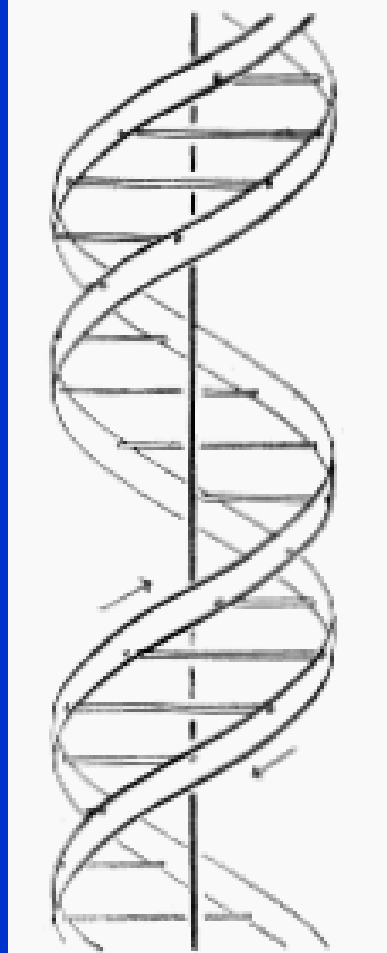


PROGETTO “DNA chiavi in mano”



PROGETTO "DNA chiavi in mano"

no. 4356 April 25, 1953

NATURE
737

MOLECULAR STRUCTURE OF NUCLEIC ACIDS A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (DNA). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey.¹ They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Frazer (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining 5-D-deoxy-ribofuranose residues with 3'-5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions.

Each chain loosely resembles Furburg's model No. 1, that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furburg's standard configuration,² the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 Å. in the α -direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so

that the two lie side by side with identical x - z coordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configuration), it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain, does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain, is given, then the sequence on the other chain is automatically determined.

It has been found experimentally,^{3,4} that the ratio of the amount of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in time following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

¹ Pauling, L., and Corey, R. B. *Nature*, 171, 348 (1953); *Proc. U.S. Nat. Acad. Sci.*, 39, 84 (1953).

² Furburg, G., *Acta Chem. Scand.*, 6, 634 (1952).

³ Chargaff, E., *See references* see Zavadoff, S., Brownstein, O., and Chargaff, E., *Biochem. et Biophys. Acta*, 5, 422 (1952).

⁴ Speyer, C. E., *J. Gen. Physiol.*, 26, 281 (1952).

⁵ Astbury, M. T., *Biophys. J.*, 1, 106 (1952).

⁶ Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, 10, 102 (1953).

This figure is purely diagrammatic. The two ribbons represent the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.



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IFOM



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Finalita'

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graph TD; A[Finalita'] --> B[Stimolare la conoscenza scientifica in campo biologico privilegiando l'approccio laboratoriale]; A --> C[Offrire un'opportunità di aggiornamento teorico e pratico agli studenti del triennio]; A --> D[Avvicinare mondo della ricerca e mondo della scuola]; A --> E[Stimolare l'interesse per le Biotecnologie e per i risvolti bioetici]; A --> F[Attuare azioni di orientamento per gli studenti delle classi quarte e quinte.];
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Stimolare la conoscenza scientifica in campo biologico privilegiando l'approccio laboratoriale

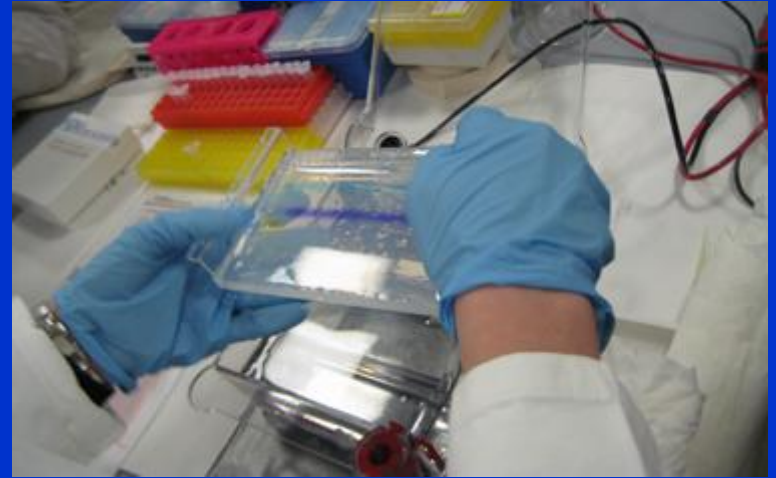
Offrire un'opportunità di aggiornamento teorico e pratico agli studenti del triennio

Avvicinare mondo della ricerca e mondo della scuola

Stimolare l'interesse per le Biotecnologie e per i risvolti bioetici

Attuare azioni di orientamento per gli studenti delle classi quarte e quinte.

Laboratorio Didattico IFOM





VORTEX
per miscelare



MICROPIPETTE
per prelevare



MICROCENTRIFUGA
per separare componenti



TRANSILLUMINATORE
per visualizzare il DNA su
gel di agarosio

I NOSTRI STRUMENTI



TERMOCICLATORE
per amplificare sequenze
di DNA



FORNO MICROONDE
per riscaldare



CELLA ELETTROFORETICA
e **GENERATORE DI CORRENTE**
per la corsa elettroforetica

Al lavoro nel nostro laboratorio



PROGETTO “DNA chiavi in mano”

Attività laboratoriale

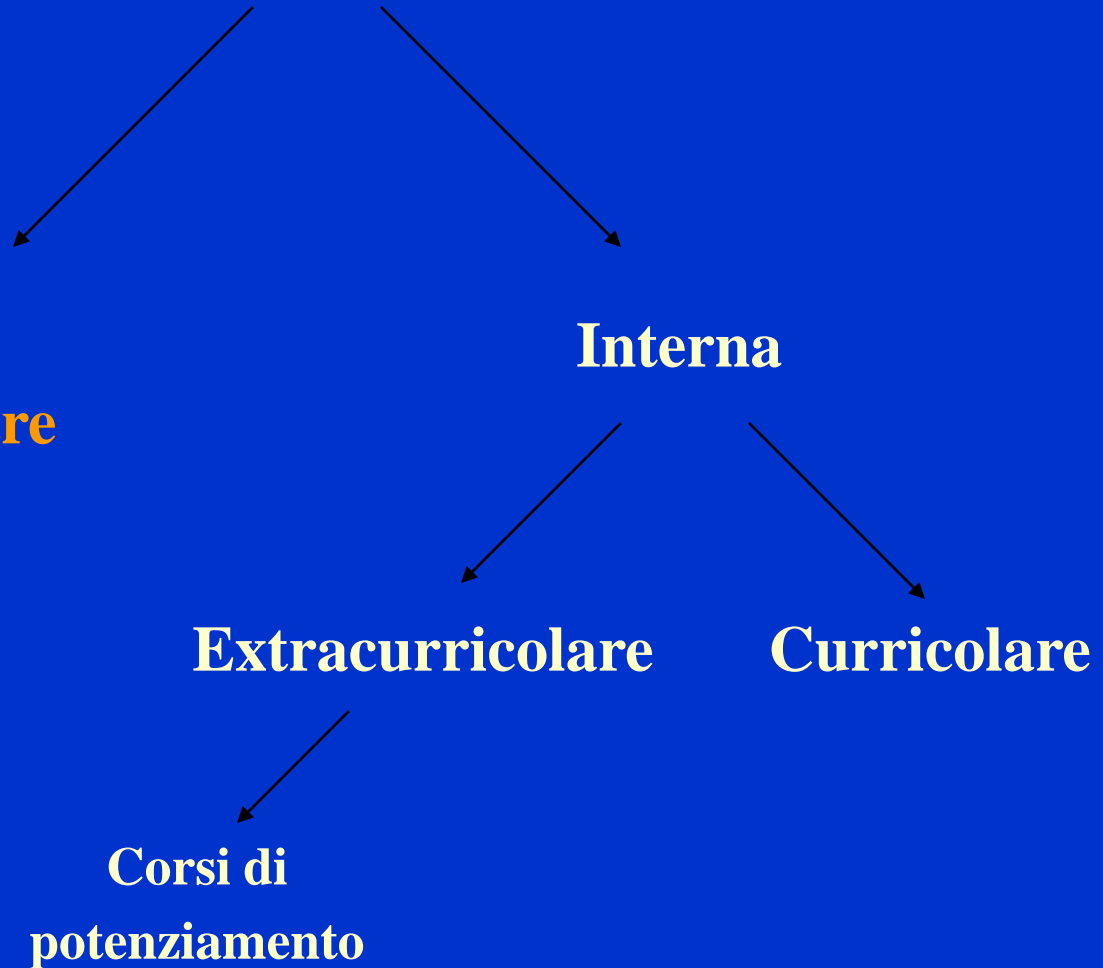
Apertura del
laboratorio ad altre
scuole

Interna

Extracurricolare

Curricolare

Corsi di
potenziamento



Protocolli sperimentali

- ESTRAZIONE DI gDNA DALLA MUCOSA BOCCALE
- DIGESTIONE ENZIMATICA DI DNA VIRALE
- VISUALIZZAZIONE DEI FRAMMENTI DI RESTRIZIONE TRAMITE
CORSO ELETTROFORETICA
- AMPLIFICAZIONE DI UNA SEQUENZA DI DNA TRAMITE PCR

ELETTROFORESI

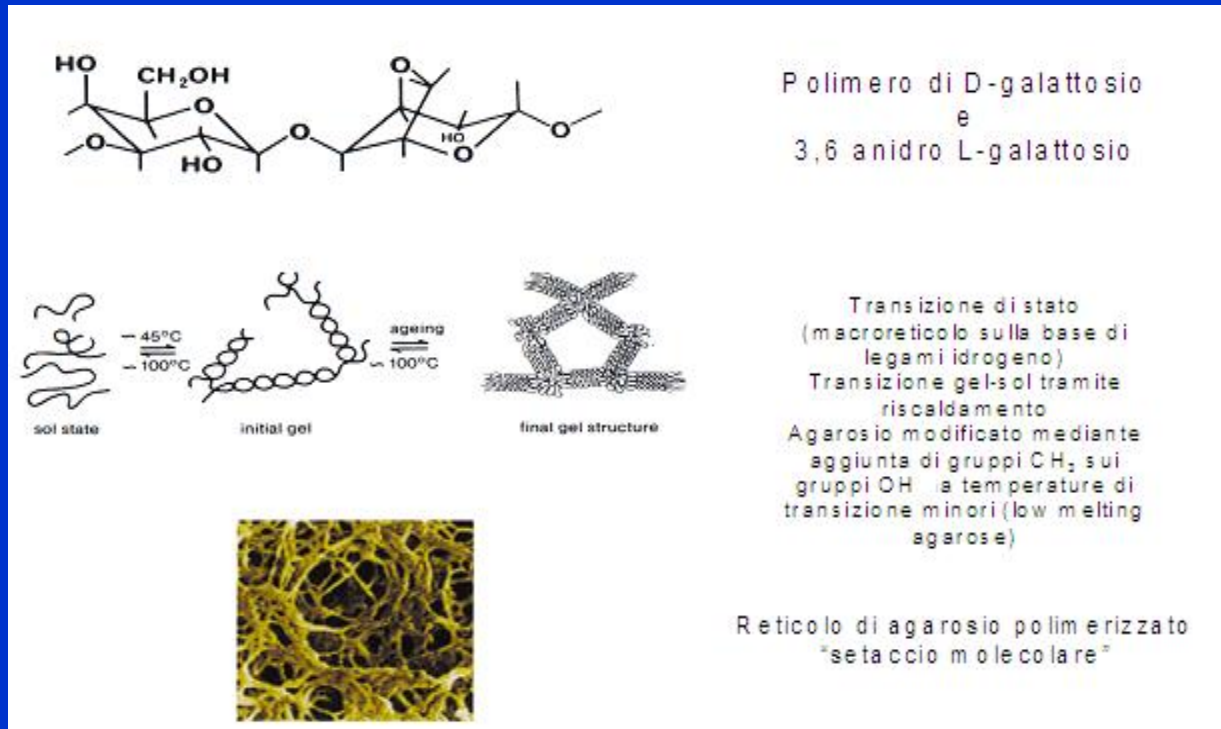
La tecnica ci permetterà di evidenziare il DNA utilizzando

- la forza di un campo elettrico
- il “supporto” di un gel di agarosio



GEL di AGAROSIO

L'**agarosio** è un polimero naturale di galattosio e 3,6 anidro-galattosio. Viene sciolto in opportuno tampone (TBE o TAE) e fatto gelificare su un supporto di vetro o di plastica.



Ecco cosa fare.....



Versa l'agarosio nel TAE

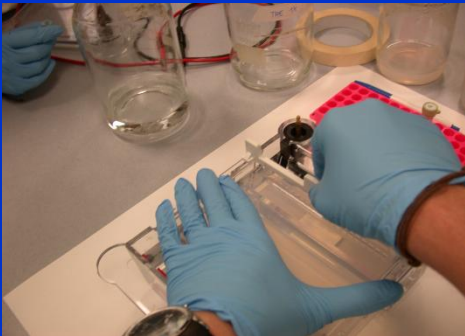


Scalda

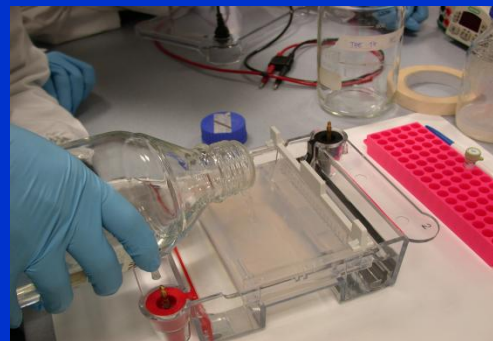


Aggiungi il Sybr Safe

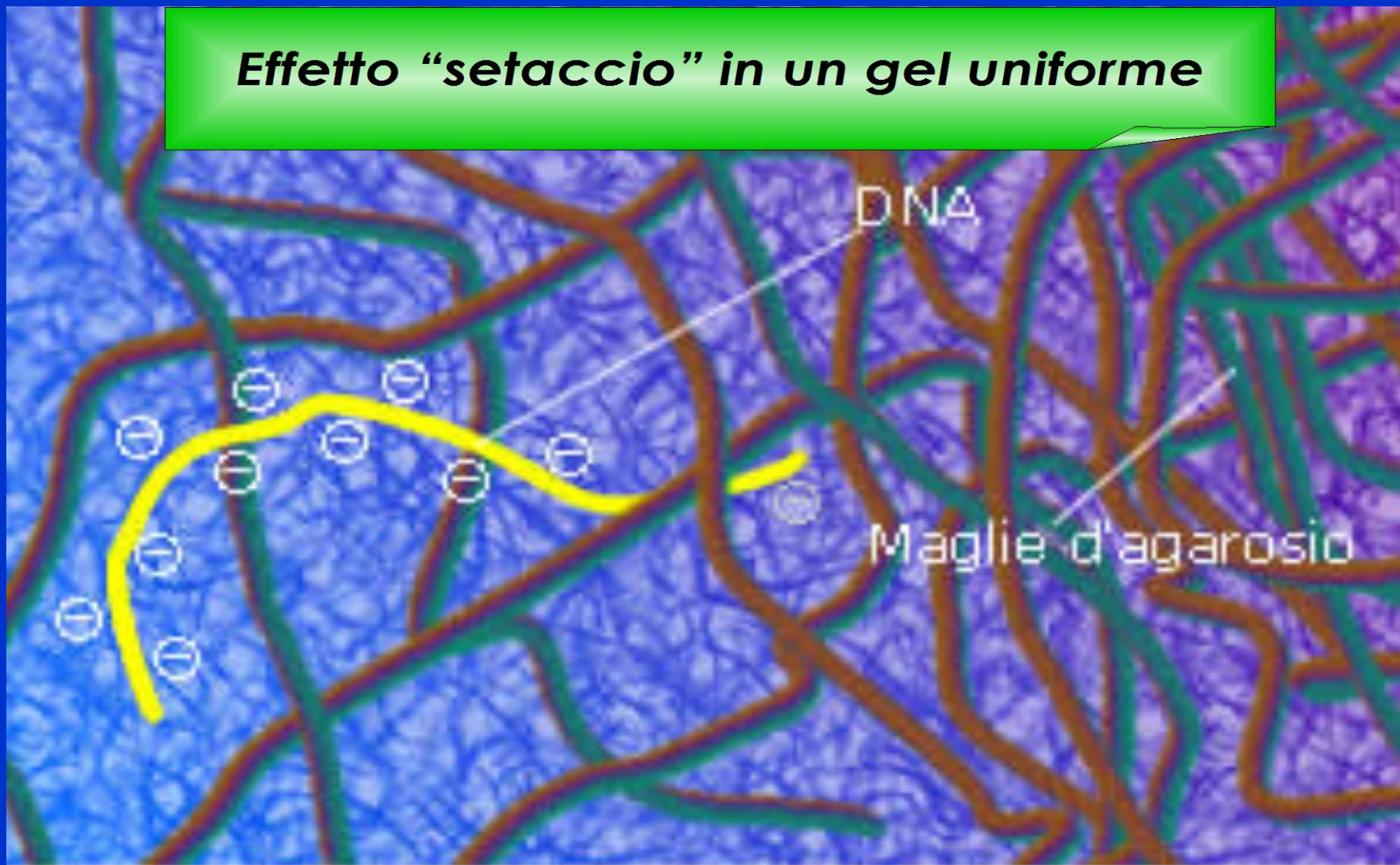
Inserisci il pettine



Versa l'agarosio nella slitta



Effetto "setaccio" in un gel uniforme

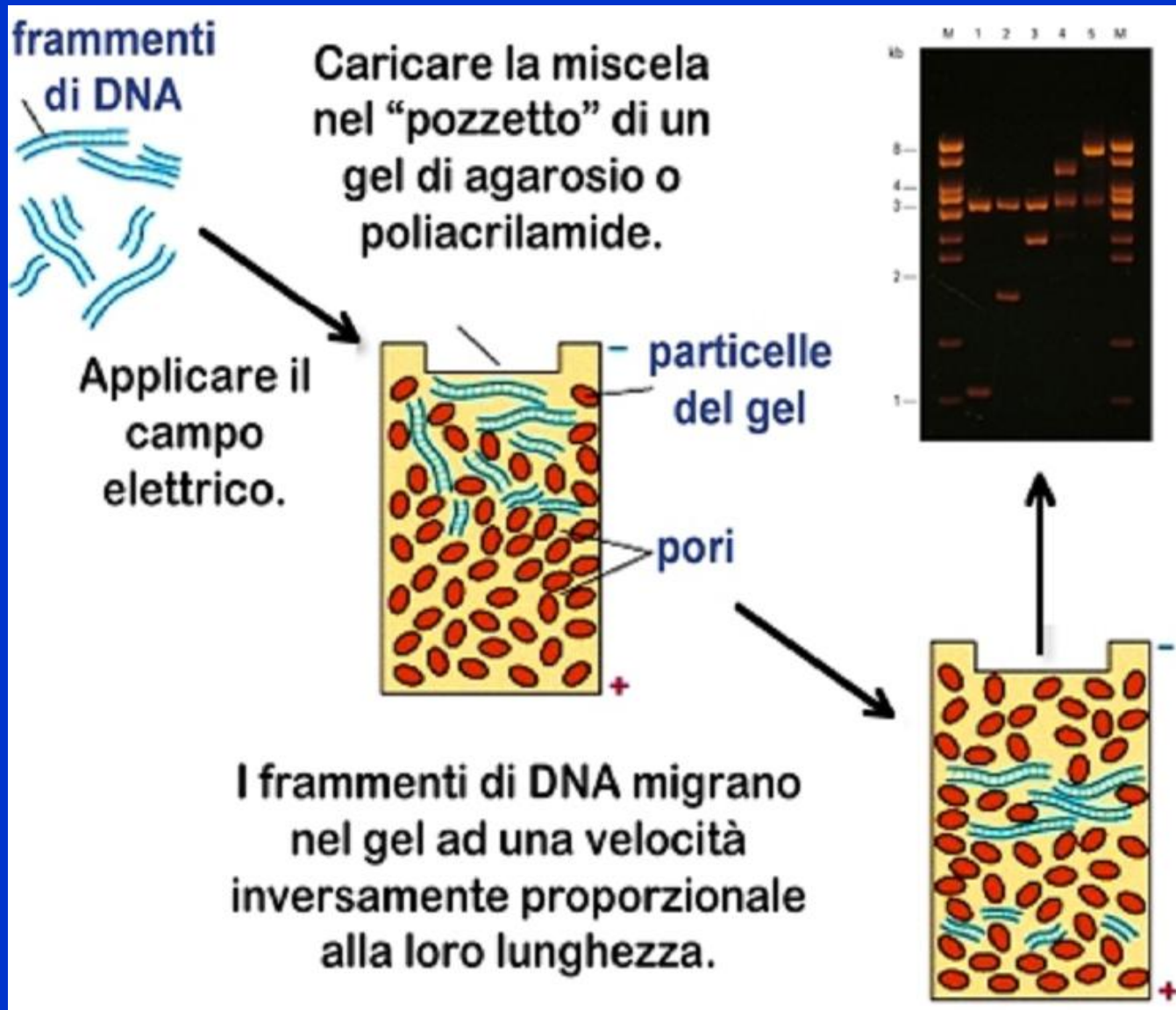


Campo elettrico

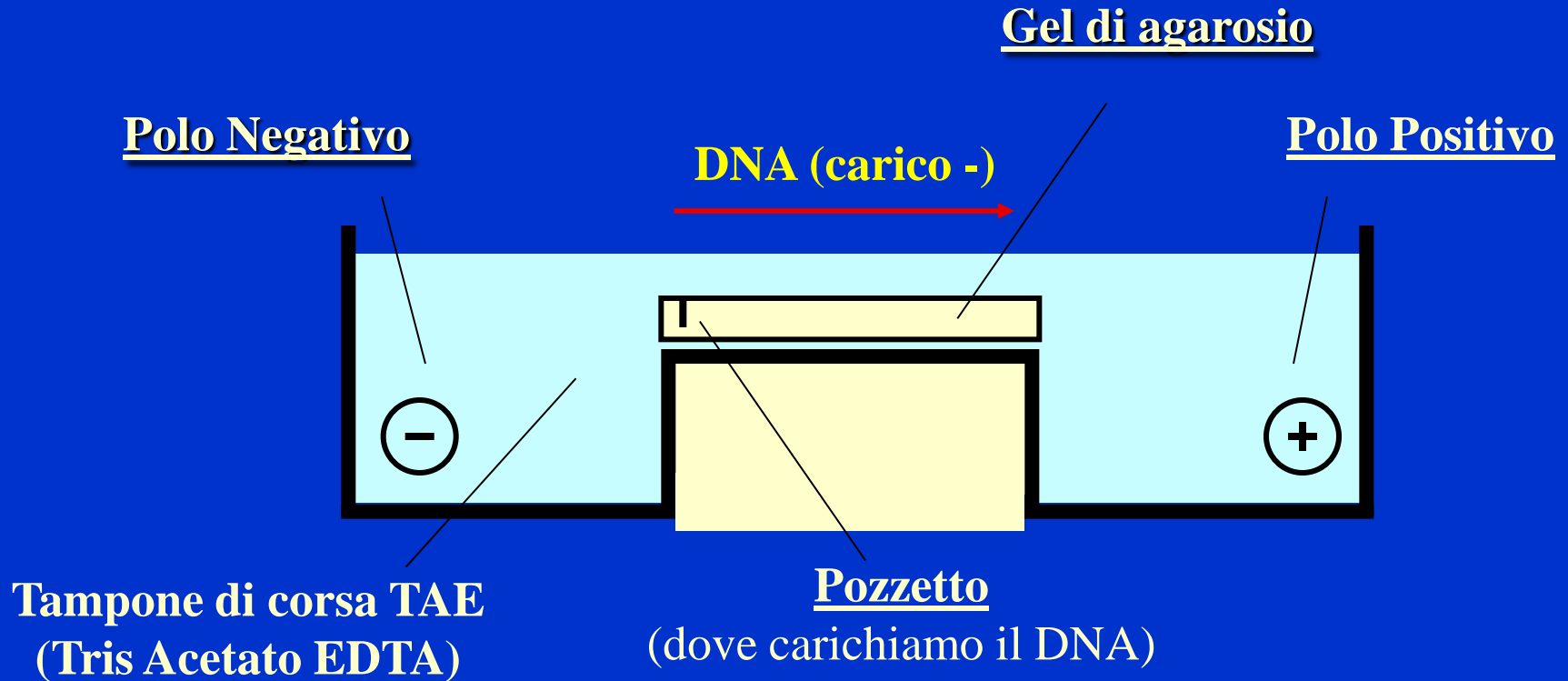
-

+

La separazione del DNA

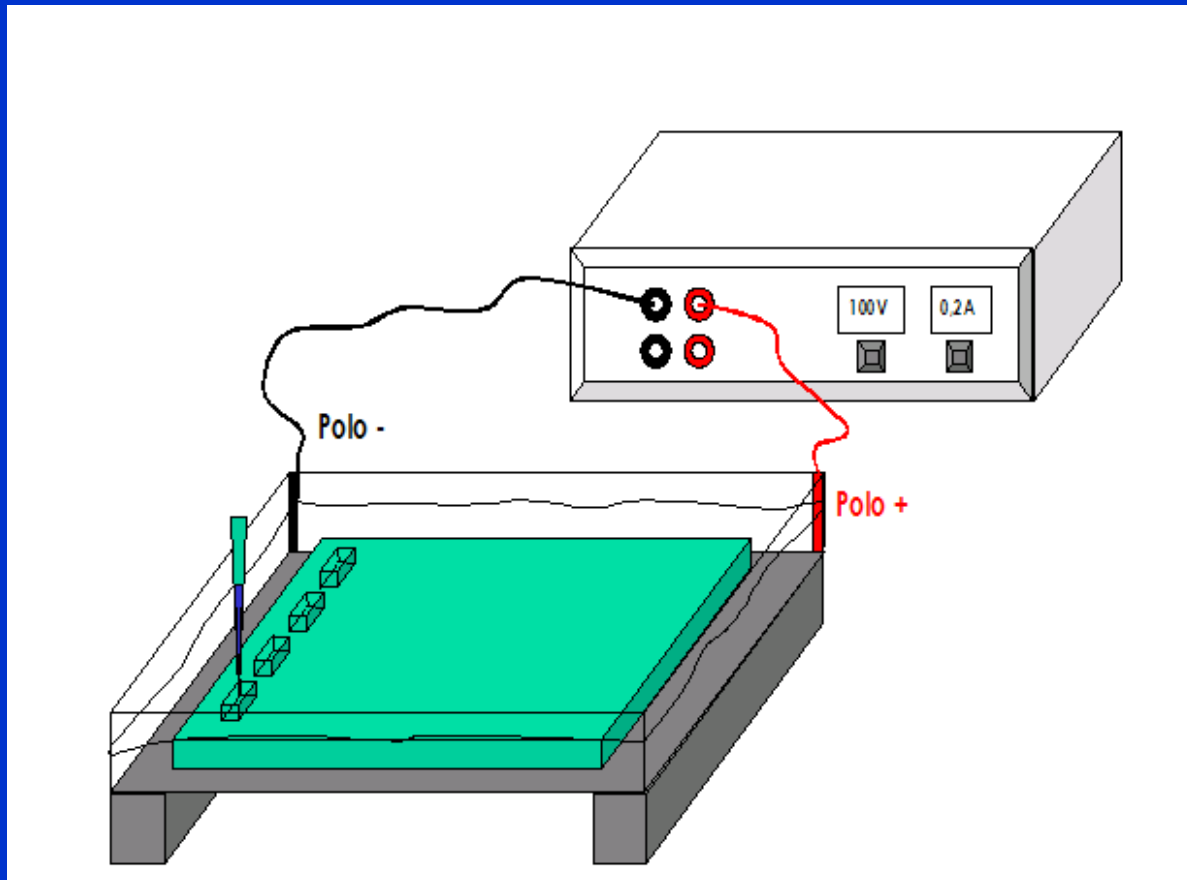


Il campo elettrico

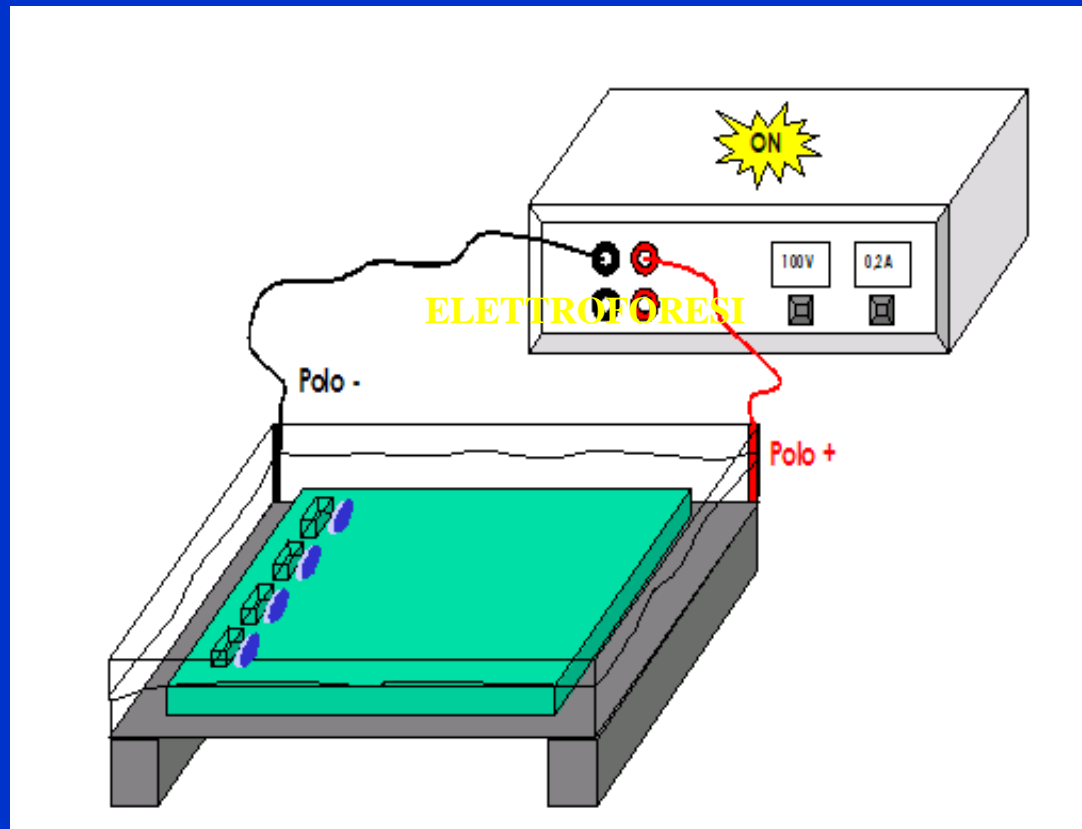


TAE: il Tris consente il mantenimento di un valore costante di pH della soluzione; l'acido Acetico fornisce l'appropriata forza ionica al tampone; l'EDTA chela i cationi bivalenti, come il magnesio (la maggior parte delle nucleasi richiede cationi bivalenti per funzionare).

ELETTROFORESI



ELETTROFORESI



BPB e XC

E' possibile visualizzare la corsa elettroforetica grazie a coloranti inerti:

- Blu di bromofenolo**
- Xilene cianolo**

che si muovono nel campo elettrico come molecole a PM noto, in relazione alla concentrazione del gel.

1%

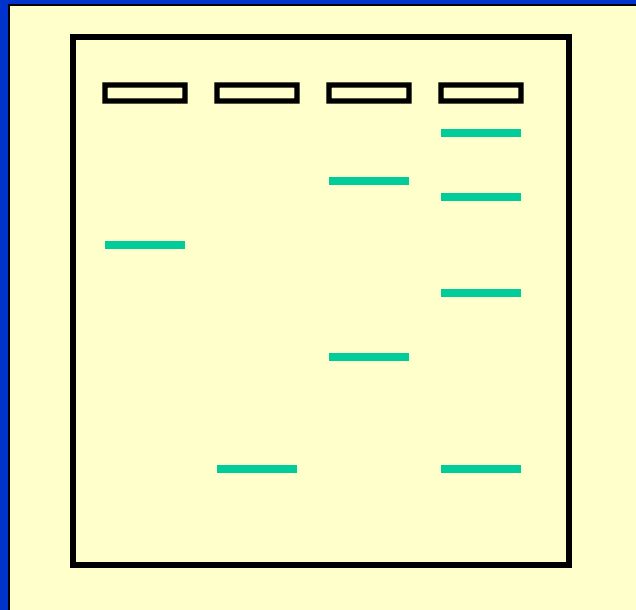
BPB 300 bp

XC 4.000 bp

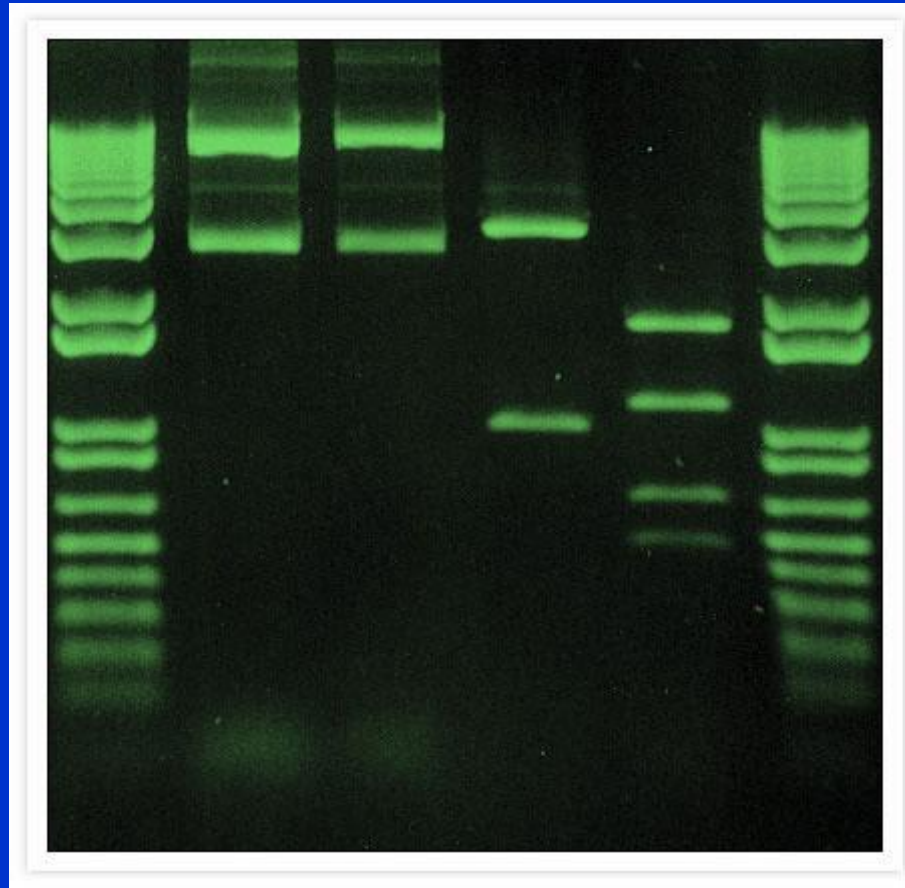
Il Syber Safe



Il DNA viene visualizzato nel gel usando un intercalante, il **Sybr Safe**, che emette luce verde sotto illuminazione con luce blu



Le bande di DNA



Una **banda** rappresenta un'area occupata da una popolazione di molecole distinguibile da un'altra area.

Ecco cosa fare.....

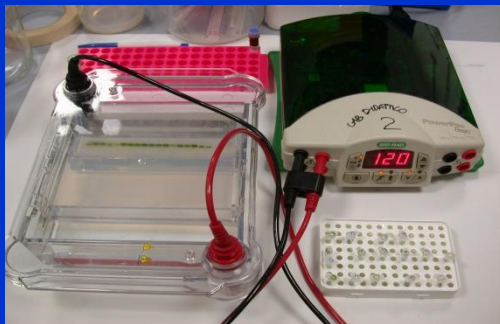
Aggiungi il Loading Dye



Carica i campioni



Inizia la corsa



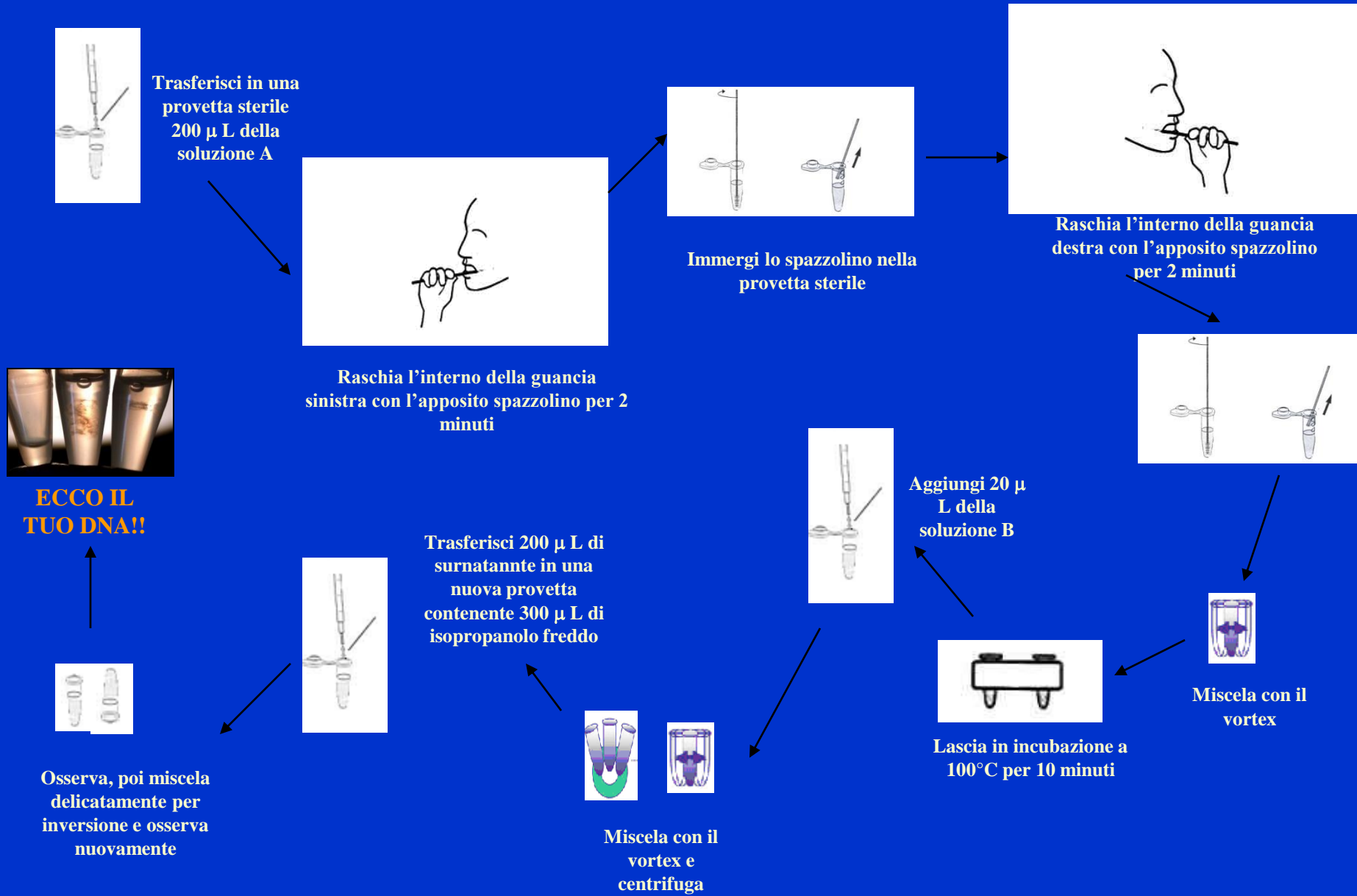
Osserva il gel



Protocolli sperimentali

- *ESTRAZIONE* *DI gDNA DALLA MUCOSA BOCCALE*
- *DIGESTIONE ENZIMATICA* *DI DNA VIRALE*
- *VISUALIZZAZIONE DEI FRAMMENTI DI RESTRIZIONE TRAMITE*
CORSA ELETTROFORETICA
- *AMPLIFICAZIONE DI UNA SEQUENZA DI DNA TRAMITE* *PCR*

Estrazione del DNA da cellule della mucosa boccale

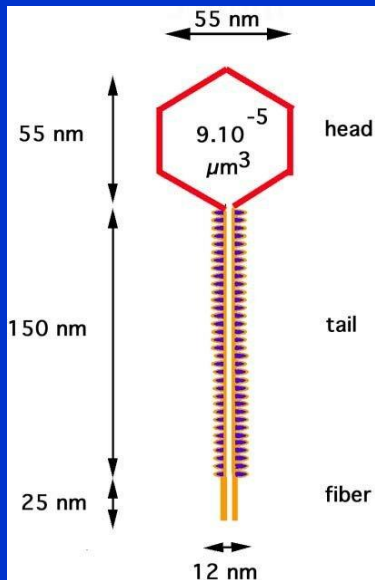


Protocolli sperimentali

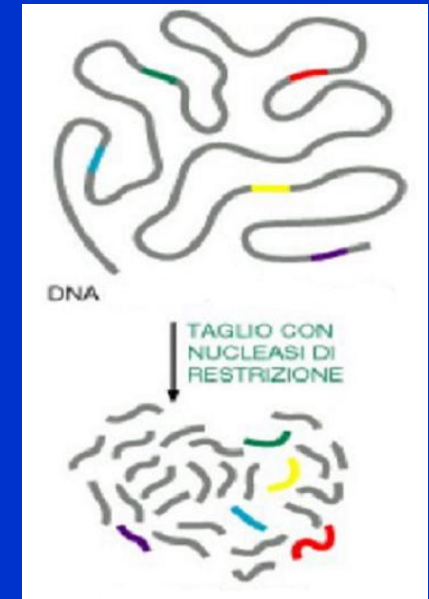
- ESTRAZIONE DI gDNA DALLA MUCOSA BOCCALE
- DIGESTIONE ENZIMATICA DI DNA VIRALE
- VISUALIZZAZIONE DEI FRAMMENTI DI RESTRIZIONE TRAMITE
CORSO ELETTROFORETICA
- AMPLIFICAZIONE DI UNA SEQUENZA DI DNA TRAMITE PCR

Digestione enzimatica ed elettroforesi

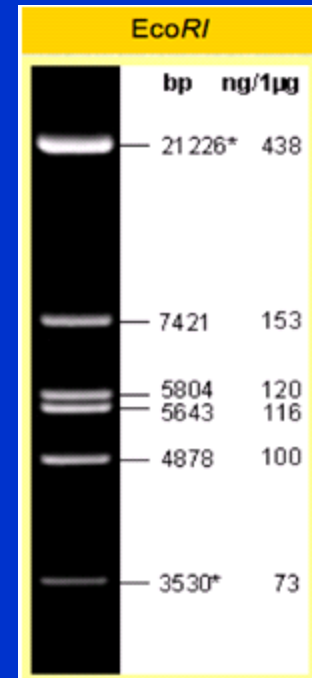
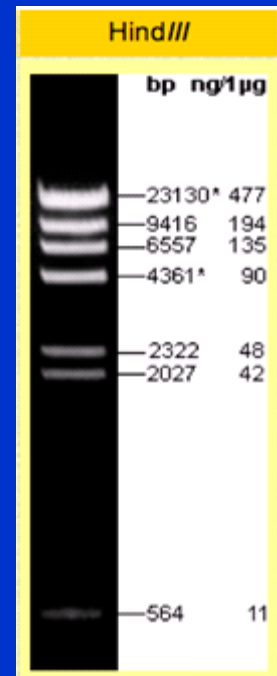
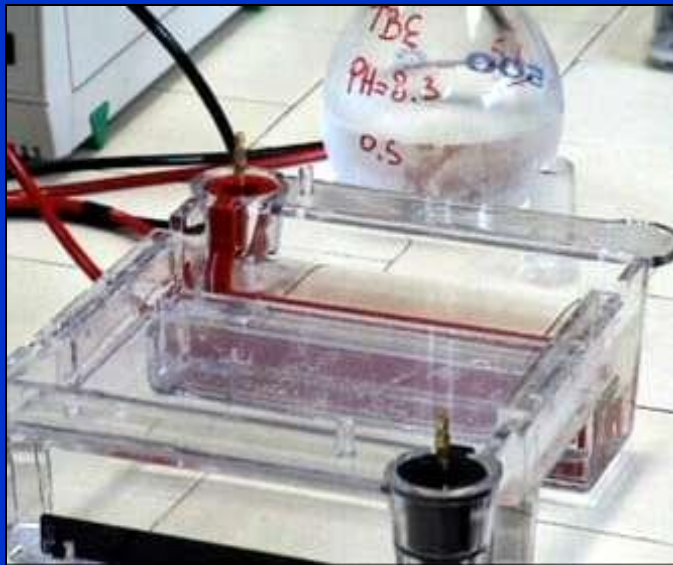
Digestione enzimatica del DNA del Fago Lambda



Enzima	Organismo di origine	Sequenza consenso	Taglio
EcoRI	<i>Escherichia coli</i>	5'---GAATTC---3' 3'---CTTAAG---5'	5'---G AATTC---3' 3'---CTTAA G---5'
BamHI	<i>Bacillus amyloliquefaciens</i>	5'---GGATCC---3' 3'---CCTAGG---5'	5'---G GATCC---3' 3'---CCTAG G---5'
HindIII	<i>Haemophilus influenzae</i>	5'---AAGCTT---3' 3'---TTCGAA---5'	5'---A AGCTT---3' 3'---TTCGA A---5'
TaqI	<i>Thermus aquaticus</i>	5'---TCGA---3' 3'---AGCT---5'	5'---T CGA---3' 3'---AGC T---5'
AluI	<i>Arthrobacter luteus</i>	5'---AGCT---3' 3'---TCGA---5'	5'---AG CT---3' 3'---TC GA---5'



Corsa elettroforetica dei frammenti di restrizione



Protocolli sperimentali

- ESTRAZIONE DI gDNA DALLA MUCOSA BOCCALE
- DIGESTIONE ENZIMATICA DI DNA VIRALE
- VISUALIZZAZIONE DEI FRAMMENTI DI RESTRIZIONE TRAMITE CORSA ELETTROFORETICA
- AMPLIFICAZIONE DI UNA SEQUENZA DI DNA TRAMITE PCR

La PCR (Polymerase Chain Reaction)

- fu inventata nel 1983 da Mullis
- permette di amplificare una sequenza di DNA (un gene o parte di esso) *in vitro*, cioè in una provetta, aggiungendo una serie di reagenti e utilizzando un termociclatore



<http://www.youtube.com/watch?v=JRAA4C2OPwg>

Che cosa serve per fare una PCR?

DNA che funge da “stampo”

Nucleotidi precursori (dNTPs: dATP, dCTP, dGTP, dTTP)

Buffer o Tampone di reazione (fornisce la giusta concentrazione di Sali, tra cui il Magnesio Cloruro e il giusto pH)

Primers Forward e Reverse

DNA polimerasi, enzima che utilizza i nucleotidi precursori per copiare un DNA “stampo” e generarne nuove copie

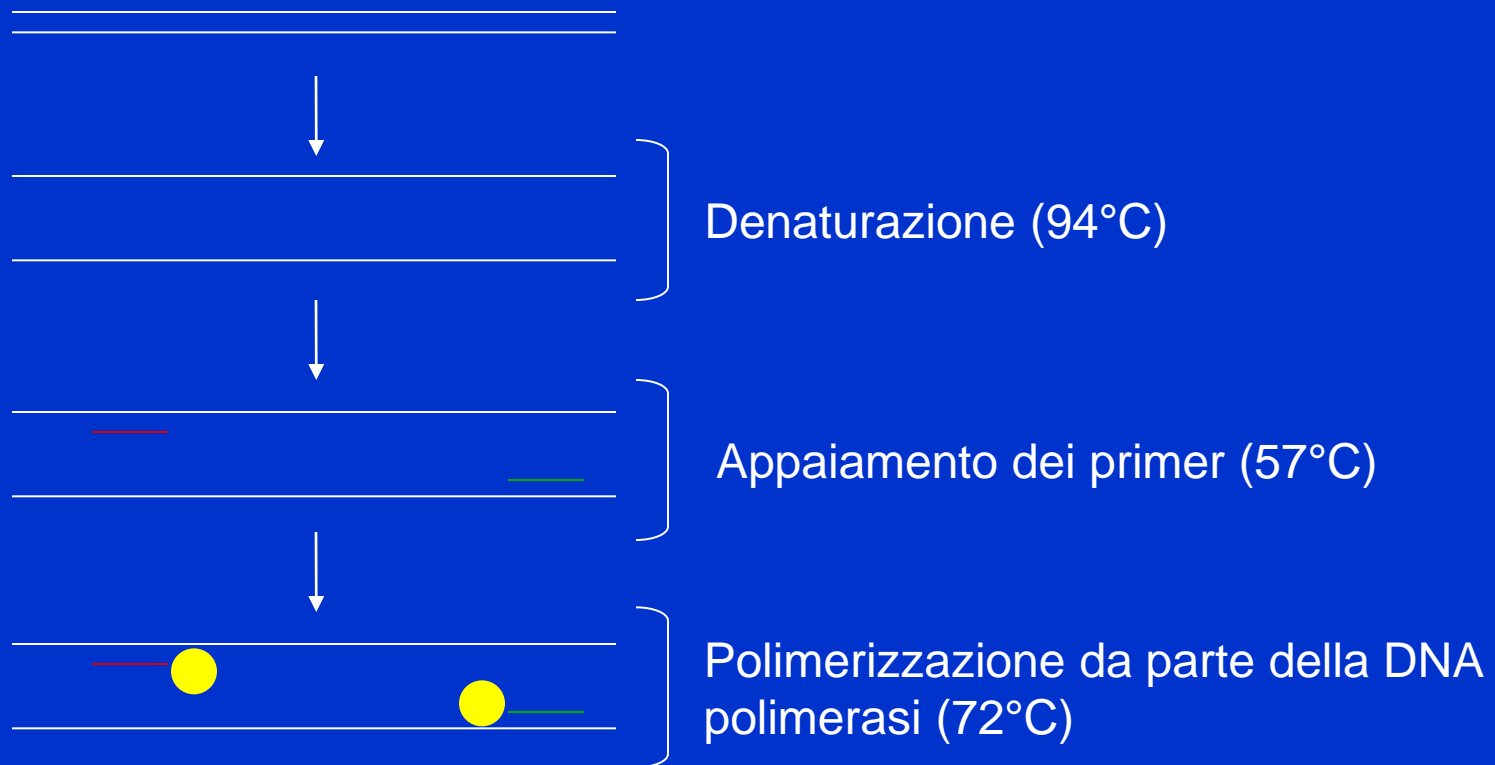
La nostra reazione di PCR

- DNA : porzione del gene umano **SRY**
- Primers: 5' GAATATTCCCGCTCTCCGGA 3'
5' GCTGGTGCTCCATTCTTGAG 3'
- Taq polimerasi

La “nostra” PCR

Il termociclatore effettua 35 cicli di

Denaturazione-Appaiamento-Polimerizzazione



Il gene SRY

FONTE UTILIZZATA:



National Center for biotechnology Information

<http://www.ncbi.nlm.nih.gov/>

□ 1: SRY sex determining region Y [*Homo sapiens*]

GeneID: 6736

updated 04-Jan-2009

Summary



Official Symbol **SRY**

provided by [HGNC](#)

Official Full Name sex determining region Y

provided by [HGNC](#)

Primary source [HGNC:11311](#)

See related [Ensembl:ENSG00000184895](#); [HPRD:08364](#); [MIM:480000](#)

Gene type protein coding

RefSeq status REVIEWED

Organism [Homo sapiens](#)

Lineage *Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo*

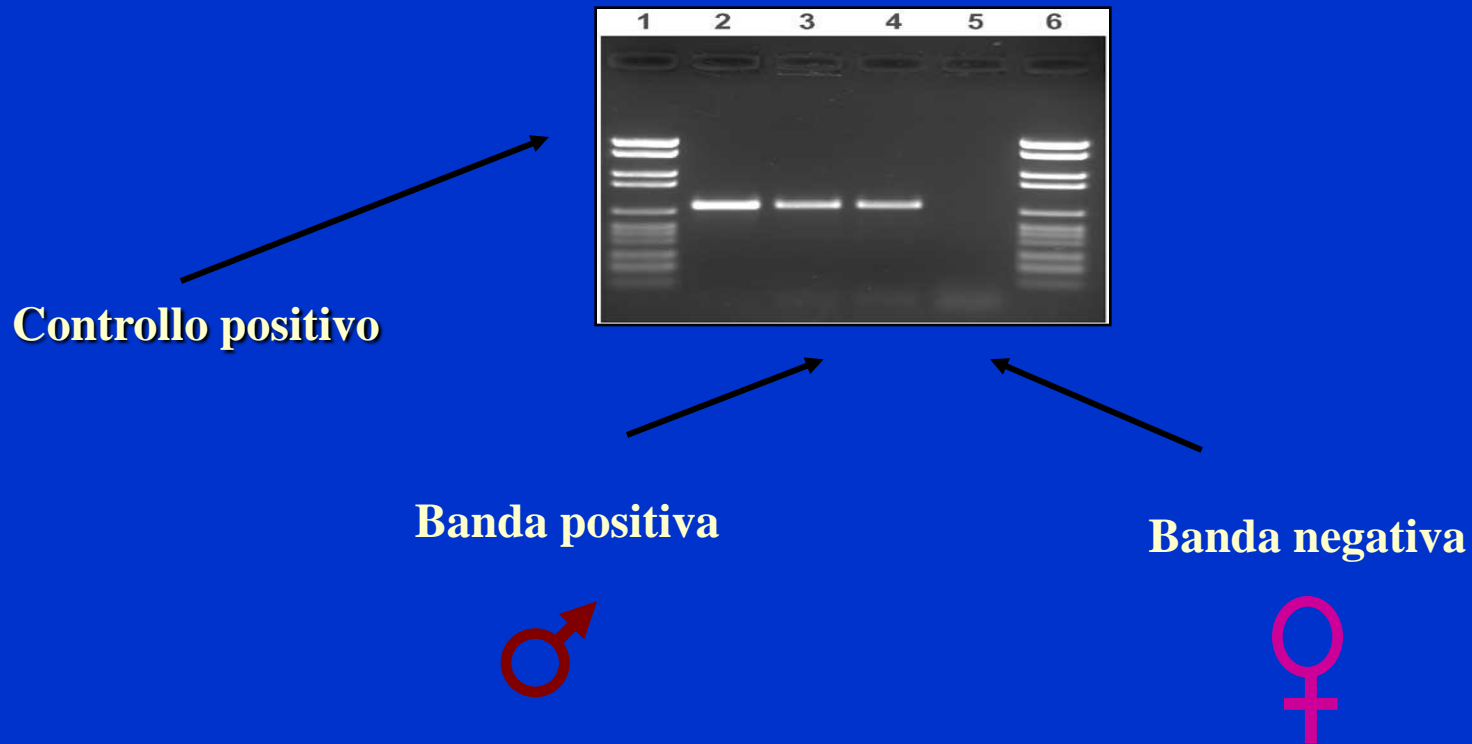
Also known as TDF; TDY; SRY

Summary

Questo gene privo di introni codifica un fattore di trascrizione che è il “fattore di determinazione testicolare” (TDF testis determining factor), che inizia la determinazione sessuale maschile.

Mutazioni in questo gene danno origine a individui femminili XY con disgenesi gonadica (sindrome di Swyer); traslocazioni di parte del cromosoma Y contenete questo gene sul cromosoma X causa individui maschili XX

Dopo la corsa elettroforetica...



Colleghe e Colleghi,

che ne pensate?

Vi interessa il nostro progetto?

Contattatemi!

paolaluisella@hotmail.com