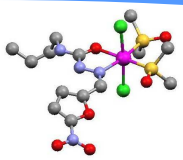
M-bioactive ligand



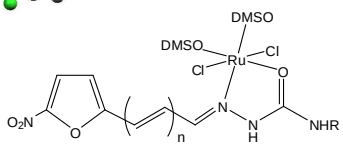


**2 parasitic targets:
dual mechanism of action**

Electrophoresis on agarose: examples



$[Ru^{II}Cl_2(DMSO)_2L]$

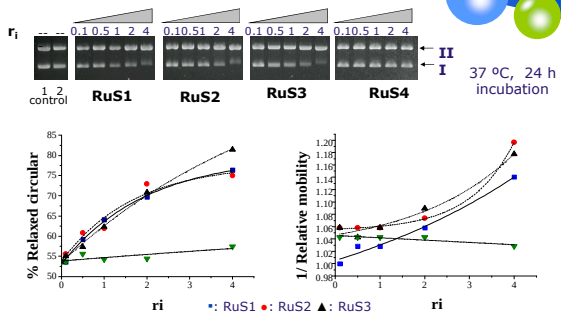


n = 0,1 R = H **RuS1**, **RuS3**
n = 0,1 R = butyl **RuS2**, **RuS4**

Plasmid DNA (pEGFP (Clontech) or
pBSK II BlueScript (Stratagene)

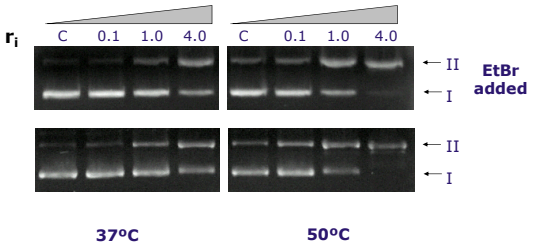
Electrophoresis on agarose: examples

r_i = mol of complex: mol of DNA base pair



Electrophoresis on agarose: examples

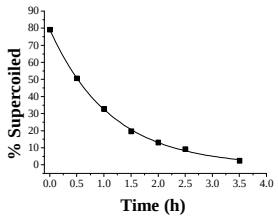
RuS_3

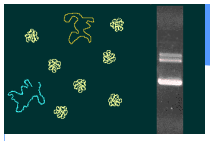


Electrophoresis on agarose: examples

RuS_3 $r_i = 4$

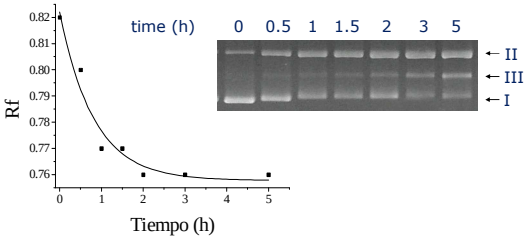
Time course



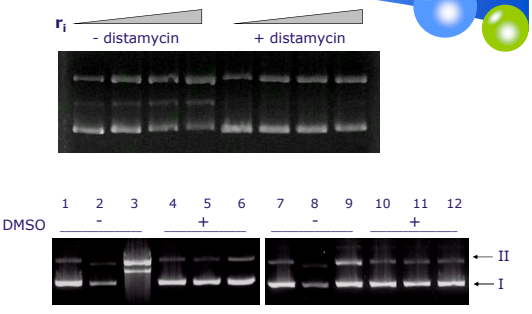


RuS_3

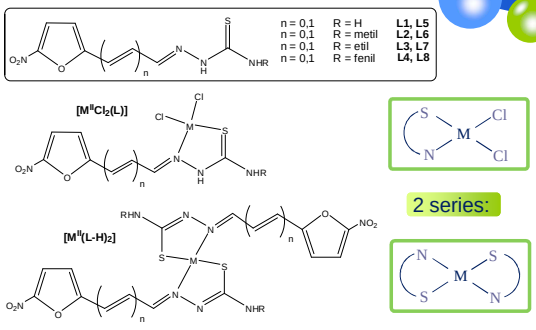
Time course



Electrophoresis on agarose: examples



Electrophoresis on agarose: examples



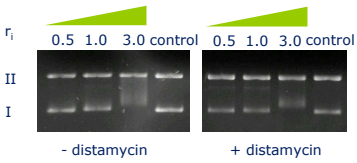
Electrophoresis on agarose: examples

r_i = mol complex/mol base pairs

[$PdCl_2L6$]

dose-dependent diminution of supercoiled DNA mobility

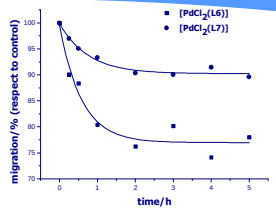
Electrophoresis on agarose: examples



[$PdCl_2L5$]

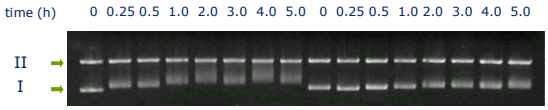
Does not bind in minor groove

Electrophoresis on agarose: examples

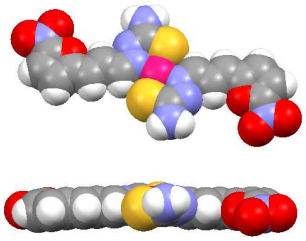


[$PdCl_2L$] complexes show a quick effect on DNA

[$PdCl_2L7$] [$PdCl_2L6$]



Electrophoresis on agarose: examples



[$Pd(L6)_2$]