1° settimana	20 - 24 febbraio 2017				
	Lunedì 20	Martedì 21	Mercoledì 22	Giovedì 23	Venerdì 24
08.30 - 09.30		Tecnologie ricombinanti (Prof. D.Balestra) Aula Boeri	Lab. di Biostatistica, Lab. Multimediale (Prof. A. Carrieri)	Tecnologie ricombinanti (Prof. D.Balestra) Aula Boeri	Ecologia Applicata (Prof. Castaldelli) Aula Boeri
09.30 -10.30		Lab. di Biostatistica, Lab. Multimediale (Prof. A. Carrieri)	Lab. di Biostatistica, Lab. Multimediale (Prof. A. Carrieri)	Tecnologie ricombinanti (Prof. D.Balestra) Aula Boeri	Ecologia Applicata (Prof. Castaldelli) Aula Boeri
10.30 - 11.30		Lab. di Biostatistica, Lab. Multimediale (Prof. A. Carrieri)	Tecnologie ricombinanti (Prof. D.Balestra) Aula Boeri	Biologia Cellulare Vegetale (Prof. L. Ferroni) aula Boeri Dip SVeB	Genetica ed Evoluzione (Prof. S. Ghirotto) Aula 11
11.30 - 12.30	Biologia Cellulare Vegetale (Prof. L. Ferroni) aula Boeri Dip SVeB	Adattamento dei vegetali all'ambiente (Prof. M. Giovanardi) Aula 11 Dip. SVeB	Adattamento dei vegetali all'ambiente (Prof. M. Giovanardi) Aula 11 Dip. SVeB	Biologia Cellulare Vegetale (Prof. L. Ferroni) aula Boeri Dip SVeB	Genetica ed Evoluzione (Prof. S. Ghirotto) Aula 11
12.30 - 13.30	Biologia Cellulare Vegetale (Prof. L. Ferroni) aula Boeri Dip SVeB	Adattamento dei vegetali all'ambiente (Prof. M. Giovanardi) Aula 11 Dip. SVeB	Adattamento dei vegetali all'ambiente (Prof. M. Giovanardi) Aula 11 Dip. SVeB		
PAUSA PRANZO					
14.30 - 15.30		Ecologia Vegetale (Prof. L. Bragazza) Aula Boeri	Ecologia Applicata (Prof. Castaldelli) Aula Boeri	Genetica ed Evoluzione (Prof. S. Ghirotto) Aula 11	
15.30 - 16.30		Ecologia Vegetale (Prof. L. Bragazza) Aula Boeri	Ecologia Applicata (Prof. Castaldelli) Aula Boeri	Genetica ed Evoluzione (Prof. S. Ghirotto) Aula 11	
16.30 - 17.30		Ecologia Vegetale (Prof. L. Bragazza) Aula Boeri	Ecologia Vegetale (Prof. L. Bragazza) Aula Boeri		
17.30 - 18.30		Ecologia Vegetale (Prof. L. Bragazza) Aula Boeri	Ecologia Vegetale (Prof. L. Bragazza) Aula Boeri		

Tipo Esame: scritto con domande aperte

Email: blsdra@unife.it

Orario ricevimento: Lunedì e Martedì dalle 12 alle 13, previo appuntamento

TESTI CONSIGLIATI

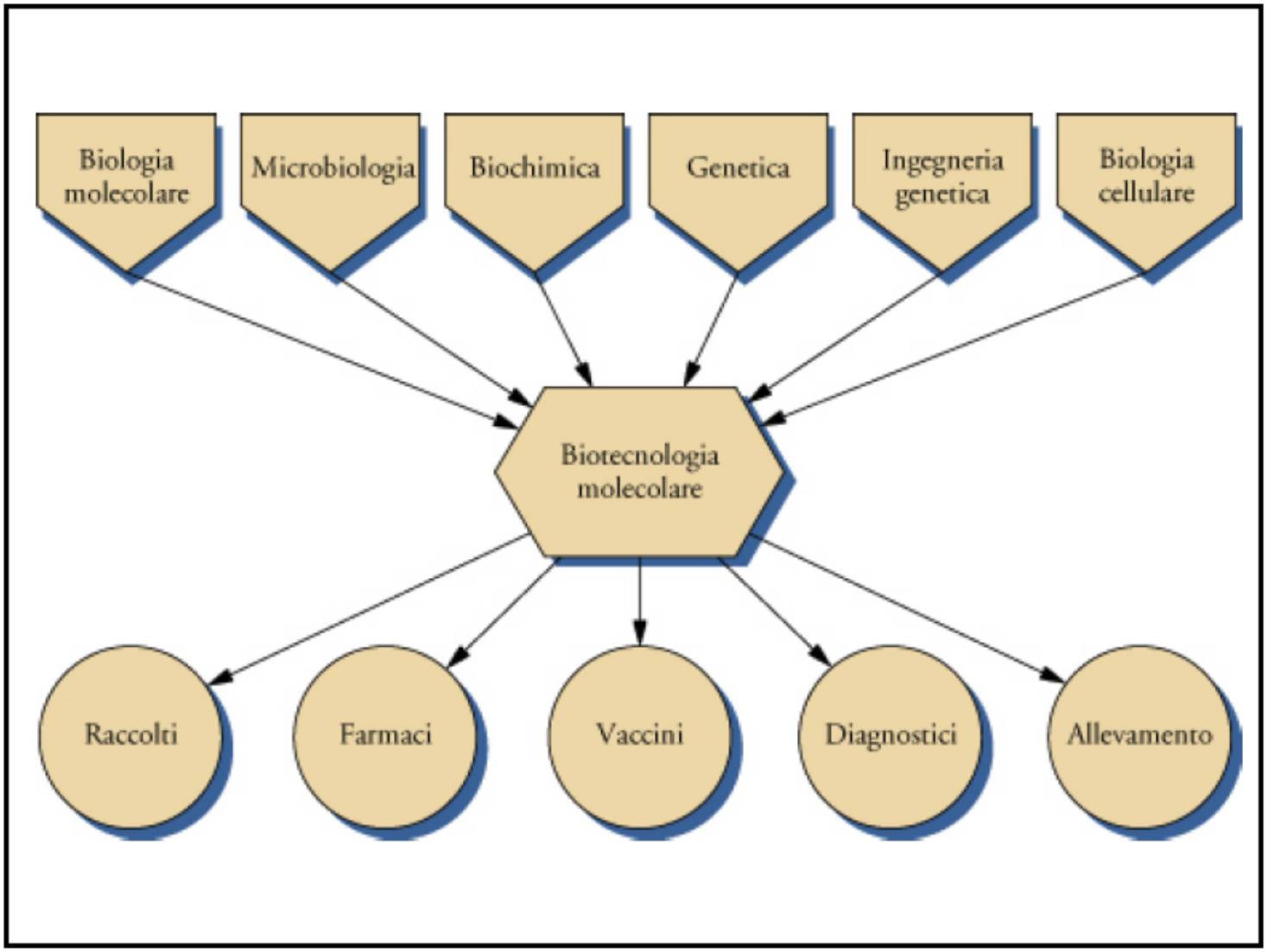
INGEGNERIA GENETICA
Sandy Primrose, Richard Twyman, Bob Old
Zanichelli

DNA RICOMBINANTE
Watson, caudy, Myers, Witkowski
Zanichelli

BIOTECNOLOGIA MOLECOLARE
B.R. Glick, J.J. Pasternak,
Zanichelli

Tecnologie Ricombinanti 2016:

- Elettroforesi, PCR, qPCR, Sequenziamento e Pyrosequencing, Mutagenesi
- Taglio e saldatura di molecole di DNA
- Vettori plasmidici e fagici, cosmidi, fasmidi, BAC, PAC, YAC, Sistemi inducibili
- Genoteche di genomi, a cDNA e analisi delle genoteche
- Clonaggio
- Espressione in cellule procariotiche
- Espressione in cellule eucariotiche ed in particolare di mammifero
- Le proteine di fusione e le loro applicazioni
- Lo studio di sequenze regolatrici attraverso l'uso di geni "reporter"
- Two-Hybrids strategy
- Phage Display
- Evoluzione Molecolare
- Vettori per Terapia Genica
- Trasferimento DNA nelle piante
- Editing genomico
- RNAinterference
- Creazione di forme viventi da zero


Biologia molecolare
Microbiologia
Biochimica
Genetica
Ingegneria genetica
Biologia cellulare
Biotecnologia molecolare
Raccolti
Farmaci
Vaccini
Diagnostici
Allevamento

BIOTECNOLOGIA

Con il termine generico di biotecnologia (tecnologia biologica) si indicano tutte le applicazioni tecnologiche della biologia.
Tra le definizioni disponibili, la più completa è senza dubbio quella stesa dalla Convenzione sulla Diversità Biologica UN, ossia:

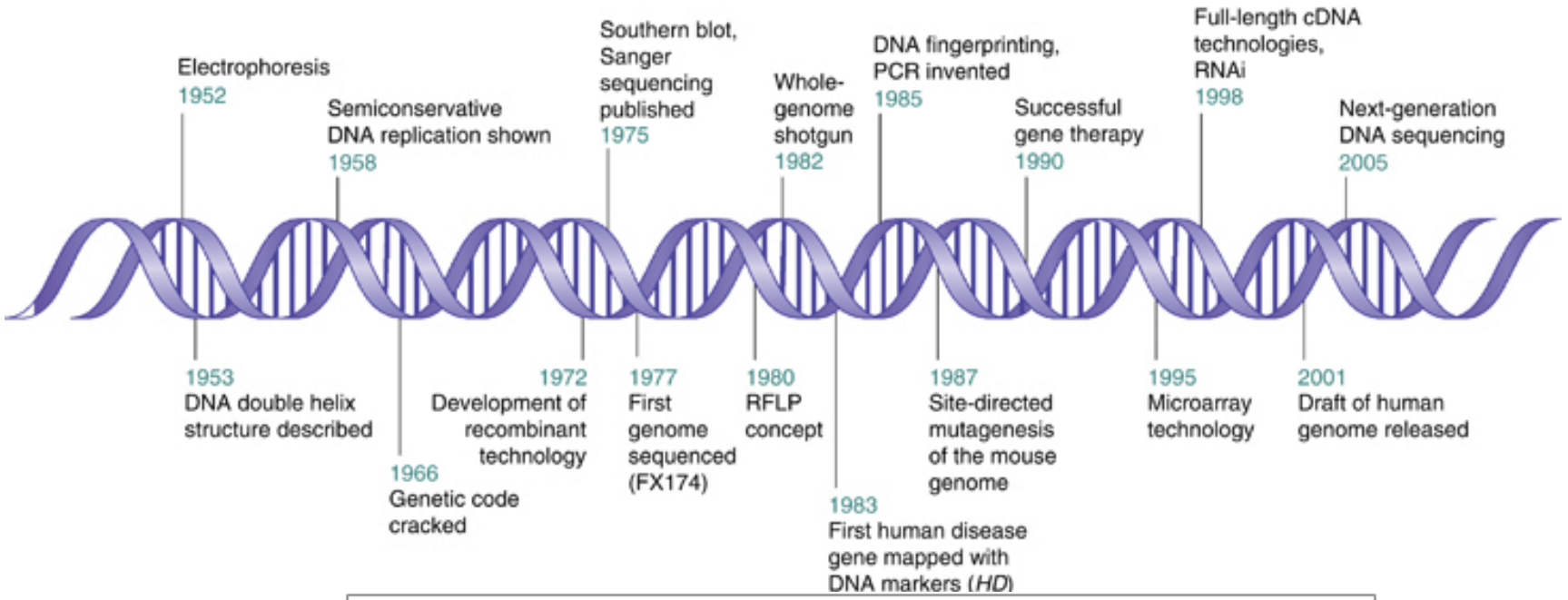
> "**La biotecnologia è l'applicazione tecnologica che si serve dei sistemi biologici, degli organismi viventi o di derivati di questi per produrre o modificare prodotti o processi per un fine specifico**".

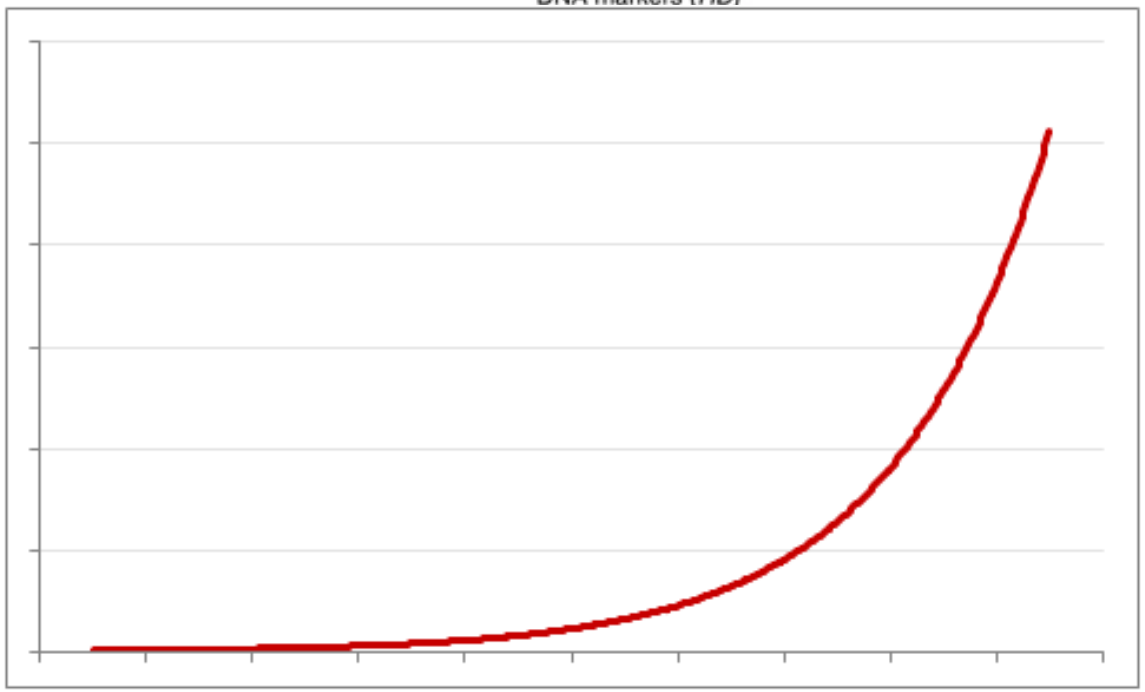
L’utilizzo di organismi viventi, di loro componenti o prodotti, sistemi o processi finalizzato alla **produzione** di **beni** o **servizi**

BREVE STORIA DELLE BIOTECNOLOGIE

6000 a.C. Sumeri e Babilonesi – birra
4000 a.C. Egizi - pane lievitato
1675.- Leeuwenhoek scopre i protozoi ed i batteri.
1855.- Pasteur comincia a lavorare sul lievito, dimostrando per la prima volta che si tratta di organismi viventi.
1863.- Mendel, nel suo studio sui piselli, scopre che le caratteristiche sono state trasmesse dai genitori alla progenie da unità indipendenti, denominate successivamente geni. Le sue osservazioni pongono le fondamenta nel campo della genetica.
1928.- Fleming scopre la penicillina, il primo antibiotico.

- 1953.- Watson e Crick rivelano la struttura tridimensionale del DNA.
- 1961.- Per la prima volta viene compreso il codice genetico.
- 1973.- Cohen e Boyer effettuano il **primo esperimento ricombinante del DNA**, usando geni di batteri.


Electrophoresis
1952
Semiconservative DNA replication shown
1958
Southern blot, Sanger sequencing published
1975
Whole-genome shotgun
1982
DNA fingerprinting, PCR invented
1985
Successful gene therapy
1990
Full-length cDNA technologies, RNAi
1998
Next-generation DNA sequencing
2005
1953
DNA double helix structure described
1966
Genetic code cracked
1972
Development of recombinant technology
1977
First genome sequenced (FX174)
1980
RFLP concept
1983
First human disease gene mapped with DNA markers (HD)
1987
Site-directed mutagenesis of the mouse genome
1995
Microarray technology
2001
Draft of human genome released



BIOTECNOLOGIE

TRADIZIONALI

Tecnologie produttive utilizzate da millenni, quali l'agricoltura, la zootecnica e lo sfruttamento delle attività fermentative dei microrganismi.

INNOVATIVE

Ingegneria genetica: le tecniche che permettono di **identificare**, **isolare** e **trasferire artificialmente** un gene dal patrimonio genetico di un organismo a quello di un altro essere.

OGM : organismo geneticamente modificato

animale, pianta o microrganismo, in cui al DNA ereditario viene aggiunto DNA che proviene da una fonte diversa dal germoplasma parentale

⇩

Le forme viventi in cui sono presenti segmenti di DNA “estraneo” sono dette *transgeniche* (Smith, 1998)

GENETIC ENGINEERING

What is it?

What are the advantages (pros) and disadvantages (cons)?

What is your opinion?

Genetic Engineering

- Means making changes to DNA in order to change the way living things work.

- Creates new crops and farm animals
- Make bacteria that can make medicines
- Grow human body parts
- Prevent genetic diseases, change humans

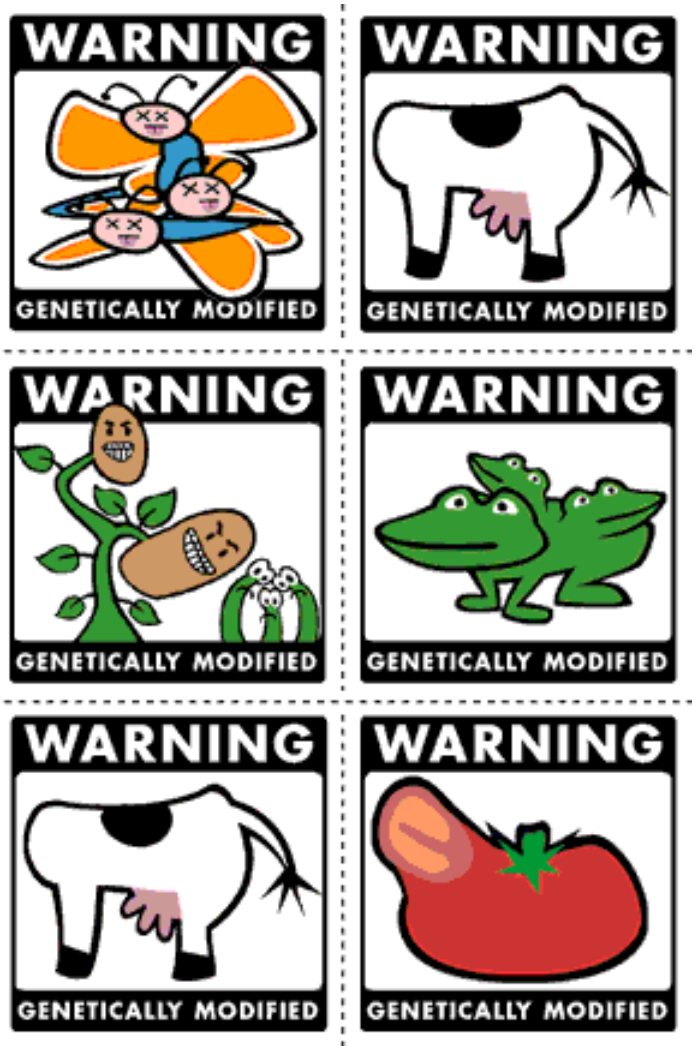
What are Genetically Modified Foods?

(“Frankenfoods”?)

You have already eaten GM foods. Some GM tomatoes, for example, have had their genes altered to stop them from going soft while they are still growing. For several years they were widely sold in tomato paste. The GM foods we eat have all been tested for safety. But some people worry about the long term effects of eating genetically modified foods!

What Have I Eaten?

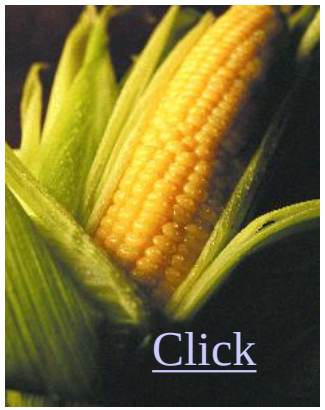
GMO food list



Genetically modified (GM) foods possess specific traits such as tolerance to herbicides or resistance to insects or viruses.

By most estimates, up to 70% of the processed foods at your local grocery store contain at least one ingredient that's been genetically altered

Genetically modified to travel better so don't have to be picked when green – better tasting!

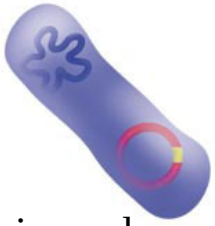


Genetically modified to reduce being eaten by insects.

Fishy Strawberries?

1. The flounder's antifreeze gene is copied and inserted into a small ring of DNA taken from a bacteria cell.

2. The DNA ring containing the flounder gene is put into a second bacterium.



3. This second bacterium is used to infect the strawberry cell. The flounder's antifreeze gene enters the strawberry's DNA.

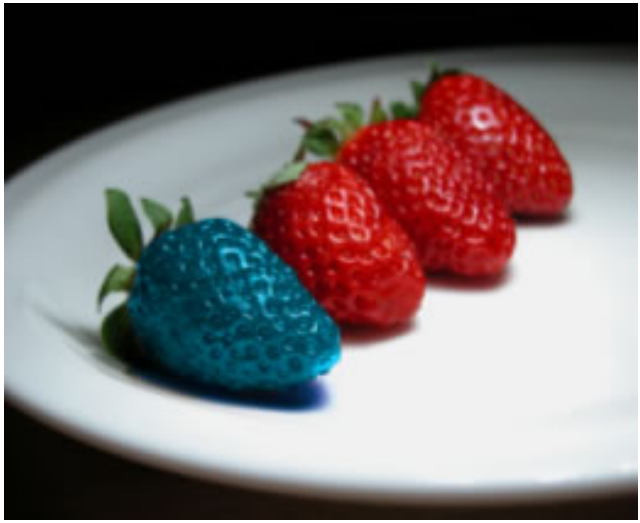
Strawberry cell with Antifreeze gene

4. The new GM strawberry cell is grown into a GM strawberry plant which can be bred many times.

This diagram shows how one type of GM food, a strawberry that resists frost damage is made.

The flounder is a fish that live in icy seas. It has a gene that stops it from freezing to death.Strawberries are soft fruits that can easily be damaged by frost.

Wonder what they used to make this one green!



Thanks to the new gene, GM strawberries make a protein which helps them resist frost. They don't contain any other fish genes and, and do not taste or smell of fish.

Going Bananas?

According to recent reports, **the world may soon be out of bananas.** Because of the starchy fruit's unique method of reproduction, it seems, banana plantations in Africa, Asia and Central America are uniquely susceptible to fungi, viruses and pests. Unless scientists can find a way to genetically enhance the banana's ability to ward off parasites, we could be bananaless in ten years.

Several agroscience companies believe they can genetically engineer such an invincible banana by copying parts of the genetic codes of other fruits and instilling them into the banana.

La storia del Golden Rice

- **La carenza di Vitamina A (VAD)**
  - **Causa cecità**
  - **Riduzione delle difese immunitarie**
  - **Aumenta la mortalità da morbillo e dissenteria**
  - **Maggior trasmissibilità di HIV da madre a figlio**

- **120 milioni di bambini soffrono di carenza di Vitamina A**

- **In molti Paesi non esistono infrastrutture adeguate alla distribuzione di pillole di Vitamine**

- **Un'alternativa è aumentare il contenuto di vitamina A nei prodotti alimentari (riso)**

Via biosintetica del β-Carotene in Pianta

Geranilgeranil difosfato

Fitoene sintasi

Fitoene

Problema:

Nel Riso mancano questi enzimi

Fitoene desaturasi

ξ-carotene desaturasi

Licopene

Licopene-beta-ciclase

β -carotene

(precursore della vitamina A)

Riso senza vitamina A

La soluzione del Golden Rice

Aggiunta dei geni della via biosintetica del β-Carotene

Geranilgeranil difosfato

Gene Narciso → **Fitoene sintasi**

Fitoene

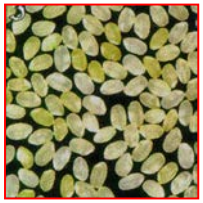
Gene batterico con entrambe le funzioni → **Fitoene desaturasi**, **ξ-carotene desaturase**

Licopene

Gene Narciso → **Licopene-beta-ciclasi**

β -carotene (precursore vitamina A)

Pathway Vitamina A completa e funzionale



Golden Rice

Pros and Cons

Crops
- Better taste and quality
- Less time to ripen.
- More nutrients, more food, and stress tolerance
- Improved resistance to disease, pests, and herbicides
- New products and growing techniques

Animals
- Increased resistance, productivity, hardiness, and feed efficiency
- Better yields of meat, eggs, and milk
- Improved animal health and diagnostic methods

Environment
- "Friendly" bioherbicides and bioinsecticides
- Conservation of soil, water, and energy
- Better natural waste management
- More efficient processing

Society
- More food for growing populations

Safety
- Potential human health impact: allergens, transfer of antibiotic resistance markers, unknown effects
- Potential environmental impact: unintended transfer of transgenes through cross-pollination, loss of flora and fauna biodiversity

Access and Intellectual Property
- Domination of world food production by a few companies
- Increasing dependence on Industralized nations by developing countries

Ethics
- Violation of natural organisms' intrinsic values
- Tampering with nature by mixing genes among species
- Objections to consuming animal genes in plants and vice versa
- Stress for animal

Labeling
- Not mandatory in some countries (e.g., U. States)
- Mixing GM crops with non-GM confounds labeling attempts

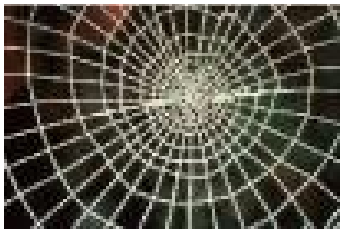
What's Been Done So Far?

- Genetically engineering chickens so they have no feathers – why?
- Genetically engineering mice so they have no fur – why?
- Genetically engineering salmon (fish) so they grow much faster than normal salmon – why?
- Glowing mice

What's Next?

Red fish, blue fish, yellow fish, GLOW FISH?

What Else?



Using the jellyfish protein to make a naturally glowing Christmas tree!

Adding a gene from insect killing bacteria to cotton so that insects who eat cotton will be poisoned!

Genetically engineered moths that pass on deadly disease genes to their relatives so they die and won't be able to destroy crops!

A gene from a spider has been inserted into some goats. Their milk now contains tiny strands of spider silk which can be made into a strong, stretchy rope.

This is only the beginning . . . !

That's Unbelievable!

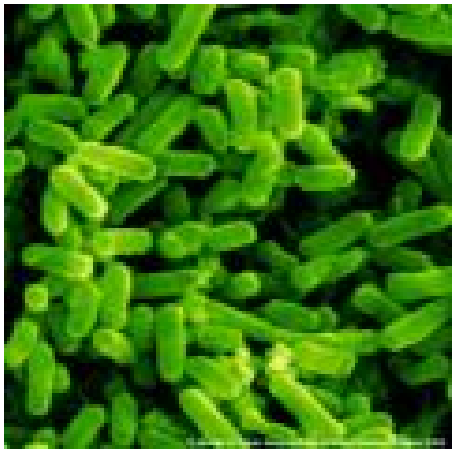
Genetic engineering examples include taking the gene that programs poison in the tail of a scorpion, and combining it with a cabbage. These genetically modified cabbages kill caterpillers because they have learned to grow scorpion poison (insecticide) in their sap.

Genetic engineering also includes insertion of human genes into sheep so that they secrete alpha-1 antitrypsin in their milk - a useful substance in treating some cases of lung disease.

Genetic engineering works because there is one language of life: human genes work in bacteria, monkey genes work in mice and earthworms. Tree genes work in bananas and frog genes work in rice. There is no limit in theory to the potential of genetic engineering!

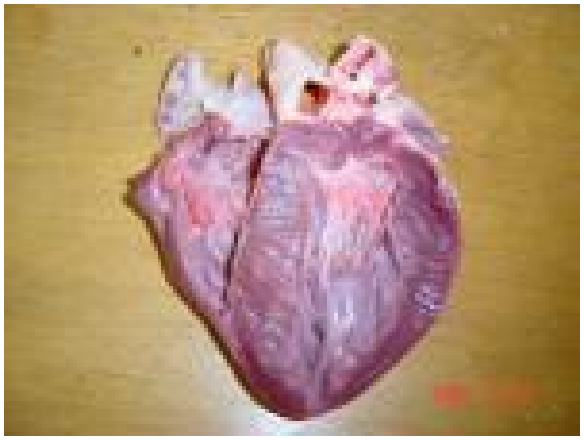
Genetic engineering has created a chicken with four legs and no wings. Genetic engineering could create crops that grow in desert heat, or without fertilizer. Genetic engineering could make bananas or other fruit which contain vaccines or other medical products.

How can we use genetically engineering to help us?



By inserting a gene for human insulin into an E.Coli bacterium, the E. coli will make lots of insulin, which scientists and doctors can collect and use.

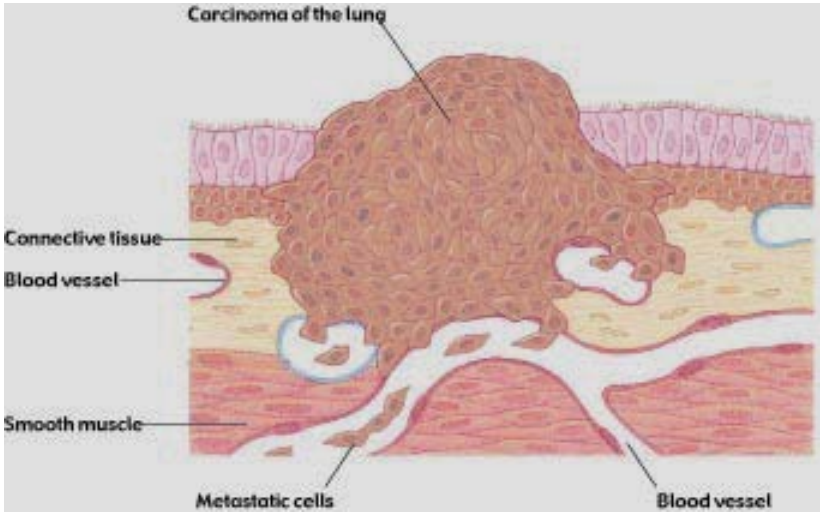
Right now, doctors are using pig hearts for transplants but there are still rejection problems. One day soon, scientists will be able to genetically engineer pigs to grow human organs for use in transplants.



Will We Be Able To Cure Cancer With Gene Therapy?

Lung cancer cells (530x). These cells are from a tumor located in the alveolus (air sac) of a lung.

Cancer happens when body cells grow out of control. Scientists have found a gene called p-53 which normally keeps cells under control. They think that in some people with cancer, the disease begins because the p-53 gene doesn't work properly – perhaps because of a mistake in the gene code. Experts are now looking for a way to cure cancer by modifying faulty DNA to make the p-53 gene work.



What’s Going On Here?

Photo of mouse growing a "human ear" - a shape made of cartilage

Mixing humans and animals

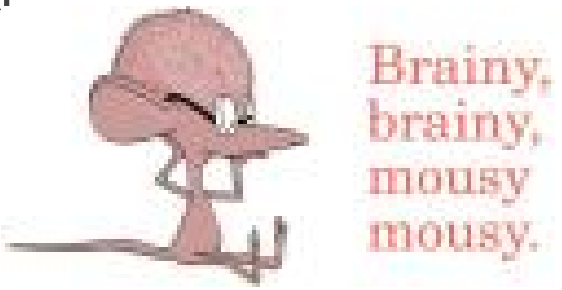
The mythic beast had a lion's body, serpent's tail, and goat's head.

Scientists have begun blurring the line between human and animal by producing chimeras—a hybrid creature that's part human, part animal.

Chinese scientists at the Shanghai Second Medical University in 2003 successfully **fused human cells with rabbit eggs**. The embryos were reportedly the first human-animal chimeras successfully created. They were allowed to develop for several days in a laboratory dish before the scientists destroyed the embryos to harvest their stem cells.

- In Minnesota last year researchers at the Mayo Clinic created **pigs with human blood** flowing through their bodies.

- And at Stanford University in California an experiment might be done later this year to create **mice with human brains**.



Scientists feel that, the more humanlike the animal, the better research model it makes for testing drugs or possibly growing "spare parts," such as livers, to transplant into humans.

The First Clone!

Johnny's next cloning experience involved writing, 'I will not copy other student's test papers', 500 times.

Cloning A Cat?

NEWS ITEM: SCIENTISTS HAVE LEARNED HOW TO CLONE CATS

Now cats may have more than nine lives. The company that funded the first successful cloning of a domestic cat two years ago has gone commercial.

The cost?

U.S. $50,000 each.

"Cc," the first-ever cloned cat shown here at seven weeks old with Allie, her surrogate mother.

The cat was cloned by transplanting DNA from Rainbow, a female three-colored (tortoiseshell or calico) cat into an egg cell whose nucleus had been removed, and then implanting this embryo into Allie, the surrogate mother.

"CC's coat color suggests that she is a clone, and a genetic match between CC and the donor mother confirms this," the researchers say.

Rat called Ralph is latest clone

Scientists have succeeded in cloning the rat.

The researchers from China and France say they managed to create several rodent copies - both male and female. The rat joins a lengthening list of animals that have been cloned from an adult cell.

These include sheep, mice, cattle, goats, pigs, cats, mules and horses.

The rat has come later than the others because of unique difficulties in controlling the development of its eggs in the early stages of the cloning process.

Rat eggs activate almost immediately they leave the ovaries, making it difficult to introduce the genetic material of the animal to be copied.

Cow Gives Birth To A Bison

Hijacking a womb by one species for another

Scientists at Massachusetts Advances Cell Technology (ACT) have succeeded in cloning a **gaur**, an ox-like animal at threat of extinction in Southern Asia. They used the "Dolly the sheep" animal cloning technique to create 81 cloned embryos after 692 attempts using gaur skin cells and cow's eggs. These cloned embryos were then implanted into **cows**, with 8 pregnancies, five miscarriages and three expected live births. (Source Guardian 7 October 2000)

The next step by ACT will be to clone the first extinct animal, the bucardo. Scientists discovered the last animal dead, but in time to freeze and preserve tissue samples for animal cloning.

Will we be able to clone extinct animals?

CLONING: Part human, part cow?

In 1998, an American company in Massachusetts, Advanced Cell Technology, **took a cell from Dr Jose Cibelli, a research scientist and combined it with a cows egg** from which the genes had already been removed. The genes activated and the egg began to divide in the normal way up to the 32 cell stage at which it was destroyed. **If the clone had been allowed to continue beyond implantation it would have developed as Dr Cibelli's identical twin.** Technically 1% of the human clone genes would have belonged to the cow - the mitochondria genes. Mitochondria are power generators in the cytoplasm of the cell. They grow and divide inside cells and are passed on from one generation to another. They are present in sperm and eggs. Judging by the successful growth of the combined human-cow clone creation it appears that cow mitochondria may well be compatible with human embryonic development.

However **the biggest piece of news is not what they did in human cloning - sensational enough - but the fact that they kept cloning secret for three years after doing it, and presumably they were trying to do it at least a couple of years before that.**

•2003, An American fertility expert has created about 200 human-cow embryos in his run-up to cloning a baby.

•Shanghai stem cell researcher Hui Zhen Sheng announced that she had created about 400 human-rabbit embryos.

Cloning HUMANS?

UK scientists clone human embryo British scientists say they have cloned the country's first human embryo. The Newcastle University team took eggs from 11 women, removed the genetic material and replaced it with DNA from embryonic stem cells.

The aim of this kind of work - the subject of fierce debate - is to make cloned embryos from which stem cells can be used to treat diseases.

Meanwhile South Korean scientists say they have created stem cells to match individuals for the first time.

Stem cell lines were created by taking genetic material from the patient and putting it into a donated egg.

The resultant cells were a perfect match for the individual and could mean treatments for diseases like diabetes without problems of rejection.

Therapeutic cloning - believed to have huge potential to treat disease and disability - is allowed in Britain.

Reproductive cloning - the cloning of human embryos with the intention of creating a baby - was made **illegal** in 2001.

To Clone or Not to Clone?

Jane is blind and has a guide dog called Bobby. Bobby has been her guide dog for 10 years but is getting old. Bobby is Jayne's best friend and she feels that without him she couldn't live. Should Jayne be allowed to clone Bobby before he dies? Should people be allowed to clone their pets?

There is only one Giant Panda left on earth. It does not have a partner to breed with so once it dies the species will be extinct. Should scientists be allowed to clone another Giant Panda to keep the species alive? Should scientists be allowed to clone endangered species?

Mrs. Jones eldest son Mark is 10 years old and is dying with cancer. Should Mrs. Jones be allowed to clone Mark before he dies? Should people be allowed to clone other humans or clone themselves?

You decide

Creating Designer Babies

Create your own designer baby!

We'll be able to choose the gender (male/female), test for genetic diseases, and possibly lots of other options!

Advanced reproductive technologies allow parents and doctors to screen **embryos** for **genetic** disorders and select healthy embryos.

In-vitro fertilisation or IVF -The fear is that in the future we may be able to use genetic technologies to modify embryos and choose desirable or cosmetic characteristics. *Designer babies* is a term used by journalists to describe this frightening scenario. It is not a term used by scientists.

Right or Wrong?

Arguments for creating designer babies

- Some couples are not able to have children because their children will have a genetic disease and die before they are born or when they are very young. Techniques used to change the genetic make-up of the embryo allow these parents to have a child.

- If we want the best for our children why shouldn't we design our own babies? Using genetic techniques we can help prevent certain genetic diseases. This both saves the children from suffering and reduces the cost and emotional strain of looking after an ill child. Will this lead to happier children and parents?

- Spare part children? In a few cases where parents have had one child with a serious blood disease, they have used IVF to select embryos so that they can have a second child that can act as a future, tailor-made blood or bone marrow donor. In these cases when the child is born he or she will be healthy and can help their older brother or sister stay well.

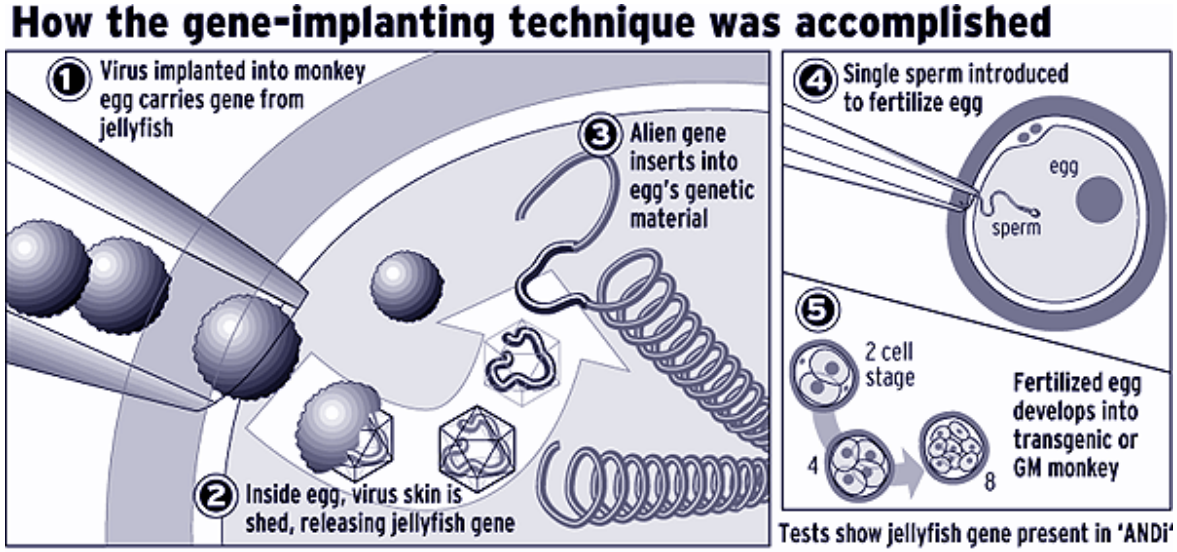
Arguments against creating designer babies

- But is this right? In these cases, parents and doctors are creating a child to act as an organ-donating factory. How will the child feel? The child may feel that they were only born to be a help to their older brother or sister. Children should be loved and cherished for themselves and not what they can do for others.

- These genetic techniques are very expensive. Why should only rich people be able to eradicate genetic diseases? This could lead to imbalances between rich and poor people.

- Alterations made by genetic engineering would be passed on from one generation to the next. What right have parents to choose what genetic characteristics are best for their children, and their children's children. Will the children react against the genetic changes that their parents have chosen for them?

- Animal studies have shown that this type of **genetic engineering is unpredictable**. There is a huge risk that we may produce physical changes, or even change the child's personality. Mice whose genes had been changed to make them more muscular, unexpectedly became very timid compared to other non-genetically engineered mice!



ANDi

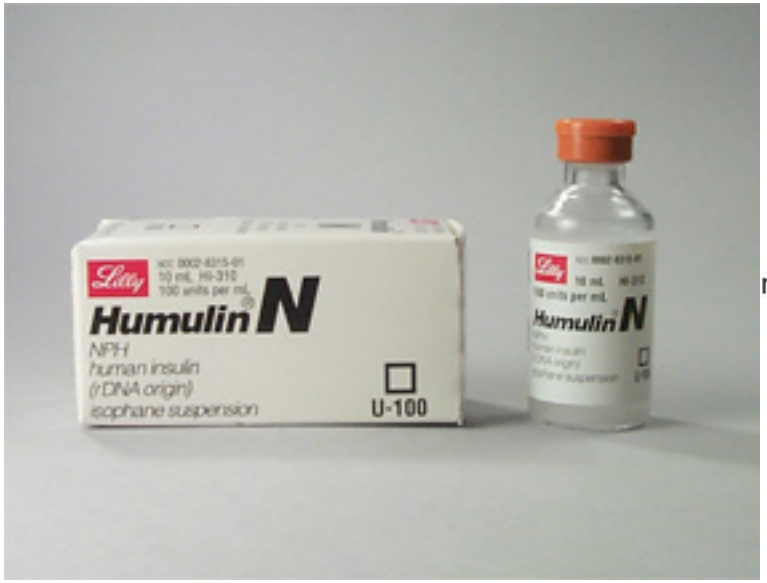
A year ago, scientists at the Oregon Regional Primate Research Center announced the birth of the first genetically engineered primate, named ANDi (for "inserted DNA" spelled backwards), a rhesus monkey whose cells contained the gene that makes jellyfish glow in the dark. The experiment was something of a flop; ANDi does not glow. (Rodents implanted with the gene do.) But imagine that one day science does acquire the skills to make "designer babies," that the connections between genes and complex traits such as intelligence or musical ability are finally known. **While only the weirdest of parents would to want to genetically engineer offspring with jellyfish genes, others would undoubtedly jump at the chance to "customize" their children with a sparkling personality, brains, and beauty**.

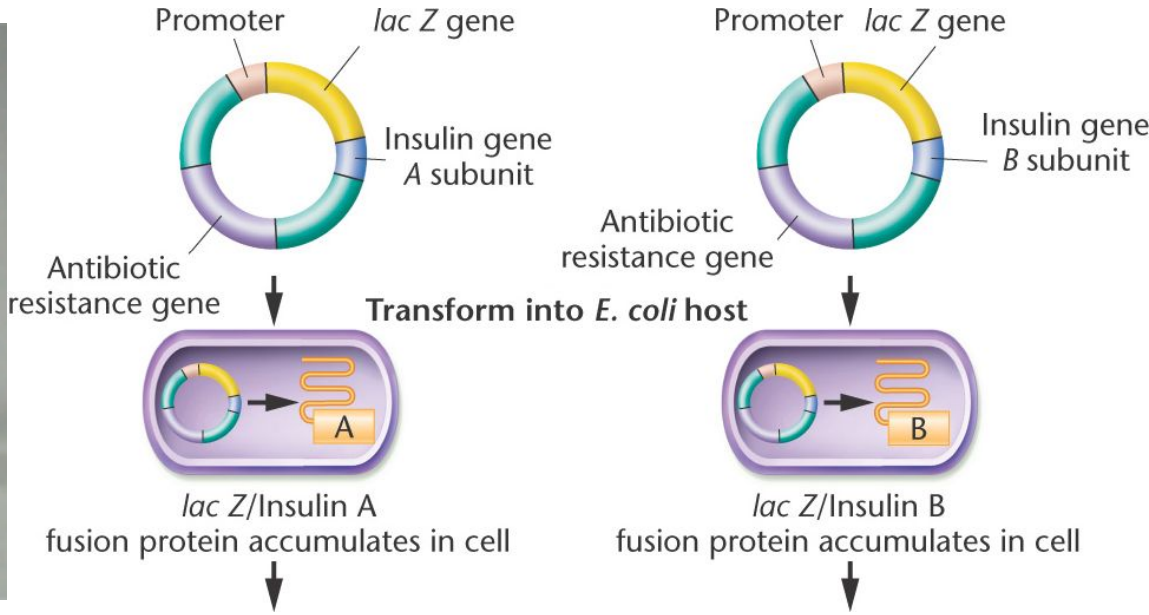
What possible evils are there?

Le applicazioni delle biotecnologie sono molteplici e recentemente sono state suddivise in **quattro categorie**.

Red biotechnology (biotecnologie rosse): vengono riferite ai settori della medicina, della veterinaria e dell'industria farmaceutica;

a) terapeutici

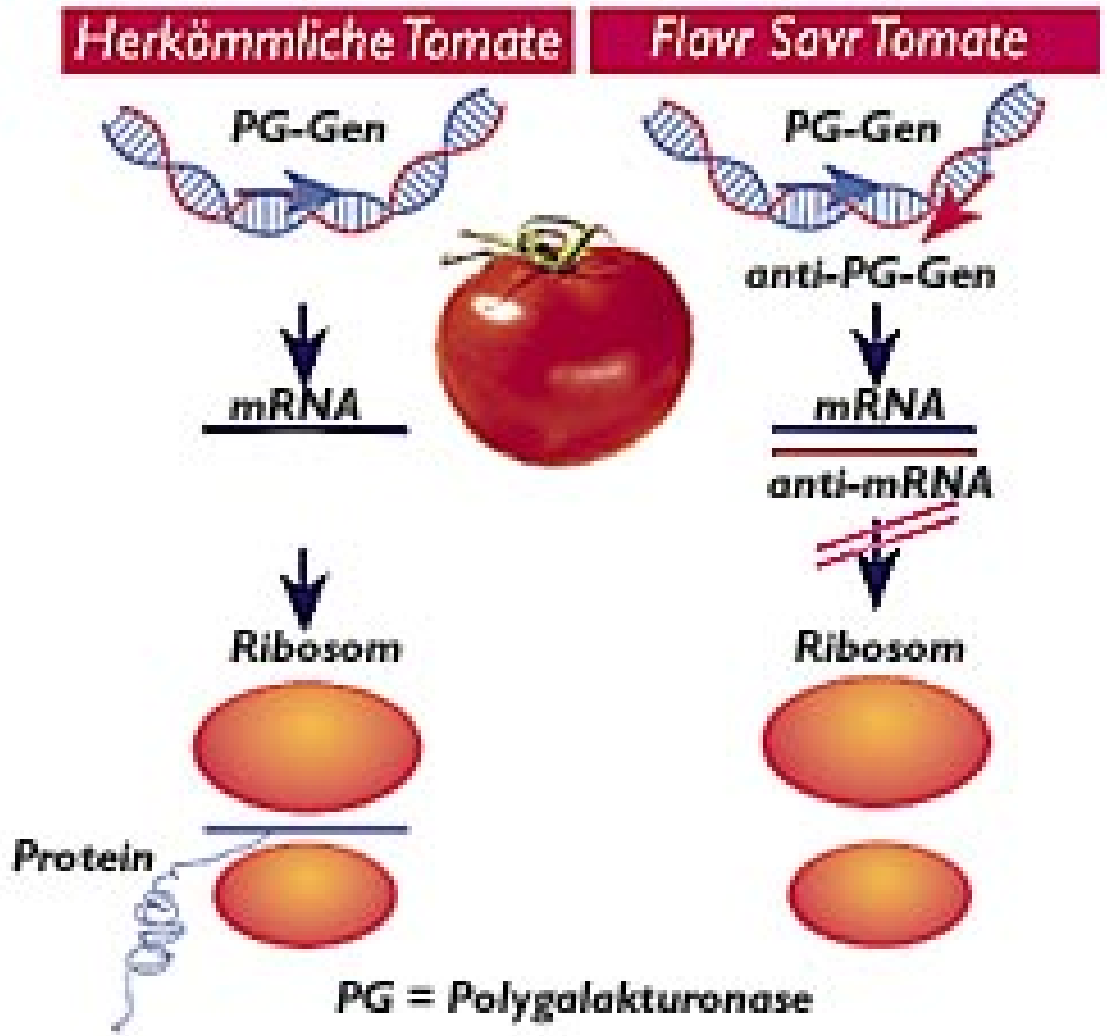




1982.- **Humulin**, l'insulina umana prodotta dalla Genentech, utilizzando batteri geneticamente modificati, è il primo farmaco biotech che viene approvato dalla FDA per il trattamento del diabete

White biotechnology (biotecnologie bianche): si riferiscono ai processi di interesse industriale, conosciute più comunemente come biotecnologie industriali. **BIOREMEDIATION**

Green biotechnology (biotecnologie verdi o agro-alimentari): vengono riferite al settore alimentare, chimico, produttivo, *pharming* molecolare

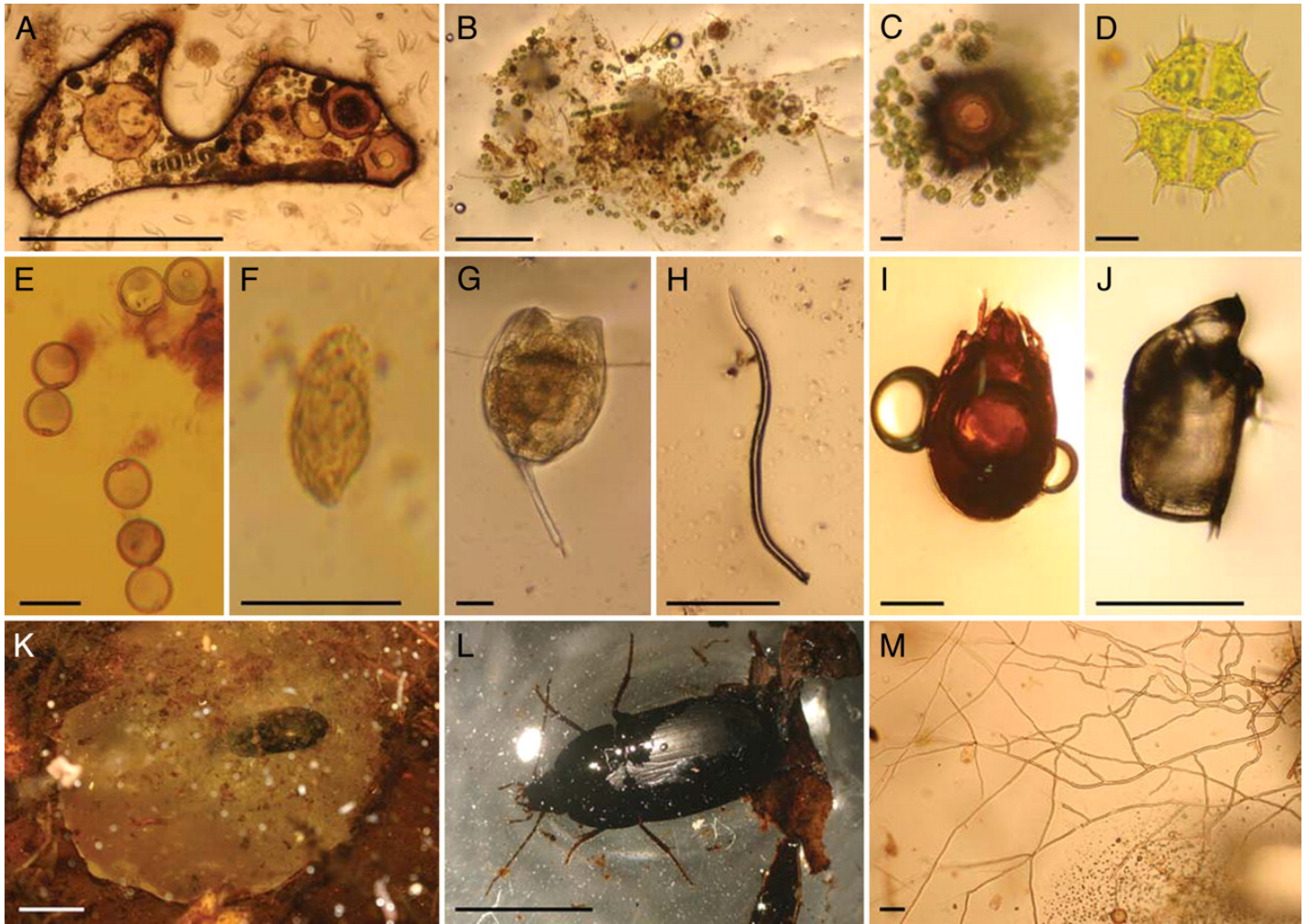


Il 18 Maggio 1994 è stata sancita la sicurezza del pomodoro FLAVR SAVR, considerato come uguale a quelli coltivati convenzionalmente e perciò non è richiesta alcuna etichetta.

L' RNA antisenso, una volta prodotto all'interno della pianta, si ibrida con il messaggero della PG naturale : viene bloccata così la traduzione e prodotta quindi meno PG.

Blue biotechnology (biotecnologie blu): di recente classificazione si applicano all'ambito marino e acquatico.

Esempi: uso di alghe e/o prodotti derivati per produrre nuovi farmaci; uso di geni di organismi acquatici per ingegnerizzare piante e renderle resistenti a particolari condizioni ambientali.



LE NUOVE SFIDE BIOTECH:

BIOLOGIA SINTETICA

Craig Venter:

-1° sequenziare genoma umano (suo)

-1° creare virus sintetico

-1° creare una batterio sintetico

Come si fabbrica una cellula

I ricercatori hanno sviluppato una tecnica di trapianto del genoma (*genome transplantation*) che può essere usata anche per fabbricare cellule sintetiche.

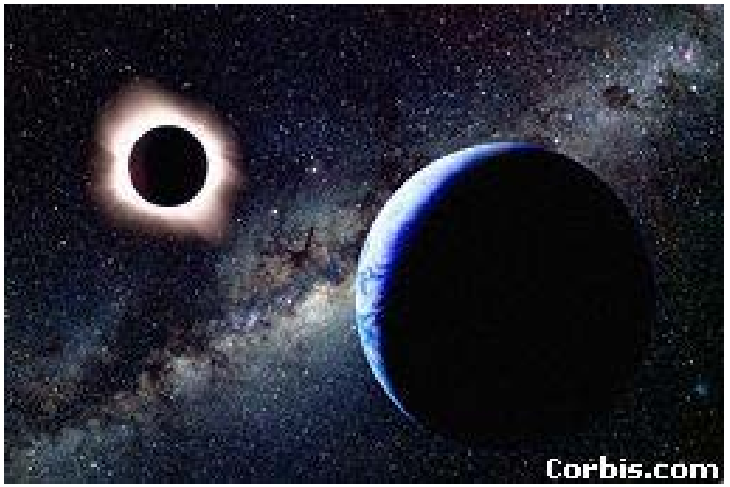
La sequenza completa del Dna (genoma) di un batterio viene prelevata e impiantata in un batterio di specie diversa.

Quando la cellula si divide, i due genomi entrano in cellule figlie differenti.

Una delle nuove cellule è identica alle cellule della specie donatrice, l'altra è la copia della cellula della specie ospite.

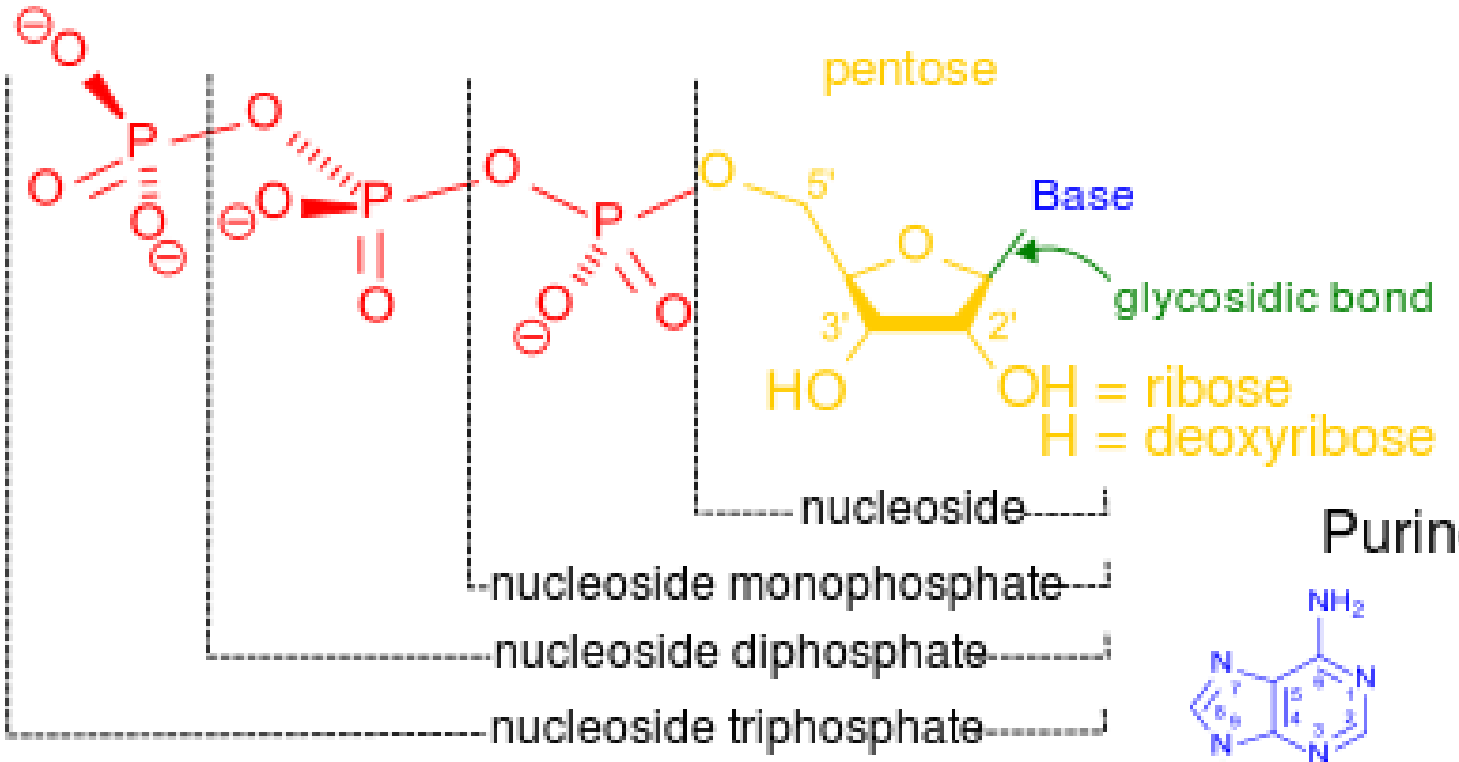
DNA by the Numbers

- Each cell has about 2 m of DNA.
- The average human has 75 trillion cells.
- The average human has enough DNA to go from the earth to the sun more than 400 times.
- DNA has a diameter of only 0.000000002 m.

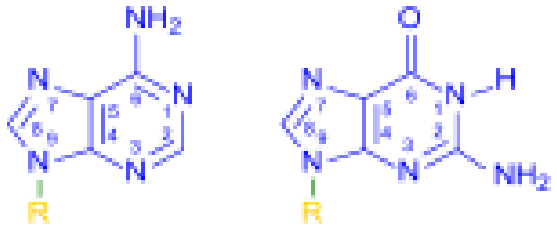


The earth is 150 billion or 93 million miles from the sun.

DNA Nucleotide

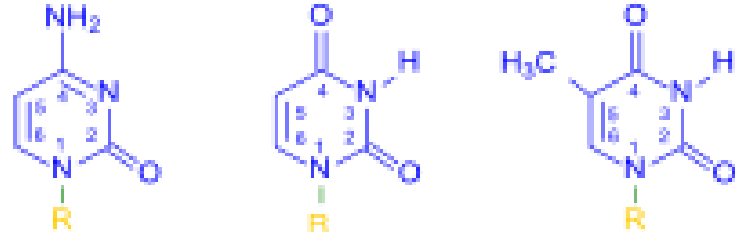


Purines



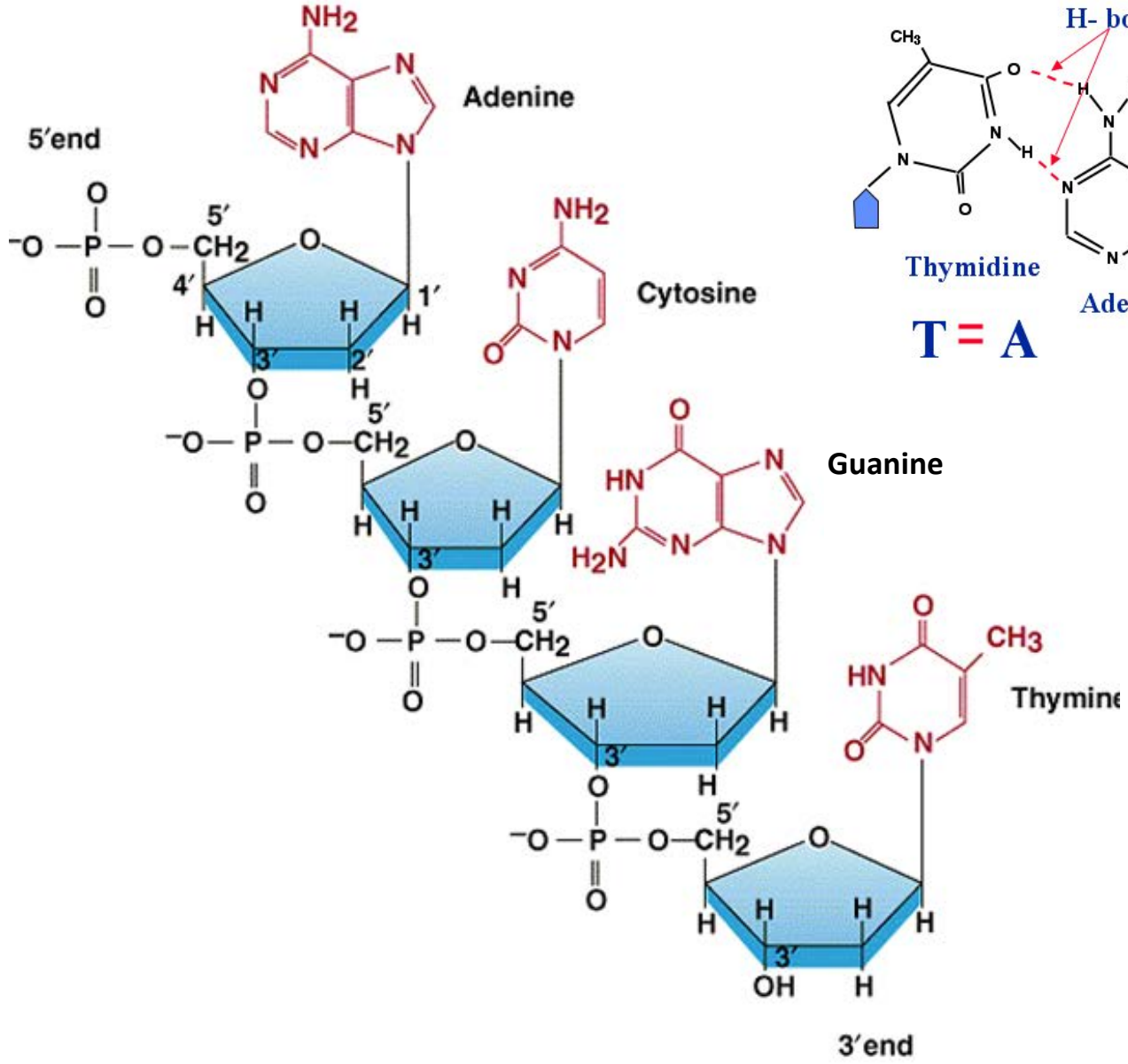
Adenine Guanine

Pyrimidines

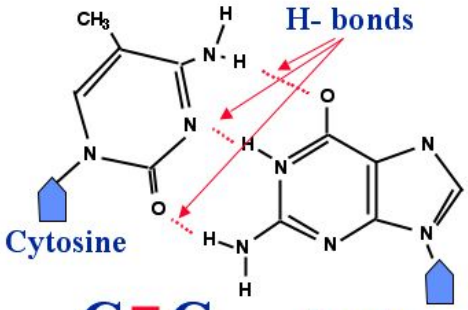


Cytosine Uracil Thymine

Nucleotide Pairing



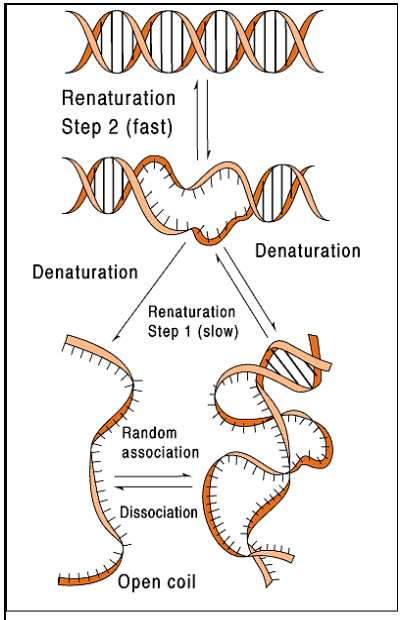
T = A



C ≡ G

Proprietà termiche

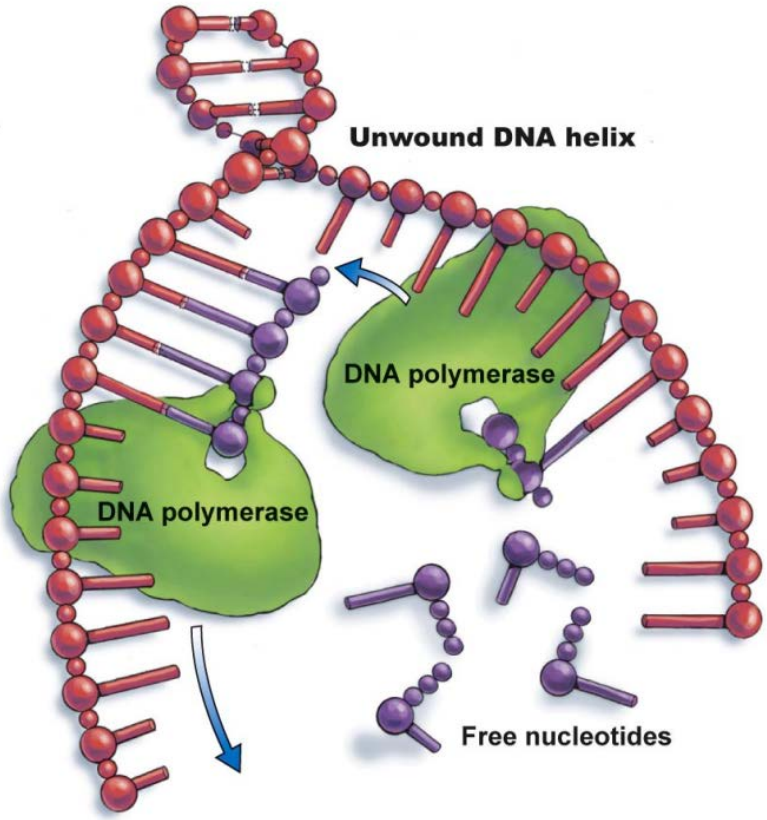
- **Se il DNA è portato ad alte temperature i legami H diventano instabili e le due catene si separano: DENATURAZIONE TERMICA.**
- **Le sequenze ricche di A e T si denaturano più facilmente di quelle ricche in G e C**
- **La temperatura alla quale il 50% del DNA è denaturato è detta Tm (Melting Temperature) dipende dalla sequenza (solvente e ioni)**
- **Dopo raffreddamento le basi si riappaiano: rinaturazione, ibridizzazione o annealing**



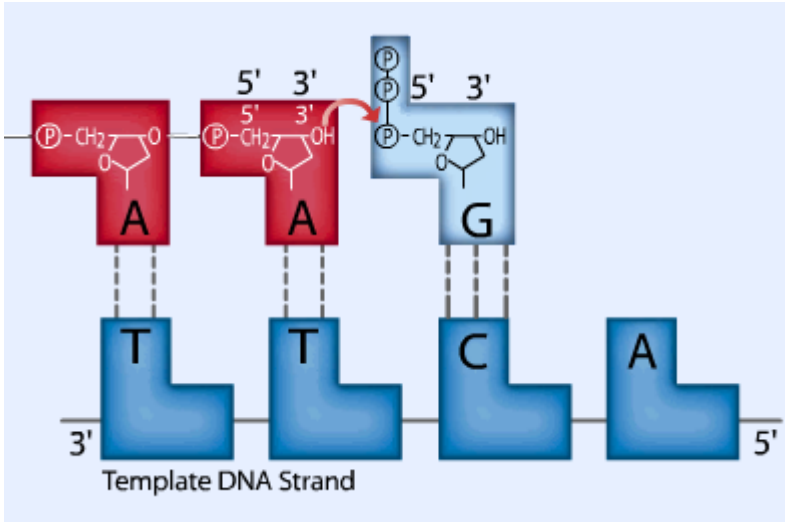
F14_15.PIC

DNA Replication

- DNA Polymerase
  - ✓ Enzyme that catalyzes the covalent bond between the phosphate of one nucleotide and the deoxyribose (sugar) of the next nucleotide



DNA Polymerization



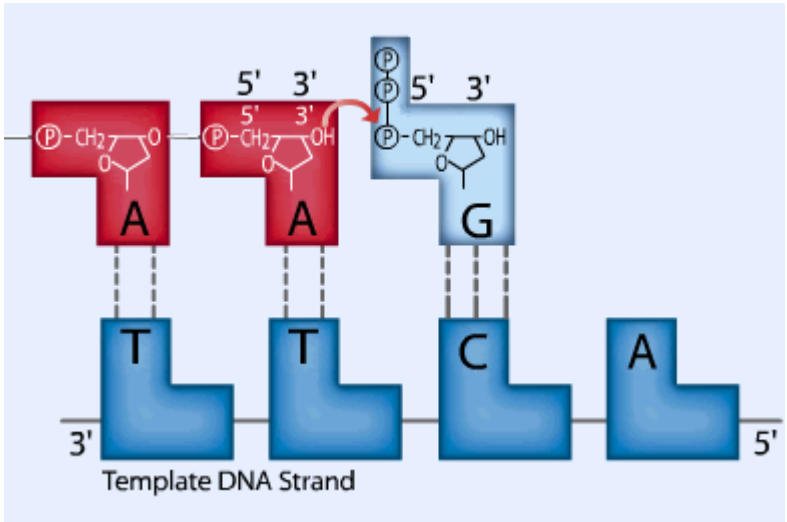
DNA Replication

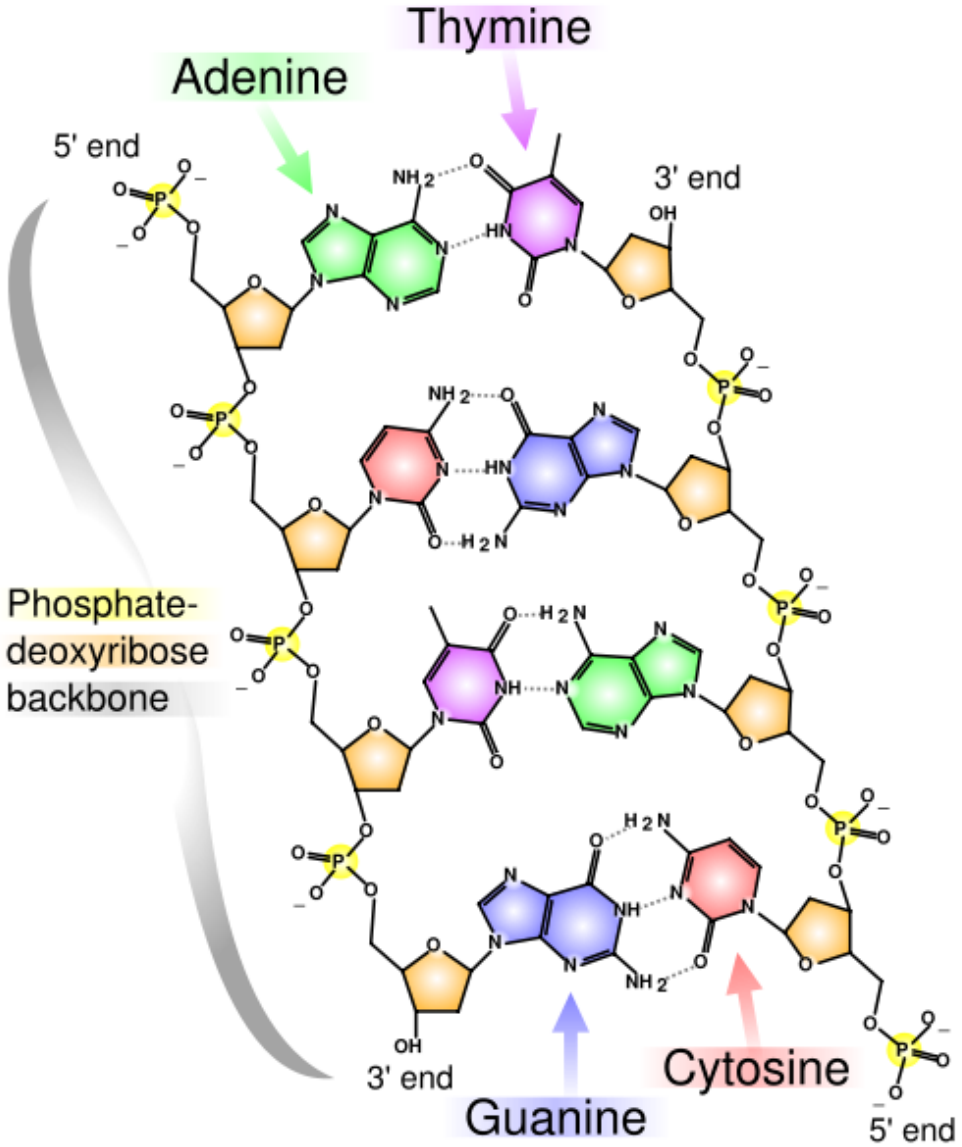
3’ end has a free deoxyribose

5’ end has a free phosphate

DNA polymerase:

- ✓ can only build the new strand in the 5’ to 3’ direction
- ✓ Thus scans the template strand in 3’ to 5’ direction

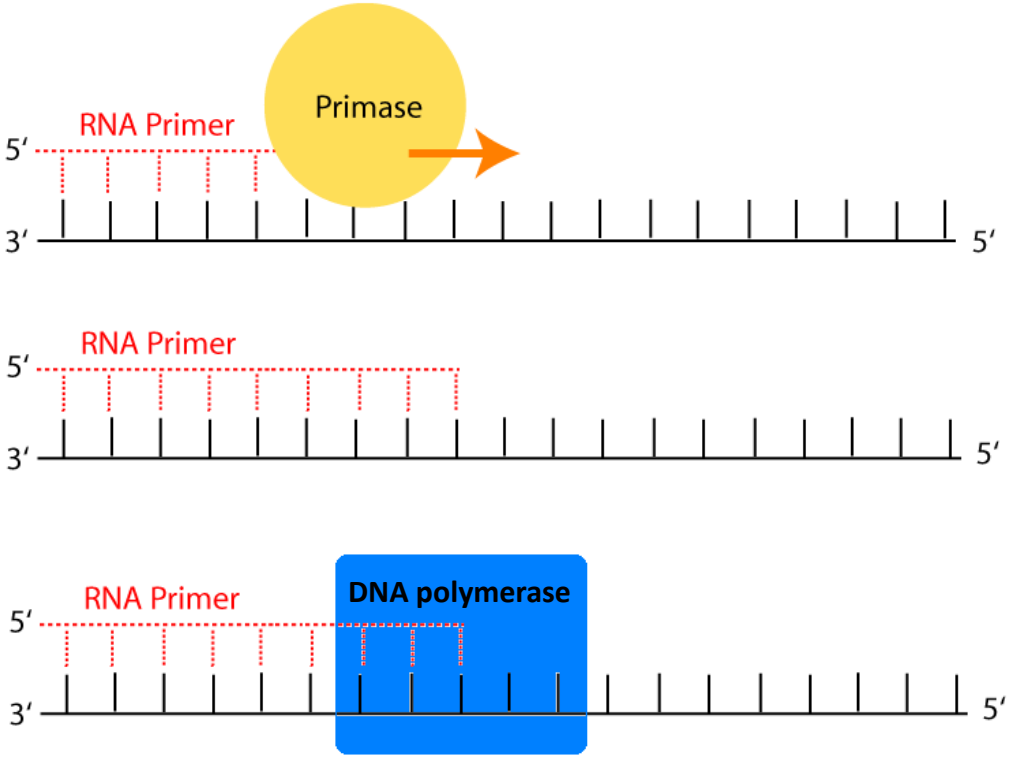




DNA Replication

Initiation

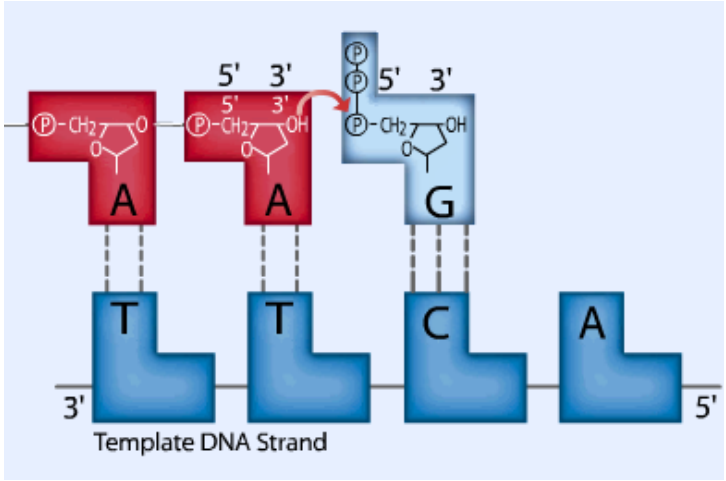
- **Primase** (a type of RNA polymerase) builds an **RNA primer** (5-10 ribonucleotides long)
- **DNA polymerase** attaches onto the 3’ end of the **RNA primer**

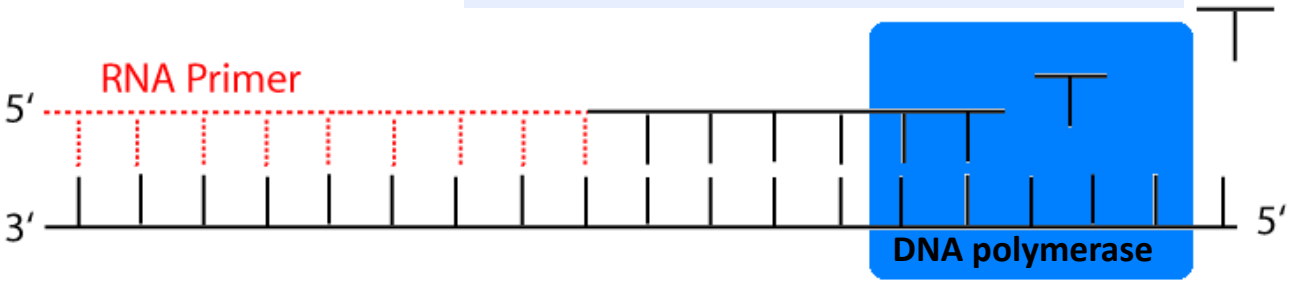


DNA Replication

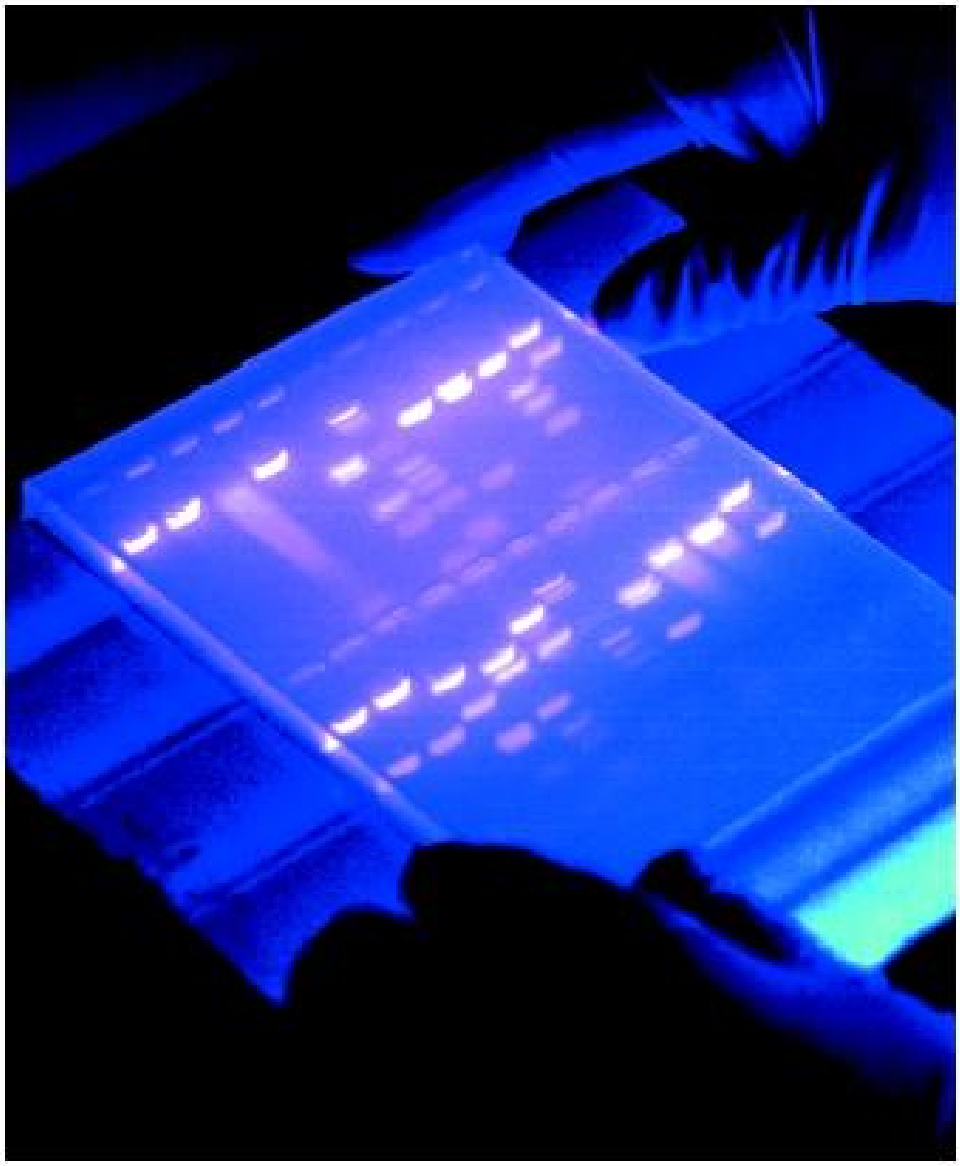
Elongation

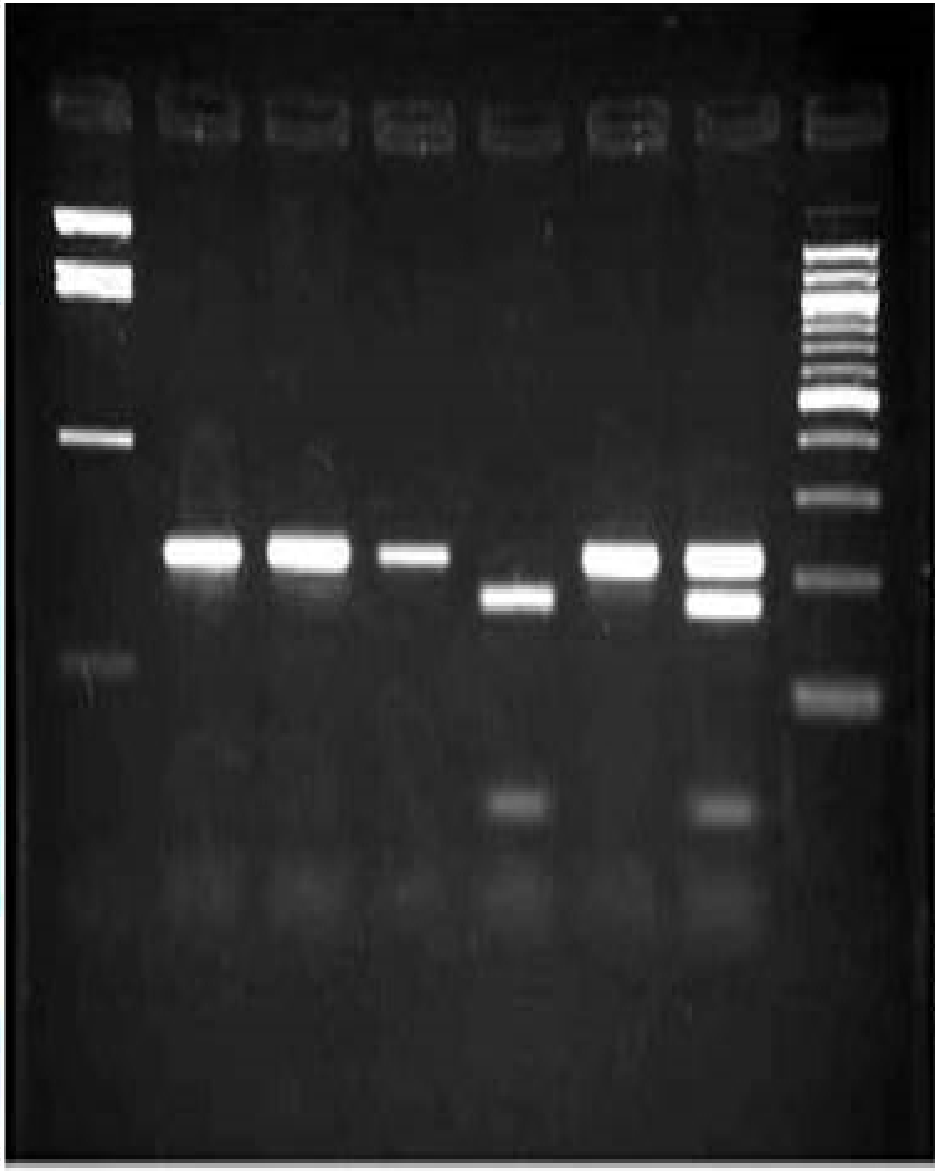
- **DNA polymerase** uses each strand as a template in the 3’ to 5’ direction to build a complementary strand in the 5’ to 3’ direction





Elettroforesi di DNA su gel di agarosio





What is it?

- Electrophoresis separates DNA and Proteins using electricity through a porous material.
  - Movement of the DNA and Protein is a function of size.
    - DNA speed is based on size.
    - Smaller is Faster and Bigger is slower.
- It's like McDonalds on a busy weekend.

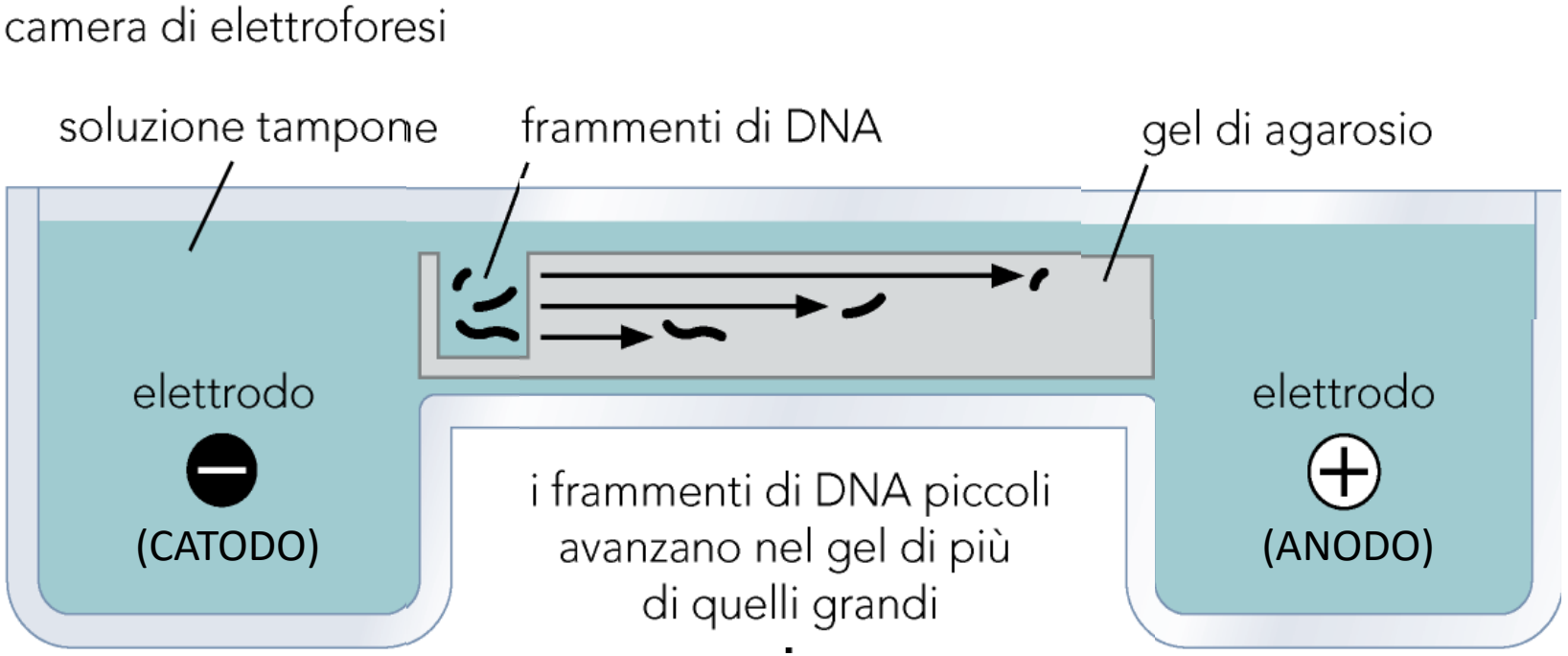
Da cosa dipende la velocità di migrazione

- Dimensione del DNA
- Concentrazione di agarosio nel gel
- Conformazione del DNA
- Voltaggio applicato → circa 5 Volt/cm (distanza anodo-catodo)
- Presenza di bromuro di etidio
- Composizione (forza ionica) del buffer →

❑ DIMENSIONE DEL DNA

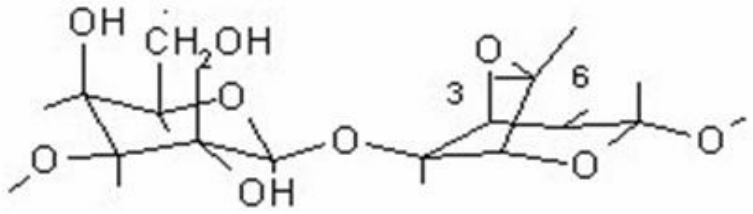
$$V = \frac{K}{\text{Log}_{10}\ bp}$$

relazione di proporzionalità diretta tra pb e PM:
- molecole grandi migrano **lentamente**
- molecole piccole migrano **velocemente**
(K varia al variare della concentrazione di agarosio nel gel)

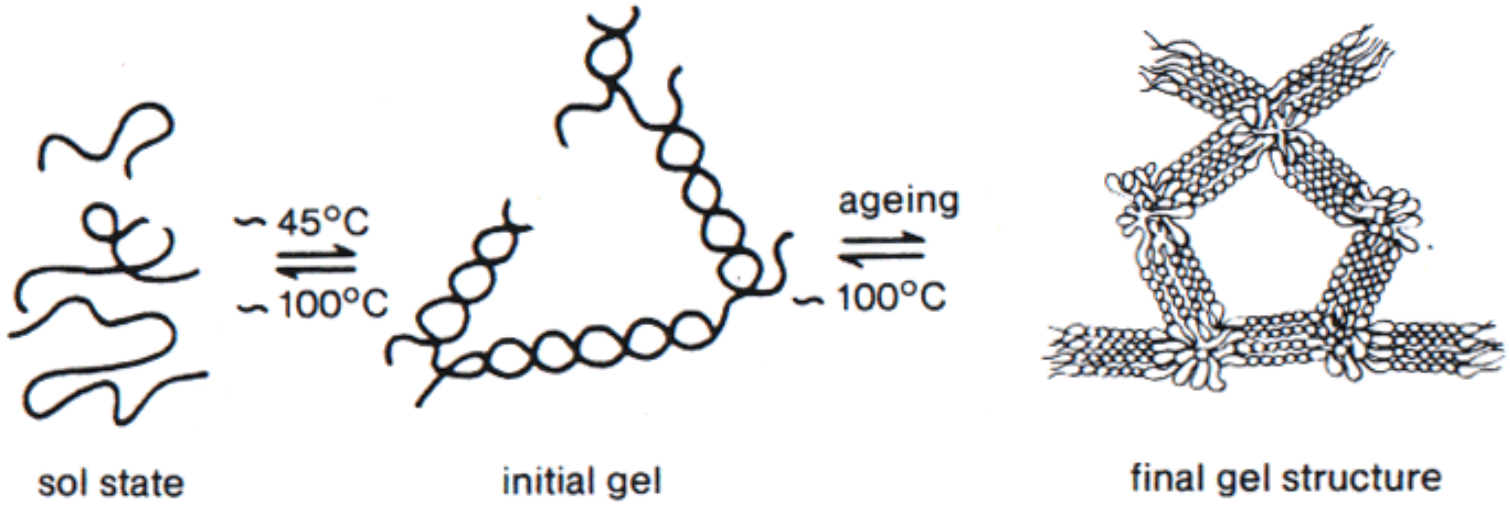


❑ CONCENTRAZIONE DI AGAROSIO NEL GEL

D-Galattoso-3,6-Anidro-L-Galattoso



- L'agarosio è un polimero lineare che forma una matrice semisolida avente pori di dimensione diversa in funzione della concentrazione utilizzata
- La sua concentrazione determina il potere risolutivo del gel



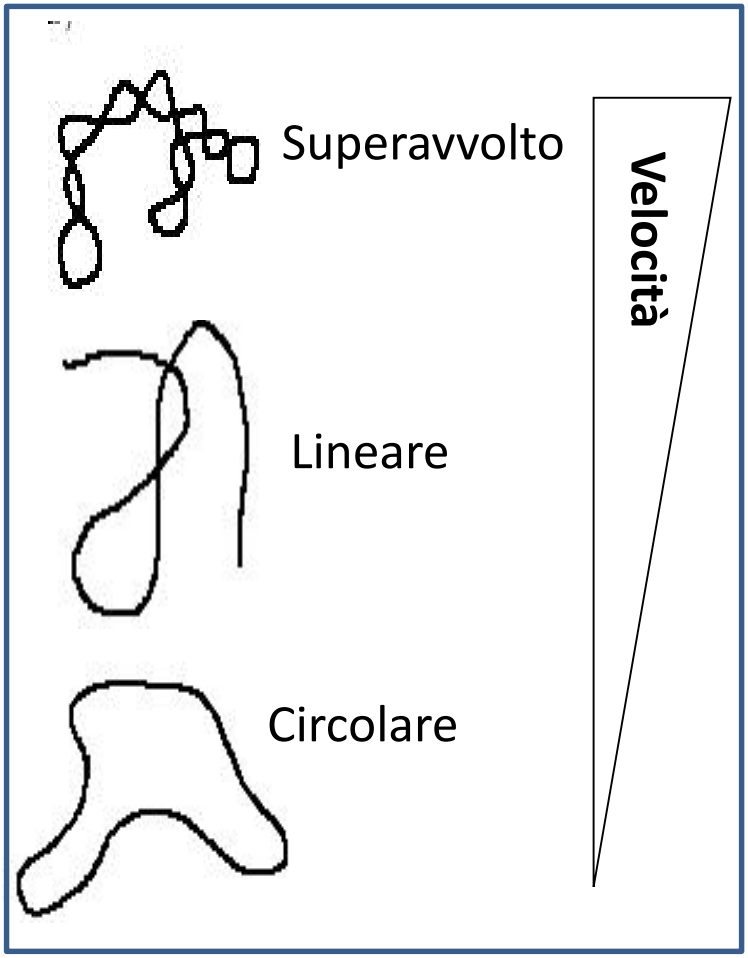
❑ CONFORMAZIONE DEL DNA

DNA superavvolto, lineare e circolare hanno velocità di migrazione diversa anche se di dimensione uguale:

- La forma SUPERAVVOLTA corre più veloce perché è più compatta;

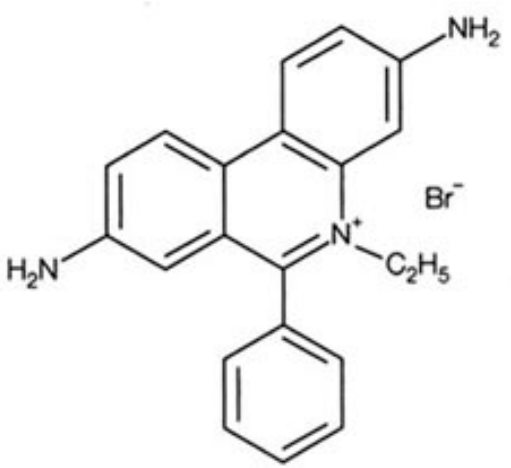
- La forma CIRCOLARE corre più lenta perché è la più "ingombrante" e fa più fatica a muoversi all'interno dei pori del gel;

- La forma LINEARE si colloca a metà (la forma lineare è, ad esempio, quella che si ritrova come prodotto nella PCR).

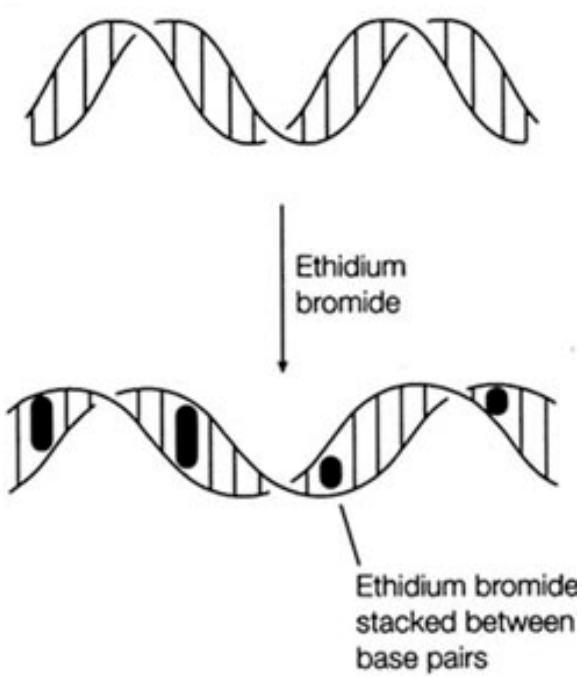


❑ PRESENZA DI BROMURO DI ETIDIO

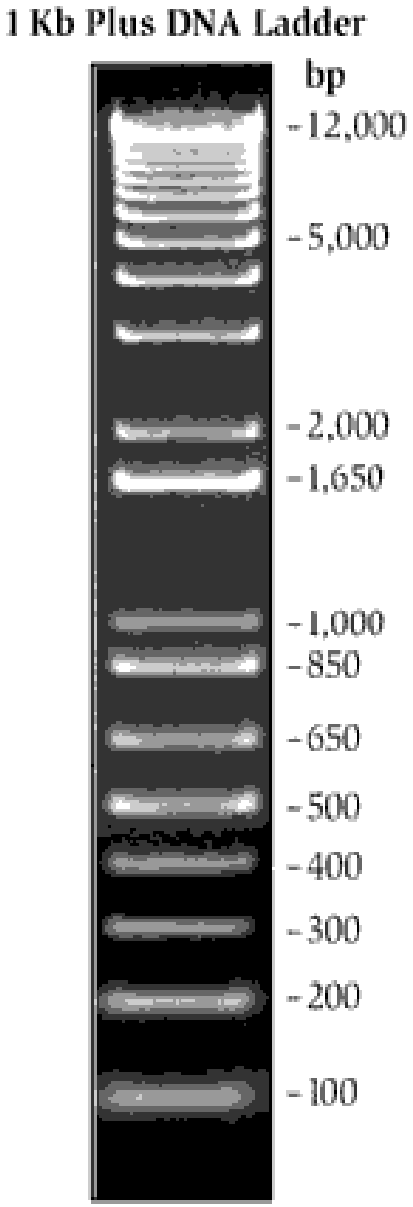
• Colorante fluorescente (intercalante) che consente di visualizzare il DNA

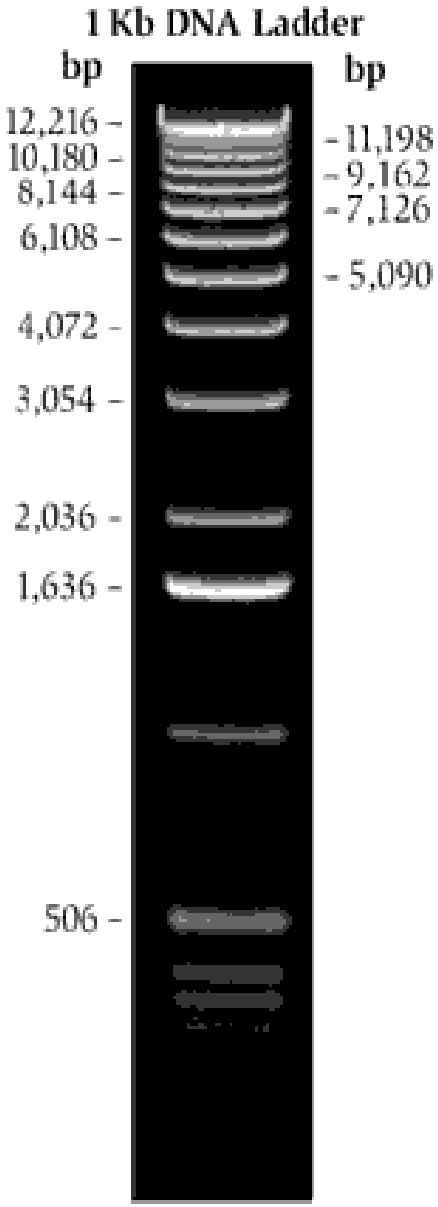


• Assorbimento a 254 nm (U.V.) ed emissione nel visibile (590 nm)



• Riduce la velocità di migrazione di circa il 15%

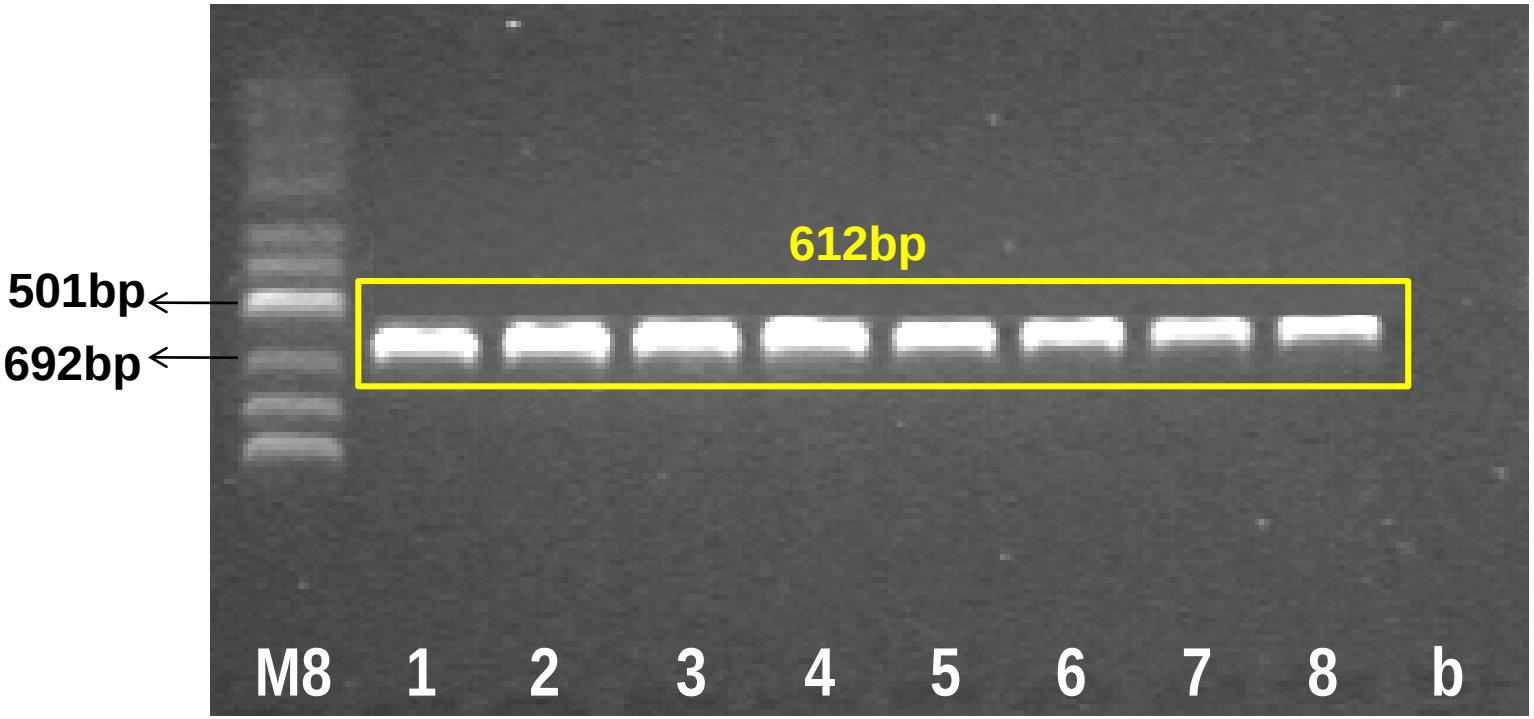




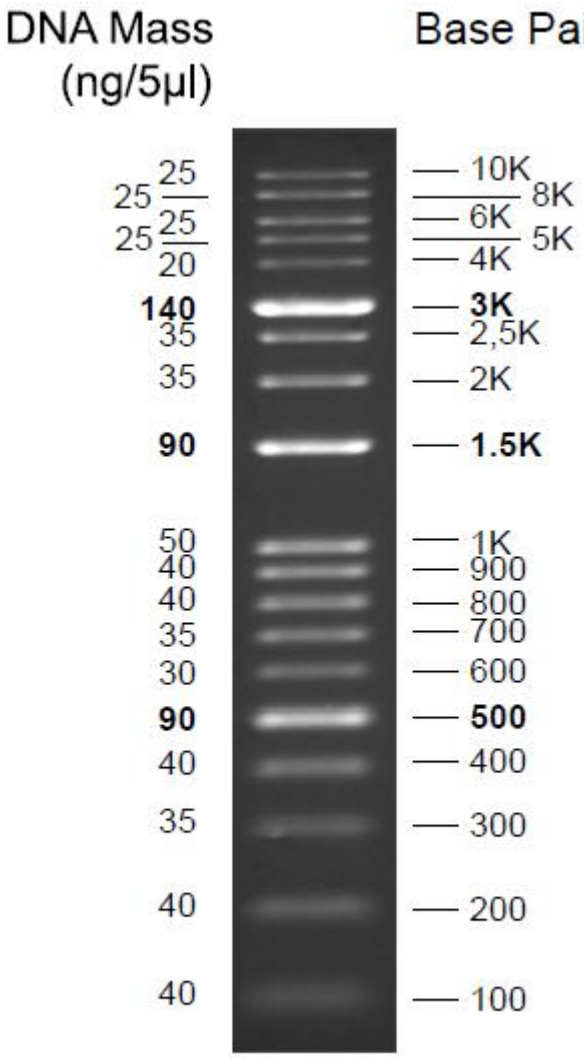
Marcatore di dimensioni:
È costituito da frammenti di DNA aventi <u>dimensioni note</u> e consente di determinare la dimensione del DNA campione

Viene fatto migrare insieme ai campioni di DNA come riferimento dimensionale

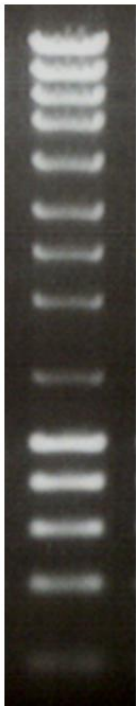
Il risultato della PCR



DNA Quantification by Gel Densitometry



1.5 % TAE agarose gel



SIZE (bp)	ng/BAND
10000	100
7500	75
6000	60
5000	50
4000	40
3000	30
2500	25
2000	20
1400	14
1000	100
800	80
600	60
400	40
200	20

Amount of DNA (ng)	Pixel Density	Density minus Background
250	111,972	83,979
500	167,958	139,965
750	223,944	195,951
1,000	242,606	214,613
background	27,993