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Estudio de la interacción de compuestos metálicos con ADN por técnicas de 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 charged 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 or nucleic acid MW

distinguish molecules by their net charge or shape

separate species quantitatively

Electrophoresis

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

electrical force

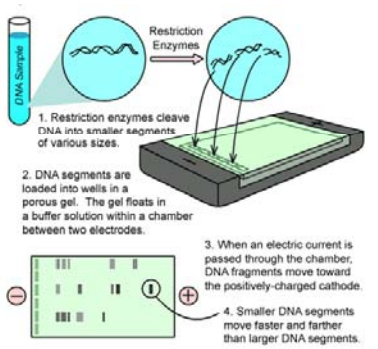
$$Eq = fv$$

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

viscous drag

f frictional coefficient
μ mobility

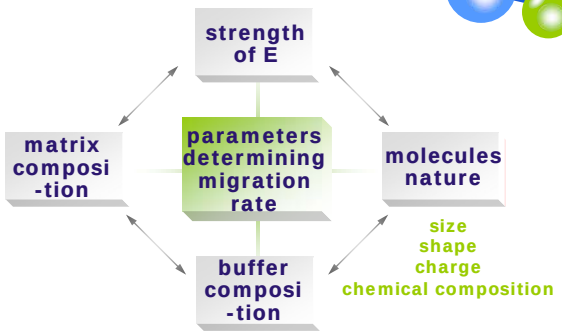
Gel electrophoresis



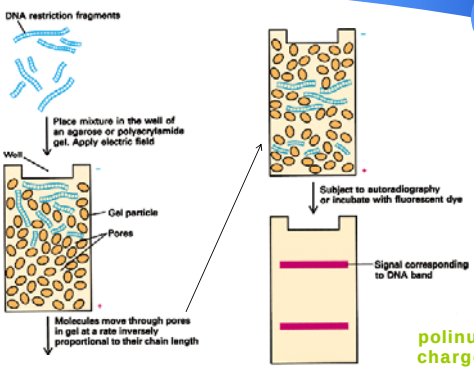
separation of charged molecules through a matrix

matrix: paper, starch, agarose, polyacrylamide

Gel electrophoresis: parameters

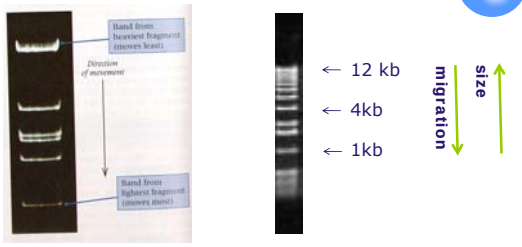


Nucleic acids gel electrophoresis



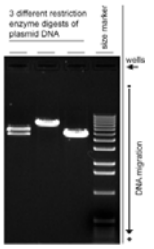
polinucleotides: charge/mass ≅ k

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.

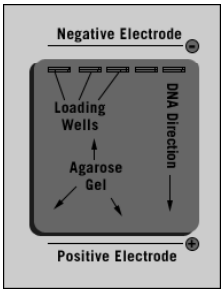
Nucleic acids gel electrophoresis

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

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

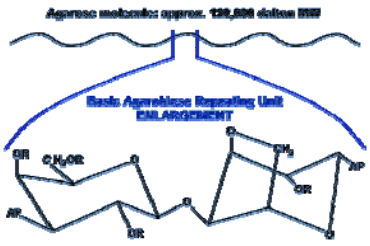
Each end of the gel is in contact with the electrophoresis buffer or the whole gel is immersed in it.

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.



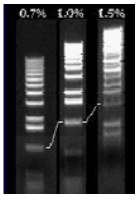
Low melting point (65ºC) agarose beds are used in Molecular Biology experiments for the analysis and separation of DNA fragments and shapes. It allows bands isolation and quantification.

Electrophoresis on agarose: % of agarose

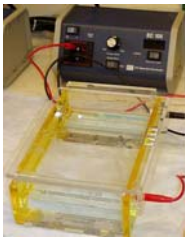
% agarose 0.7-2.0 in buffer (TBE or TAE)

resolution and speed varies with % agarose

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



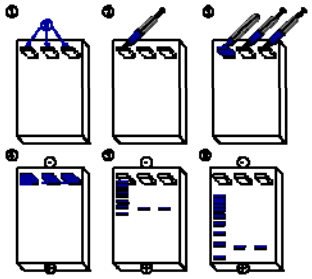
Electrophoresis on agarose gels: technique



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*
glycerol
MW marker

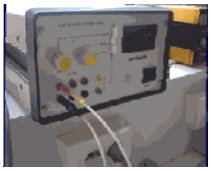


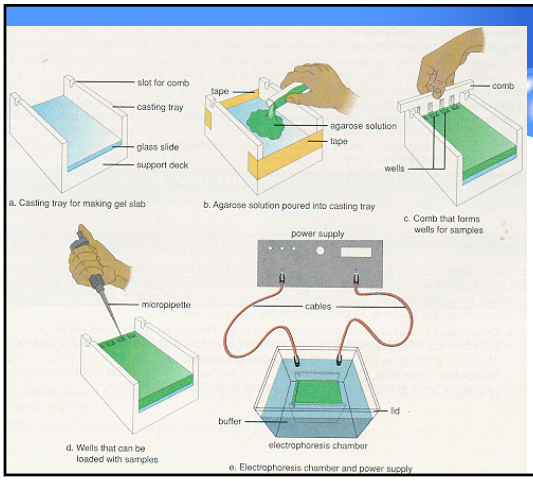
* bromophenol blue (300bp), xylencianol (4kb)

Electrophoresis on agarose: intensity

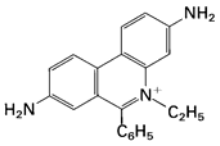
generally: 50-100 V
for a couple of hours

High voltages
diminish resolution.





Electrophoresis on agarose: NA visualization



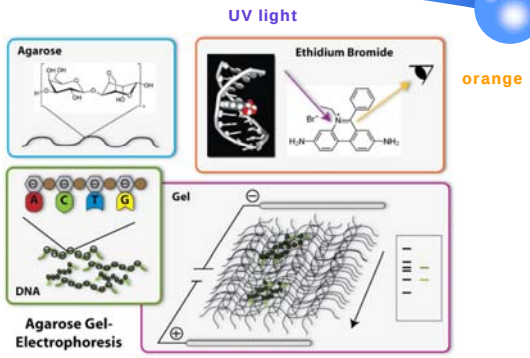
stain:
ethidium bromide

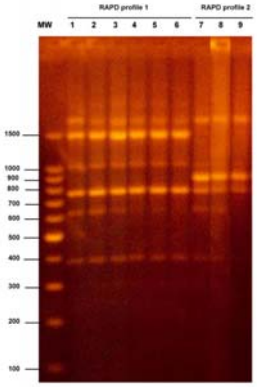
fluorescent intercalator: absorbs UV light and emits in the visible region (orange); its quantum yield is enhanced when bound to DNA

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

a transiluminator and a camera are needed

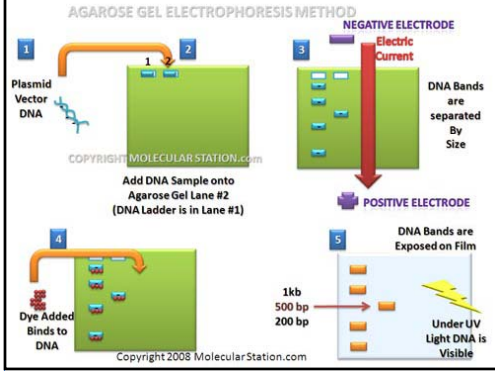
Electrophoresis on agarose: NA visualization





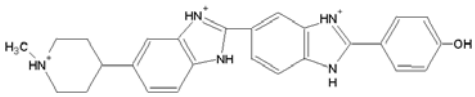
fluorescence shield increases 50 to100 times when bound to DNA

Electrophoresis on agarose: NA visualization



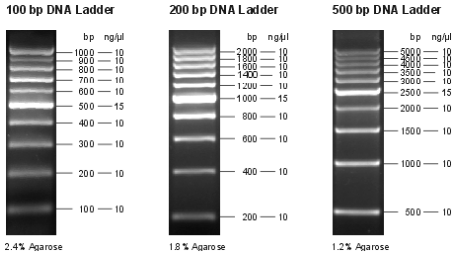
Electrophoresis on agarose: NA visualization

ethidium bromide: carcinogenic, mutagenic

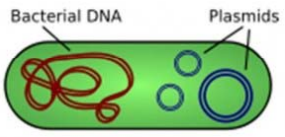


Hoechst 33258

Electrophoresis on agarose: NA quantification

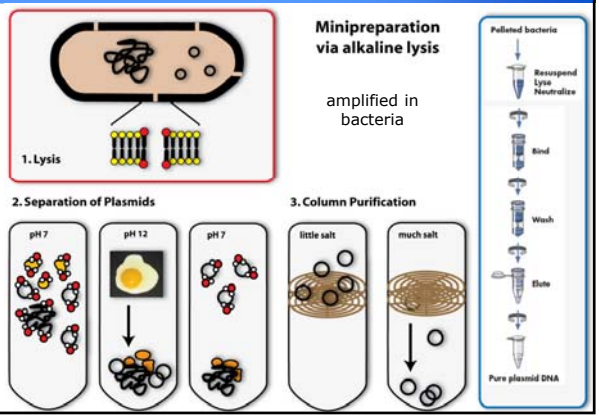


Electrophoresis on agarose: plasmid DNA

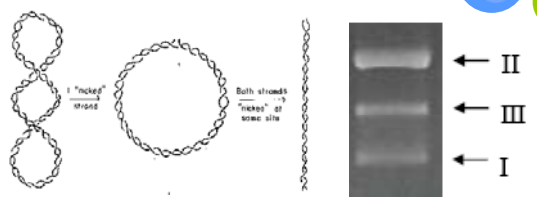


Bacterial non-chromosomal DNA

Electrophoresis on agarose: plasmid DNA



Electrophoresis on agarose: plasmid DNA

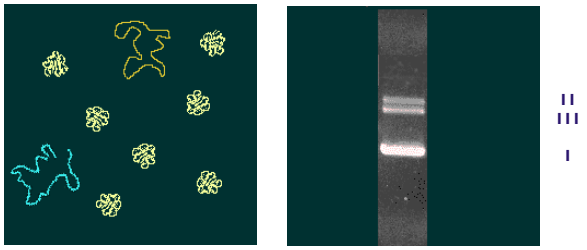


form I Supercoiled (SC)

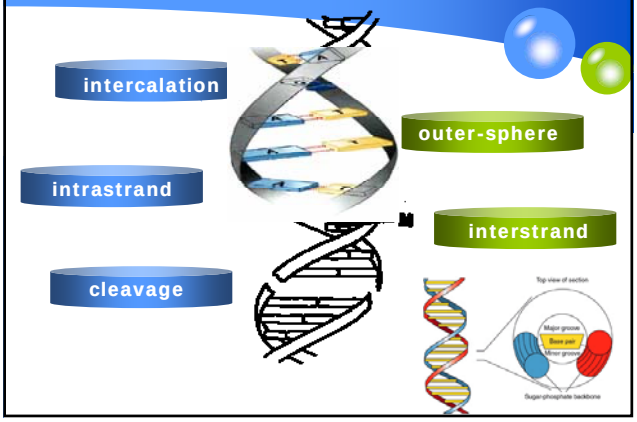
form II relaxed circular (OC)

form III linear

Electrophoresis on agarose: plasmid DNA



Electrophoresis on agarose: interaction



Neglected parasitic diseases

- Malaria
- Schistosomiasis
- Leishmaniasis
- Trypanosomiasis

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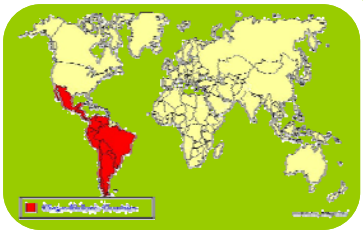
Chagas disease: American Trypanosomiasis

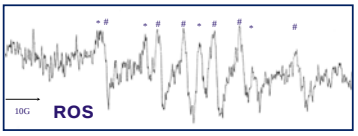
parasite:
Trypanosoma cruzi



vector:*Triatoma infestans*

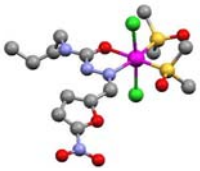
Bioactive metal complexes: Mechanism of action

M-bioactive ligand

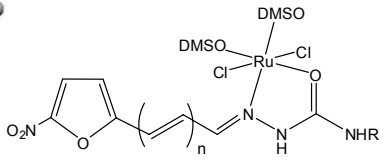


2 parasitic targets:
dual mechanism of action

Electrophoresis on agarose: examples



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



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

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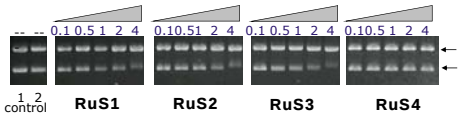
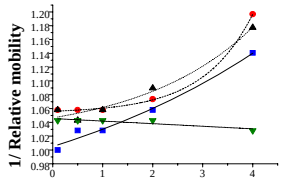
Plasmid DNA (pEGFP (Clontech)
pBSK II BlueScript (Stratagene)

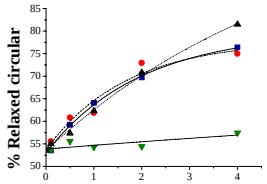
Electrophoresis on agarose: examples

r_i = mol of complex: mol of DNA base pair



37 ºC, 24 h
incubation



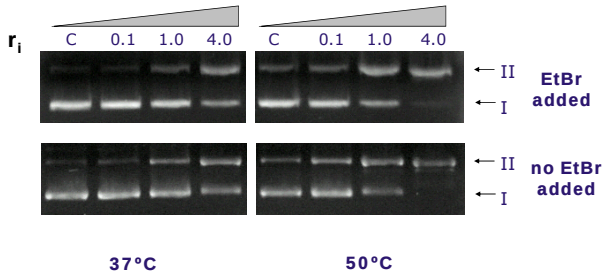


$R_{fi}/ R_{fcontrol}$

▪: RuS1 ●: RuS2 ▲: RuS3

Electrophoresis on agarose: examples

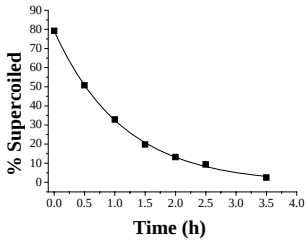
RuS_3

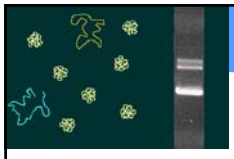


Electrophoresis on agarose: examples

RuS_3 $r_i = 4$

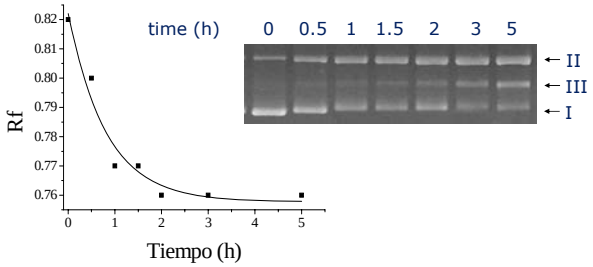
Time course





RuS_3

Time course



Electrophoresis on agarose: examples

Mechanism of cleavage: hydrolytic *vs.* oxidative

Effect of oxidizing agents- oxone (potassium peroxomonosulphate), presence or abscence of O_2

Effect of reducing agents- mercaptopropionic acid

Effect of OH radical scavengers- D-mannitol, ethanol, DMSO, sodium azide

Effect of light- photocleavage

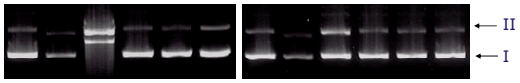
Minor or major groove interaction:

distamycin (binds to minor grove)

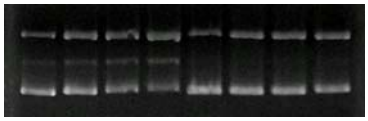
Electrophoresis on agarose: examples

Lanes 2, 5, 8, 11 RuS3
Lanes 3, 6, 9,12 $Fe(EDTA)^{2-}$ /DTT
O_2 Ar

1 2 3 4 5 6 7 8 9 10 11 12
DMSO - + - +



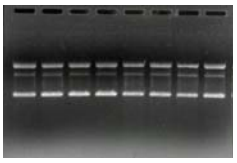
r_i - distamycin + distamycin



binds minor groove

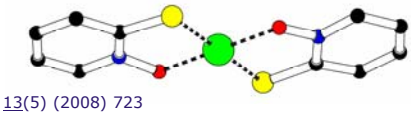
Electrophoresis on agarose: examples

0.5 1.0 2.0 4.0 0.5 1.0 2.0 4.0



Agarose gel electrophoresis (0.7%), plasmid DNA (pBSK II), 24 h incubation at 37ºC, 10 mM Tris-HCl pH 7.4, ri (mol of complex/mol of DNA base pair) 0.5 – 4.0, non ethidium bromide added. Gel was stained after the run.

$[Pd(mpo)_2]$ $[Pt(mpo)_2]$



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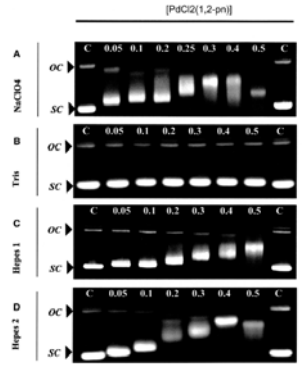
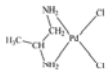
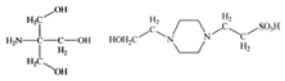
Electrophoresis on agarose: examples







Tris Hepes

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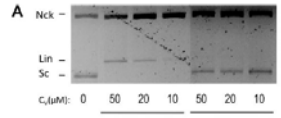
Electrophoresis on agarose: examples



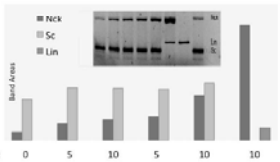
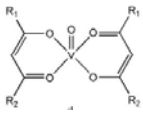
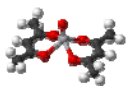


Fig. 4. Effect of the nature of the pH buffer on DNA cleavage by **1**. (A) Incubation under phosphate (PO_4) or hepes buffers and (B) DNA cleavage efficiency of **1** (50 μM) after incubation in solutions buffered with mixtures of phosphate and tris buffers. The inset contains the corresponding agarose gel, which also shows the controls for linear and native DNA on the two rightmost positions.

$[VO(acac)_2]$





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