BIOTECHNOLOGY

MODULO CLIL

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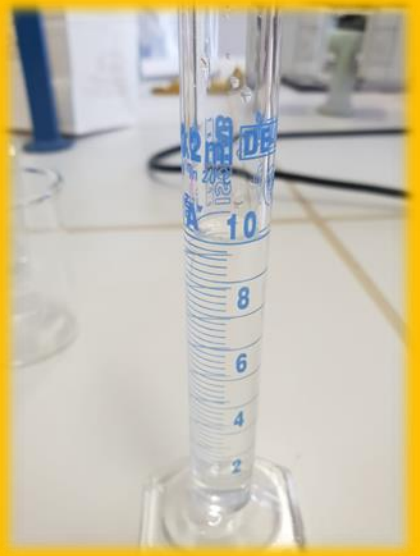
Extracting DNA from a banana

All living things are made up of trillions of microscopic building blocks called cells. cells contains a nucleus that holds a stringy substance called DNA, which is like a set of blueprints, or instructions. DNA contains a code for how to build a life-form and put together the features that make that organism unique.

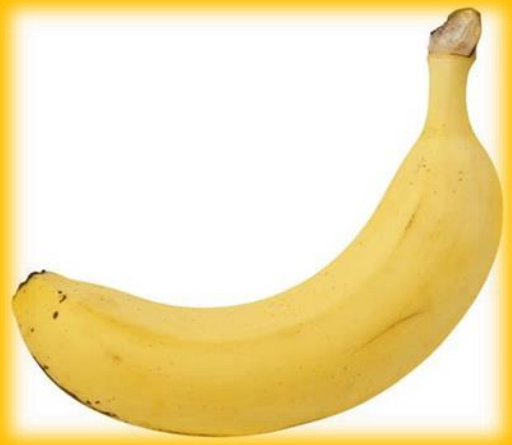
What do we need:

½ banana
Hot water (10 ml)
A teaspoon of salt (3g)
A teaspoon of liquid soap (10ml)
Alcohol-ethanol
A filter
A beaker
A plastic sealable bag
Test tubes
A gratuated cylinder
A wash-bottle
A funnel
A lab-coat



First step

We first peeled the banana, we put it in a resealable zip-top bag and closed the bag. We crushed the banana in the bag (to separate the cells) for about two minutes until it had a fine, pudding like consistency and until all lumps are gone.

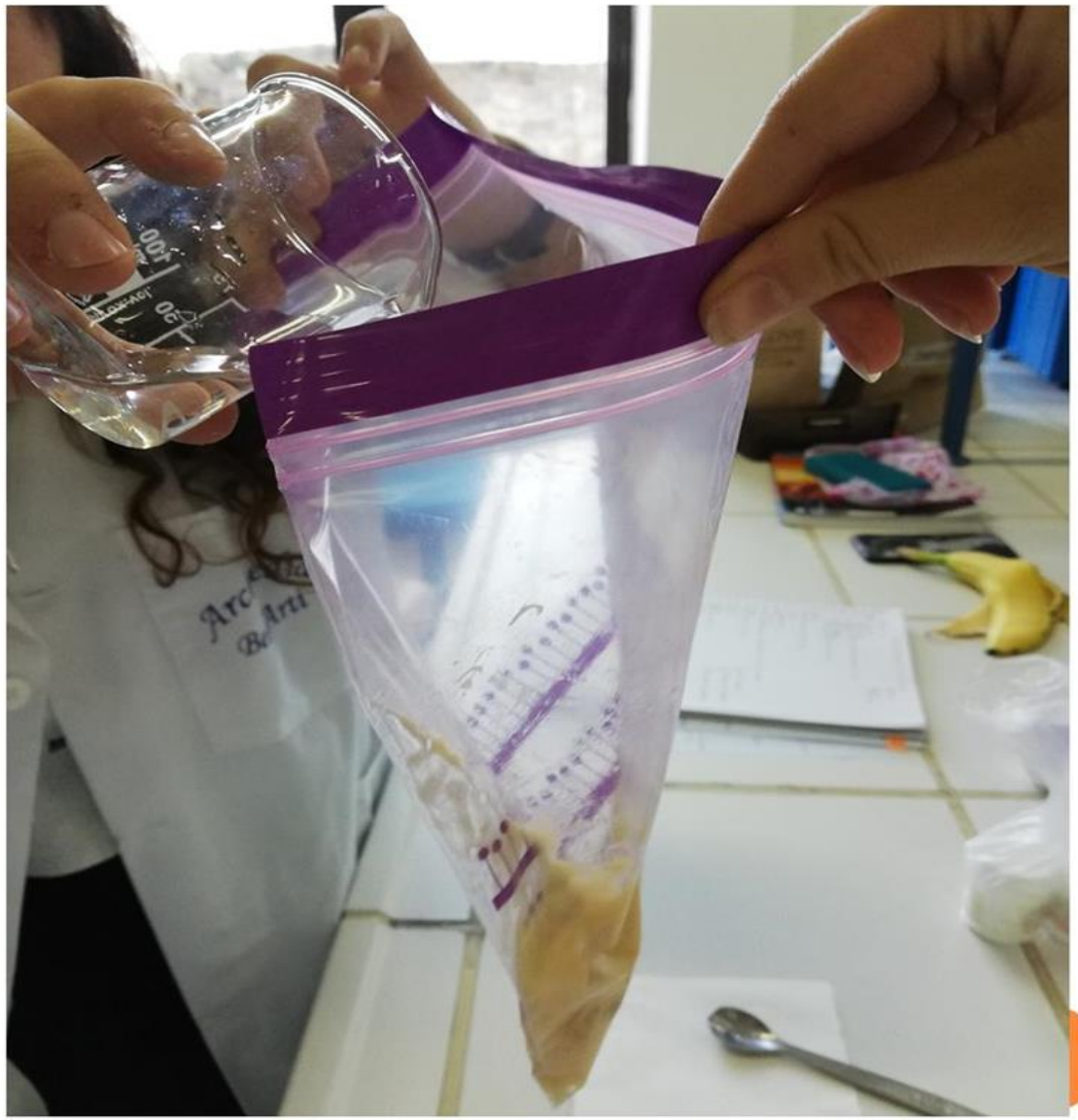


STEP 2

Dissolve a ¼ tsp of cooking salt in a graduated baker with 10ml of distilled water

STEP 3

Pour the salt solution in the plastic bag with the mashed banana and mix them. The salt helps to separate the DNA from other materials in the cell. And because the DNA doesn't dissolve in alcohol, this substance helps the DNA clump together in a separate layer

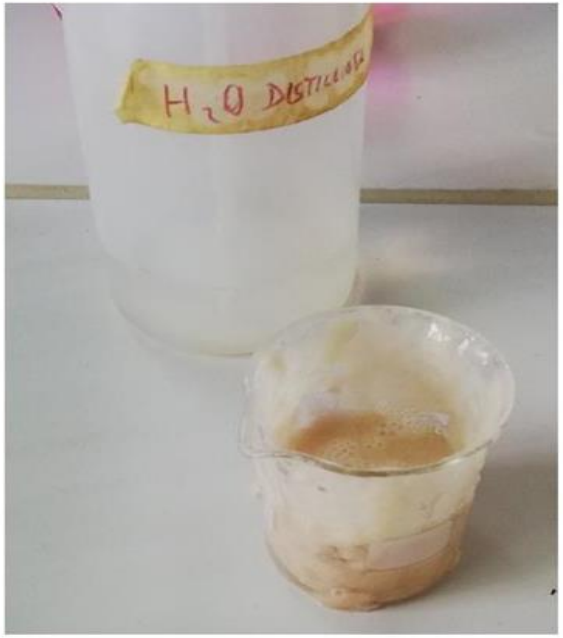


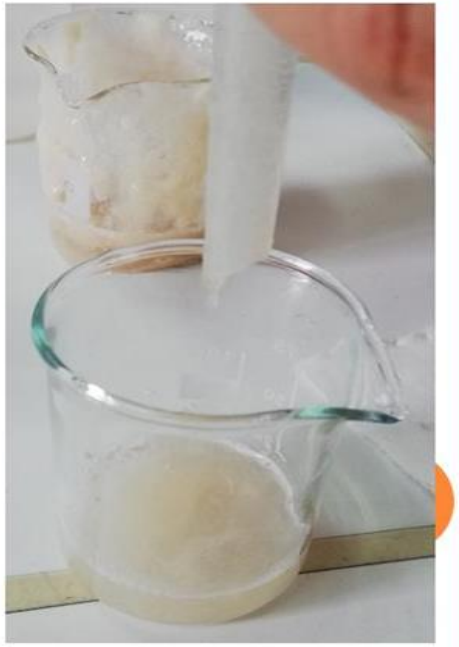
STEP 4

Add 1 tsp of dish detergent in the bag and mix. The detergent helps to break down the cell's outer membrane

STEP 5

Pour contents in a beaker, filter it using a funnel and paper filter to get the extract (we used a taper filter to have a better result) and let it drain into the beaker for few minutes





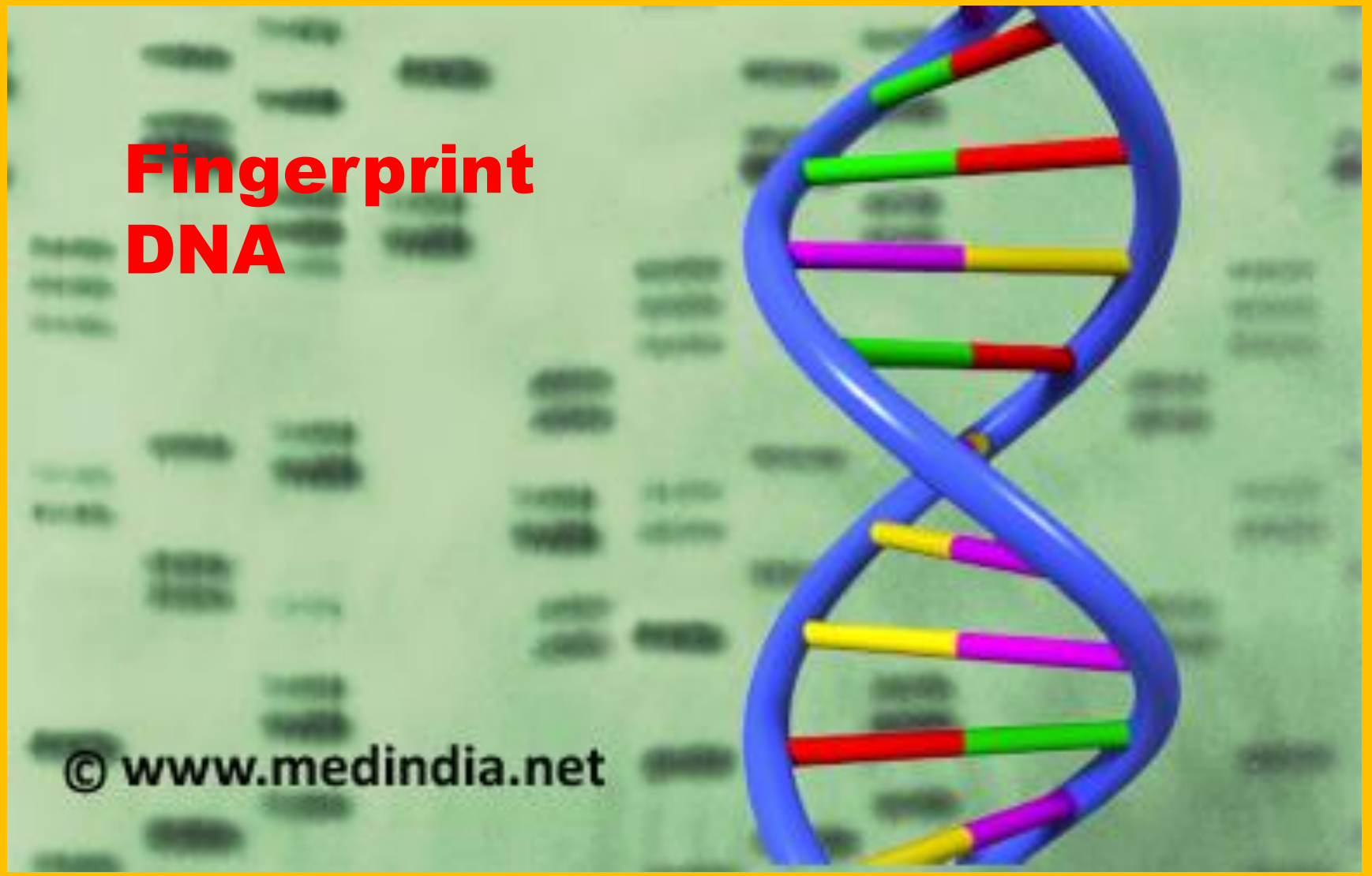
Step 6

Throw away the filter and pour the extract in a test tube adding 5ml of ethyl alcohol. The DNA clumps are soluble (can be dissolved) in some liquids, but not in alcohol. So adding alcohol helps the clumps of DNA to form and let it sit for about 8 minutes. The DNA should be to clump together.

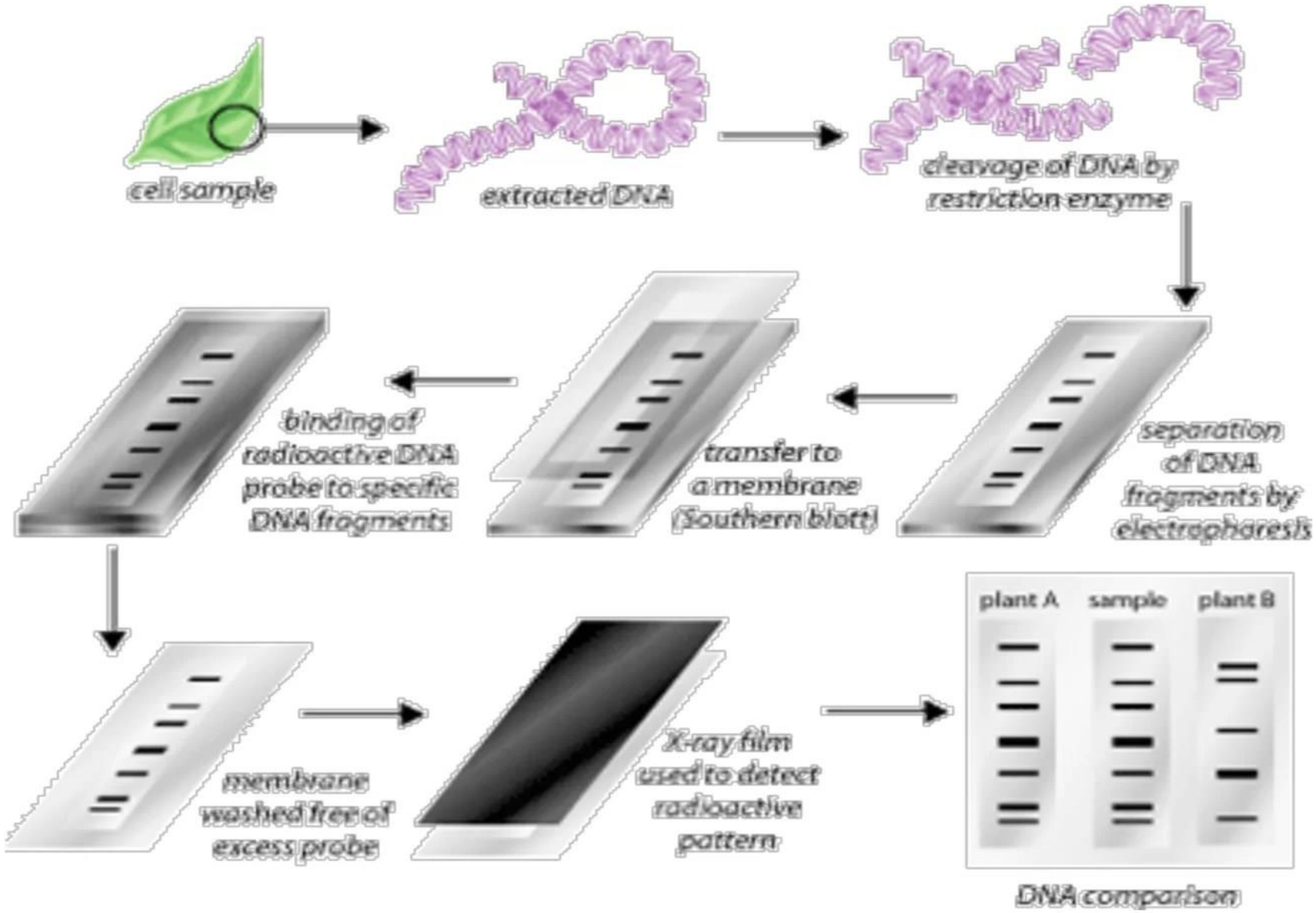
After 8 minutes, the cloudy that has formed is the DNA!

Conclusion

The cloudy that we see is DNA. It has been removed from the millions and millions of cells that make up the banana as we had proposed in the objective.


Fingerprint
DNA
© www.medindia.net

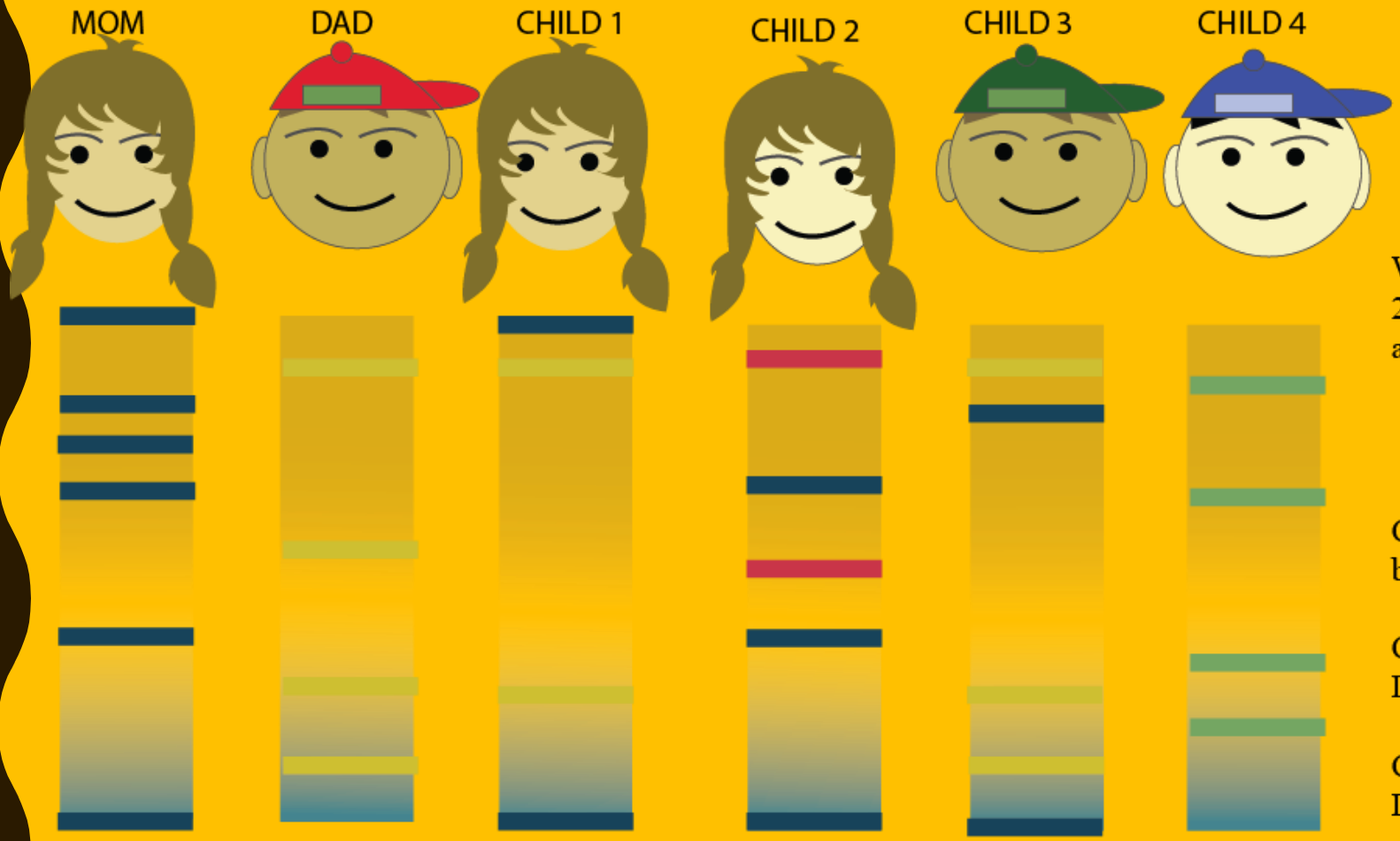
Stages in DNA fingerprinting



DNA Fingerprinting Real World Applications

- Crime scene
- Human relatedness
- Paternity
- Animal relatedness
- Anthropology studies
- Disease-causing organisms
- Food identification
- Human remains
- Monitoring transplants

DNA FINGERPRINTING



When a child is born, they inherate 23 chromosones from the mother and 23 chromosones from the father.

Child 1 and 3 are the childrent of both Mom and Dad.

Child 2 is the child of Mom, but not Dad.

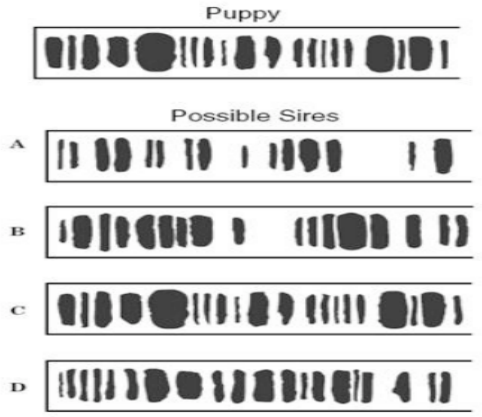
Child 4 is not the child of Mom or Dad.

NAME DATE PERIOD

DNA Fingerprinting / worksheet

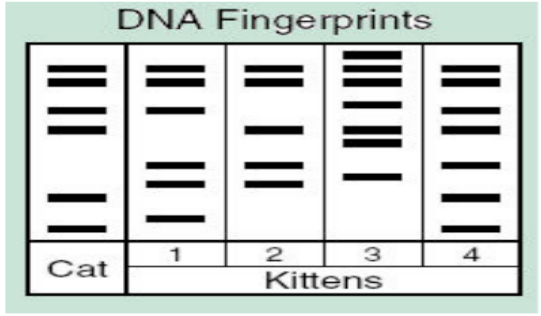
1. The DNA fingerprints were made from blood samples taken from a puppy and four possible sires of this puppy in an effort to determine the puppy's pedigree. According to this information, which sire was probably the father of this puppy? CIRCLE YOUR ANSWER
.

   a. A
   b. B
   c. C
   d. D

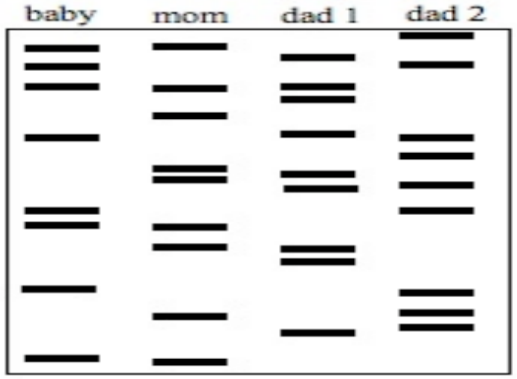


2. The picture shows a segment of DNA from a cat. Which of these is most likely the kitten of this cat? CIRCLE YOUR ANSWER.

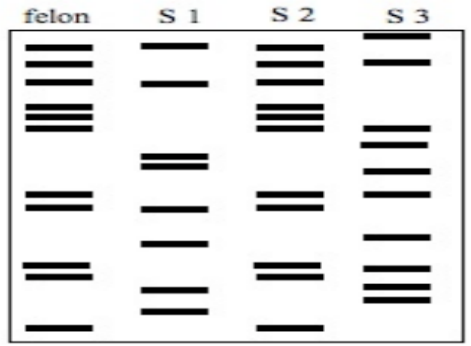
   a. 1
   b. 2
   c. 3
   d. 4



3. Mrs. Smith has a baby named Tyra. She believes one of two men can be the father of her child. A paternity test is done and the results are shown above. Which of the 2 men are baby Tyra's father? _____

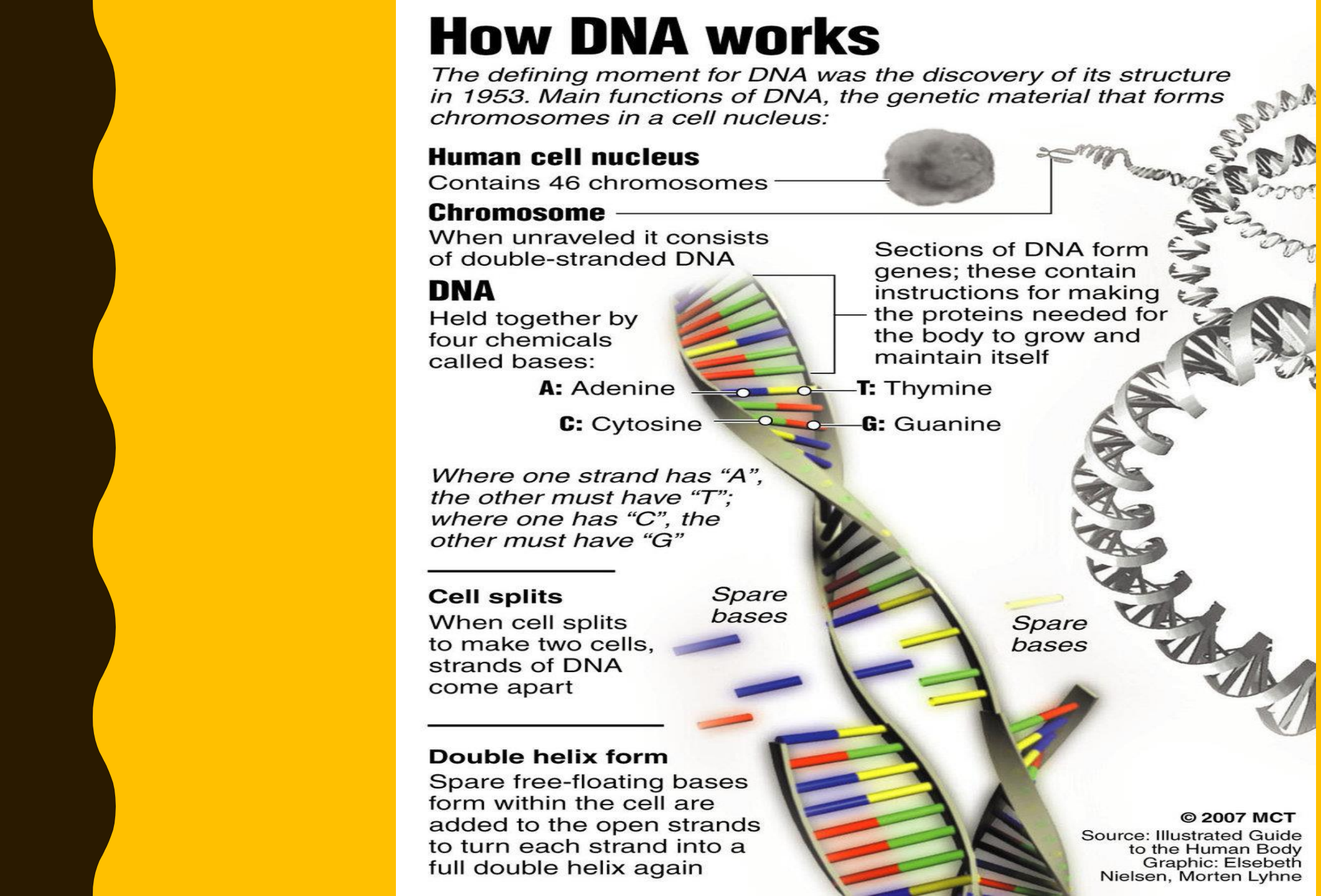


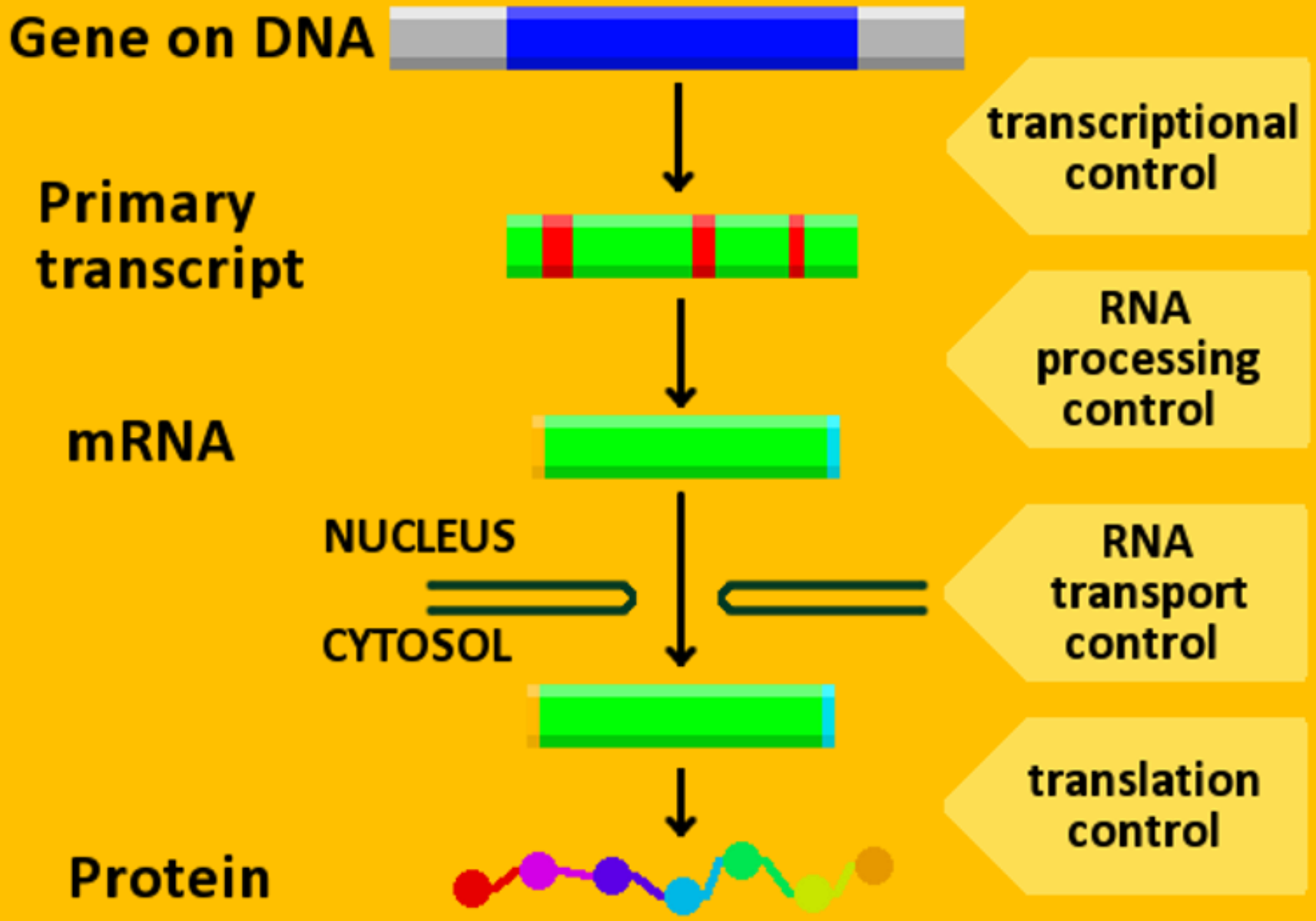
4. Lt. Russ is investigating a murder scene. The felon was scratched by his victim & some of his skin cells were found under the victim's fingernails. A DNA test was performed. Which of the suspects is the murderer? _____



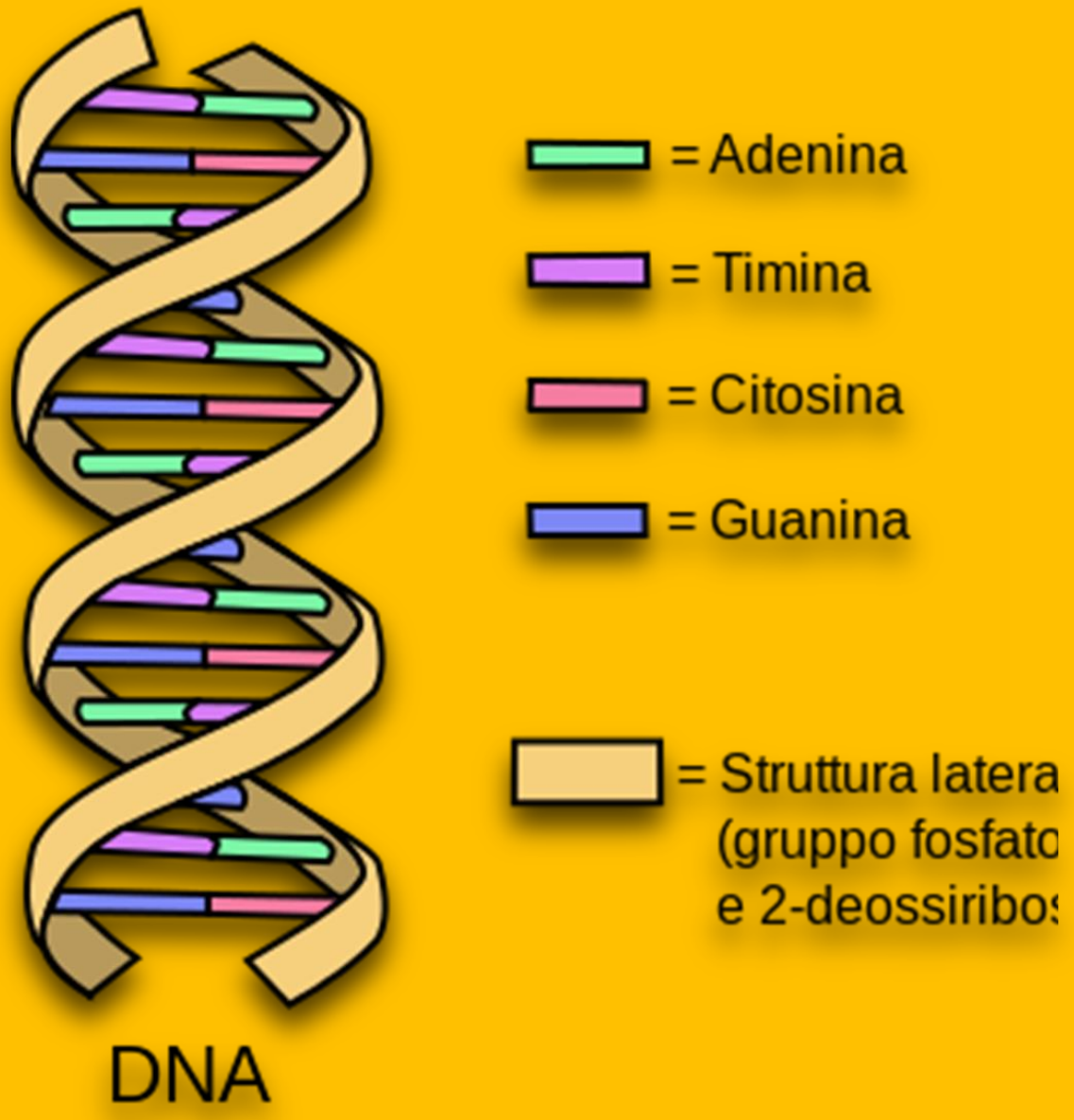
How DNA works

The defining moment for DNA was the discovery of its structure in 1953. Main functions of DNA, the genetic material that forms chromosomes in a cell nucleus:

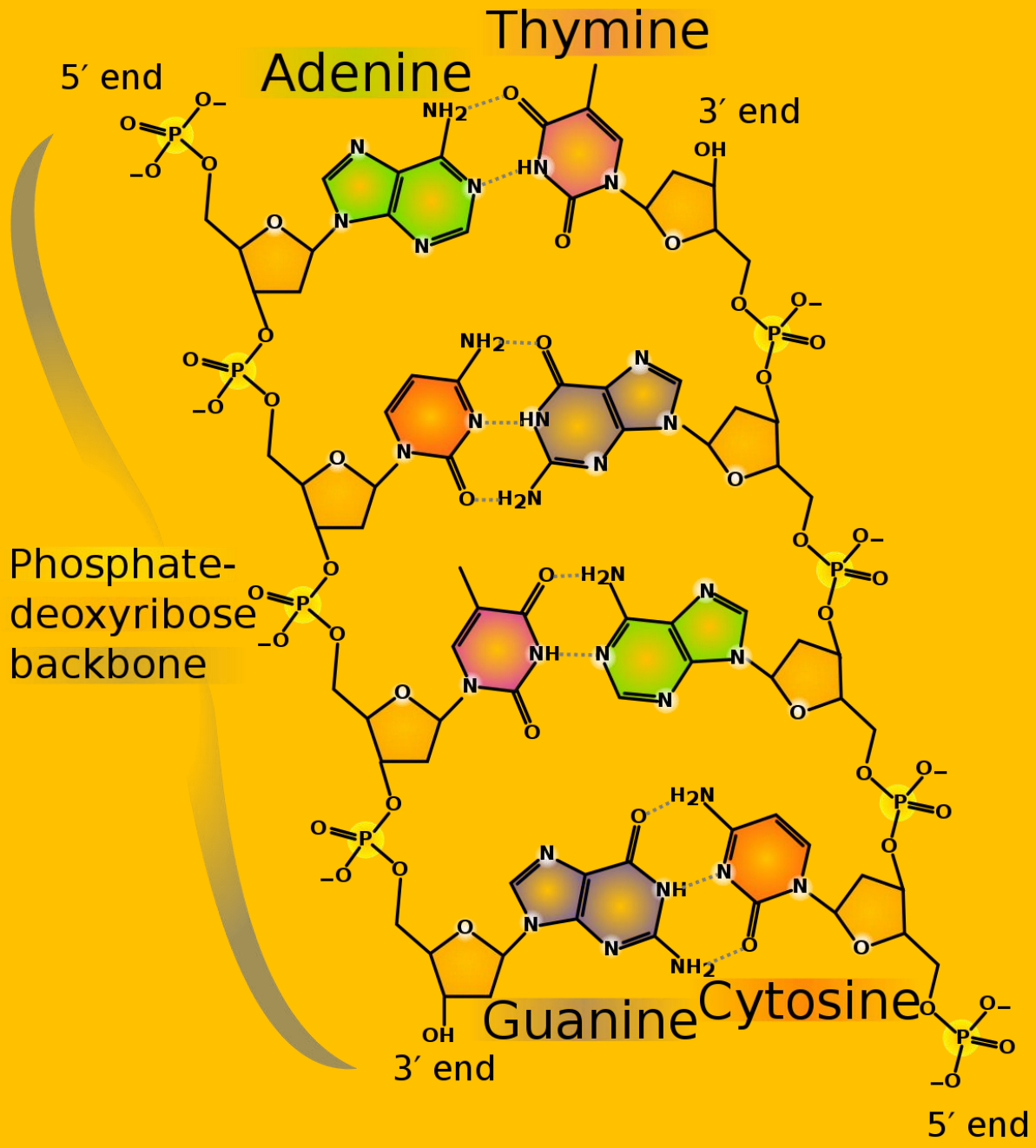


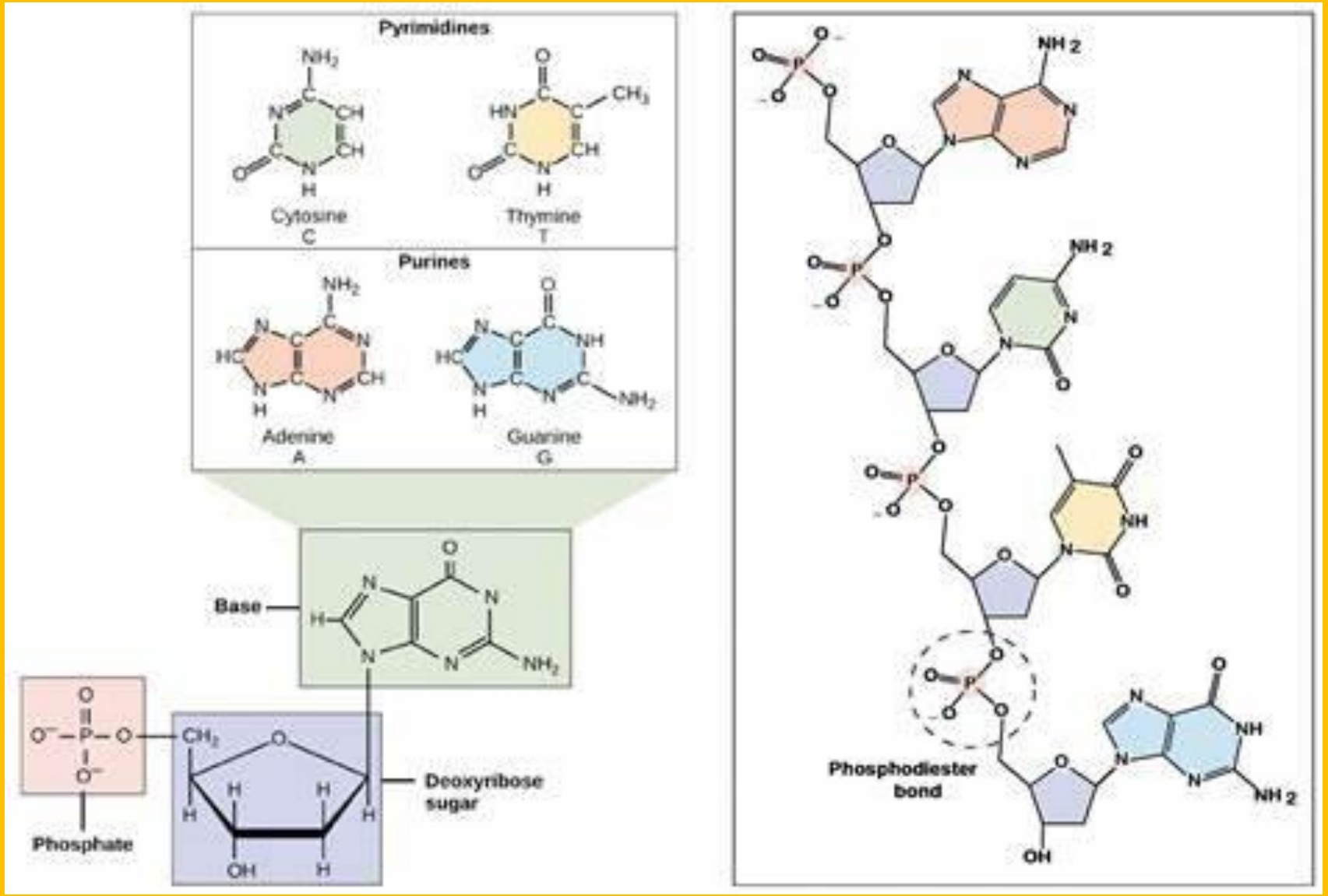

Gene on DNA
Primary transcript
mRNA
NUCLEUS
CYTOSOL
Protein
transcriptional control
RNA processing control
RNA transport control
translation control

What is DNA ?


= Adenina
= Timina
= Citosina
= Guanina
= Struttura latera
(gruppo fosfato
e 2-deossiribos
DNA

DNA Structure



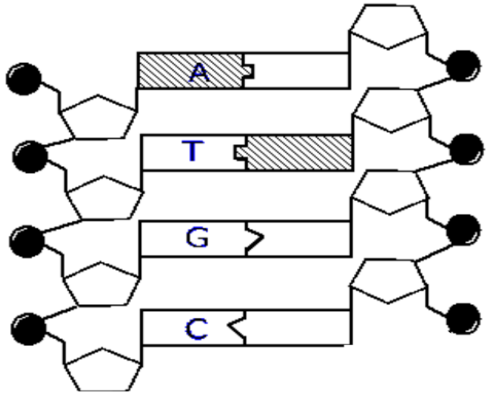

Pyrimidines
Cytosine
C
Thymine
T
Purines
Adenine
A
Guanine
G
Base
Phosphate
Deoxyribose sugar
Phosphodiester bond

DNA Structure, DNA Replication, and Protein Synthesis Review

1. A nucleotide is made of three parts: a _____ group, a five carbon _____, and a nitrogen containing _____
2. In a single strand of DNA, the phosphate group binds to the _____ of the next group.
3. The 5' end of a single DNA strand contains a free _____, while the 3' end contains a free _____.
4. Purines have _____ rings, and pyrimidines have _____ ring.
5. Write out the complete name for DNA: _____

On the diagram:

- Label the 3' and 5' ends.
- Circle a nucleotide.
- Label the sugar and phosphate.
- Label the bases that are not already labeled.



6. The two sides of the DNA helix are held together by _____.
7. The purines are _____ and _____; the pyrimdines are _____ and _____.
8. The term used to describe how the two strands of DNA are oriented is _____, which means _____.
9. In a strand of DNA, the percentage of thymine is 30 %. What is the percentage of cytosine? _____ Adenine? _____ Thymine? _____

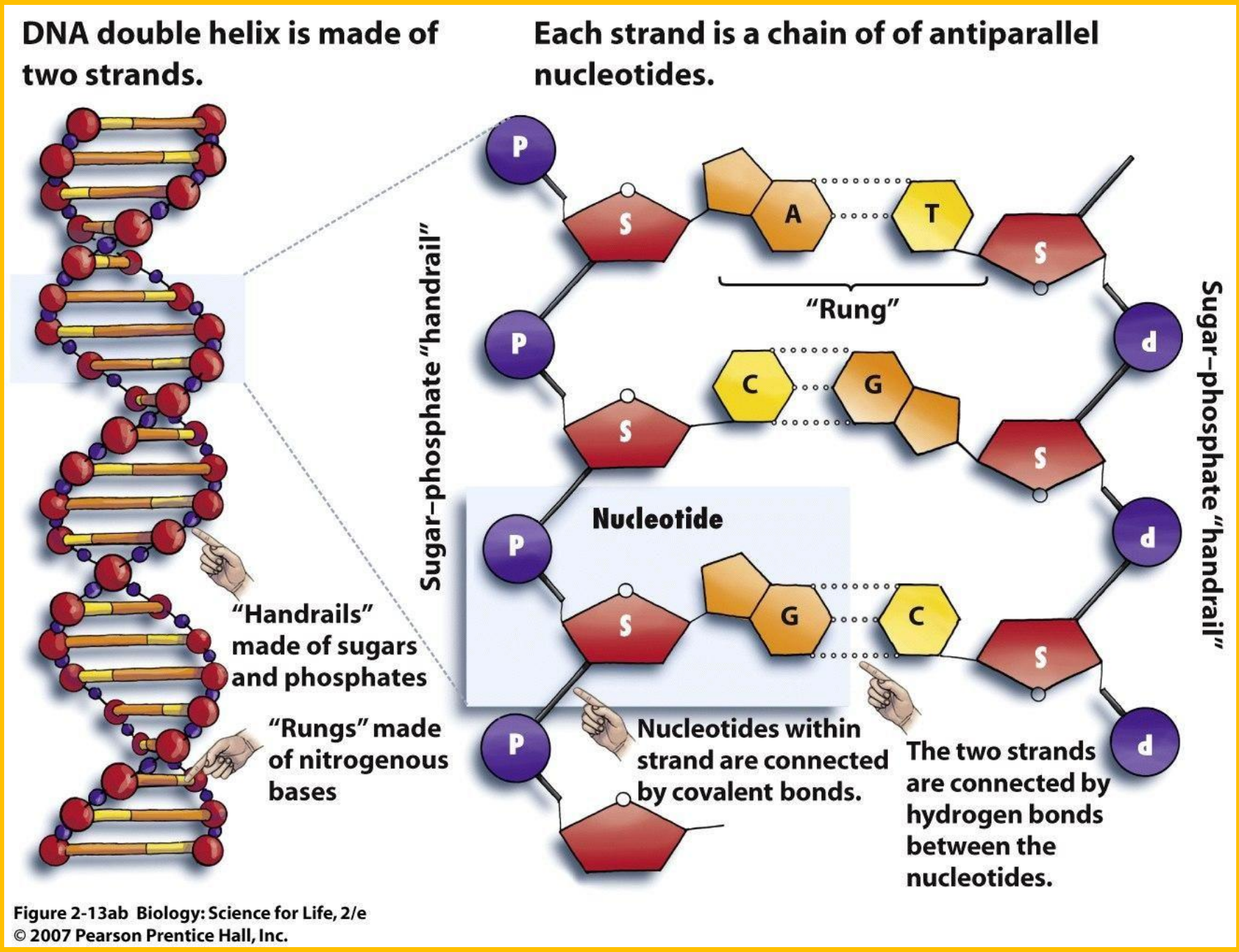
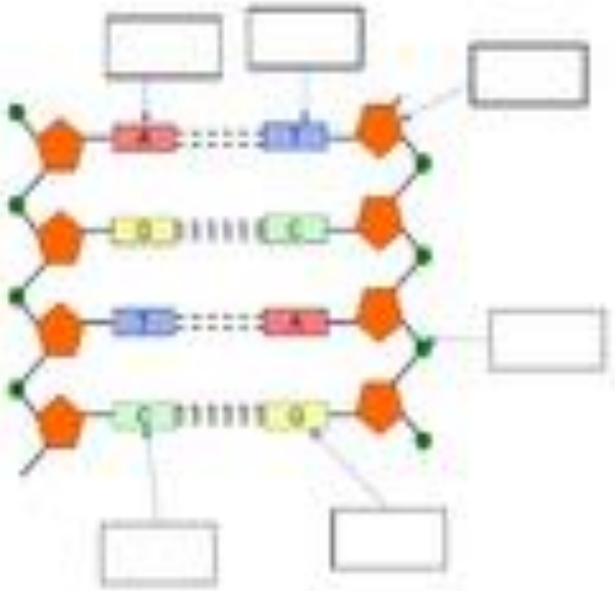


Figure 2-13ab Biology: Science for Life, 2/e

The Structure of DNA

1. Why is the structure called a double helix?

2. Name the bases



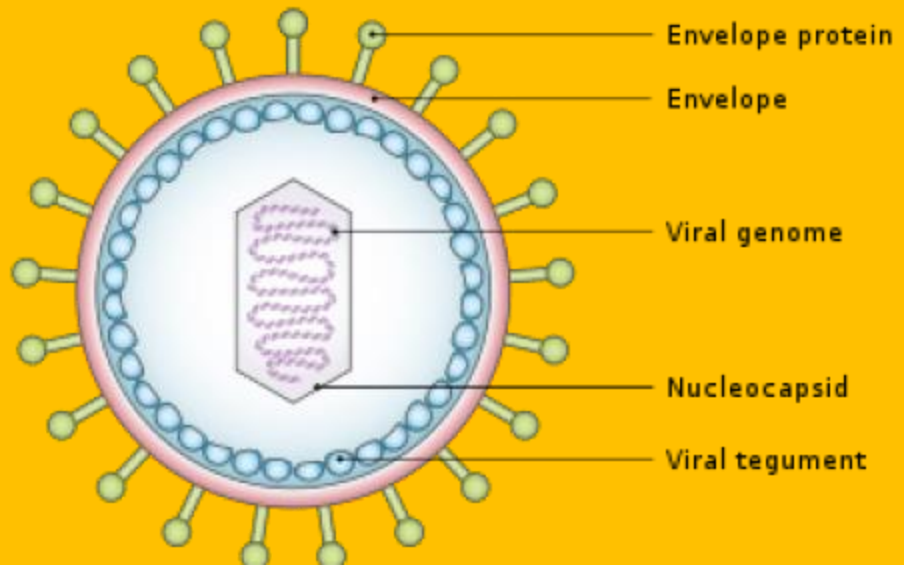
3. List the base pairs

4. What is the backbone of DNA made from?

5. What is a nucleotide?

VIRUS

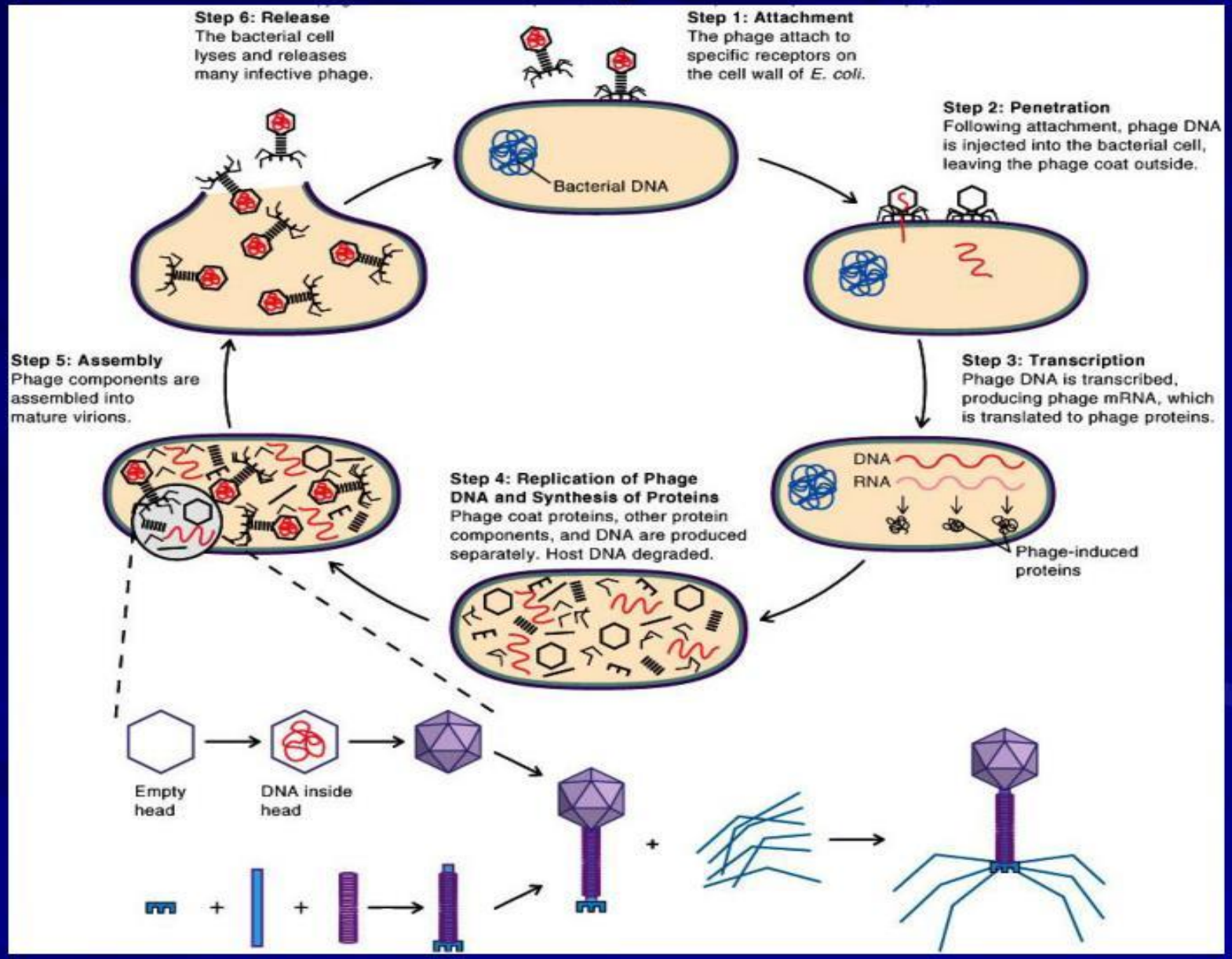
- They are not living organism but microscopic particles. Consisting of a inner core made up of a nucleid acid (DNA or RNA) surrounded by a protein coat (Capsid). Certain viruses are termed «enveloped viruses», their capsid is surrounded by a membrane coat. They replicate only within the living cells of bacteria, animals or plants . They are obligate intracellular parasities .



VIRUS REPLICATION

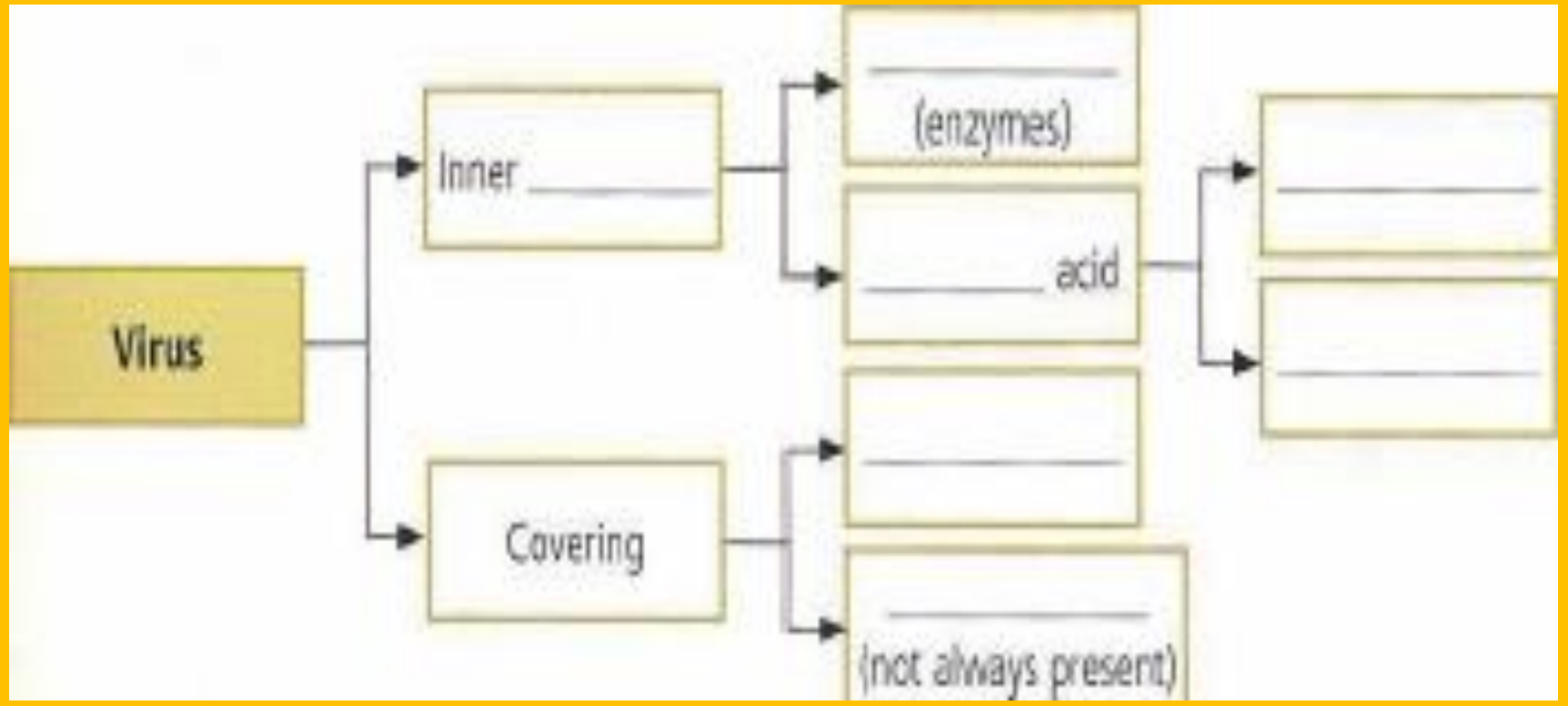
- Viruses recognize specific receptors on the cell membrane and release only their nucleic acid into the host cell cytoplasm, whereas the capside remains outside (bacteriophages). The enveloped viruses enter the host cell entirely. Inside the host cell, the nucleic acid directs the synthesis of new viruses, using the cell biochemical apparatus to synthesize various copies of the capsid proteins and replicate the viral nucleic acid, then to produce new virus particles which are released from the infected cell.

Viral Replication

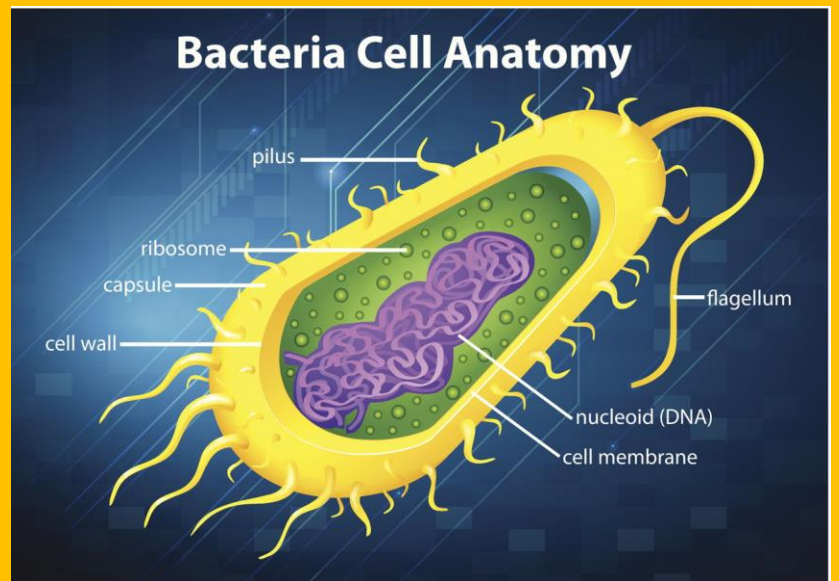


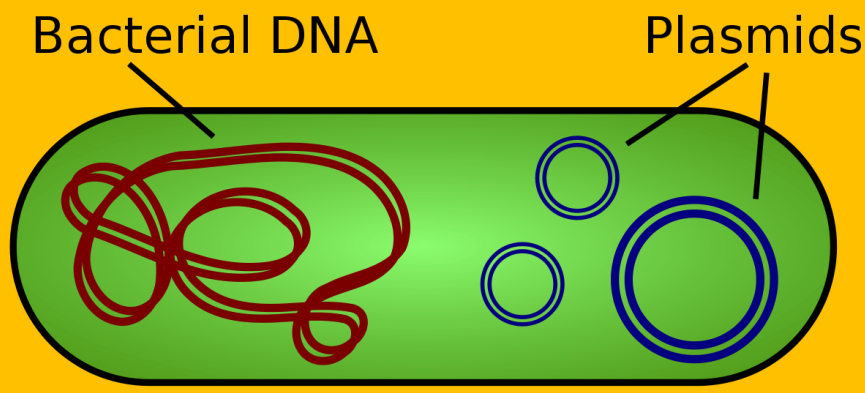
- VIRUS AND BIOTECHNOLOGY
- Since viruses introduce their DNA and RNA into cells as part of their replication cycle, they can be used as cloning vectors, to clone large fragments of DNA, or to produce viral vaccines. The ability of certain viruses to integrate into the cell genome makes these viruses suitable as vehicles, in order to deliver a therapeutic gene directly into the target cell, in gene therapy

EXSERCISES:
IN GROUPS, COMPLETE THE FLOW CHART BELOW ABOUT THE STRUCTURE OF VIRUSES, USING THE WORDS LISTED. THEN COMPARE YOUR CHOICE WITH THE OTHER GROUPS.
VIRUS-NUCLEIC-DNA-RNA--PROTEINS-ENVELOPE-CAPSID-CORE

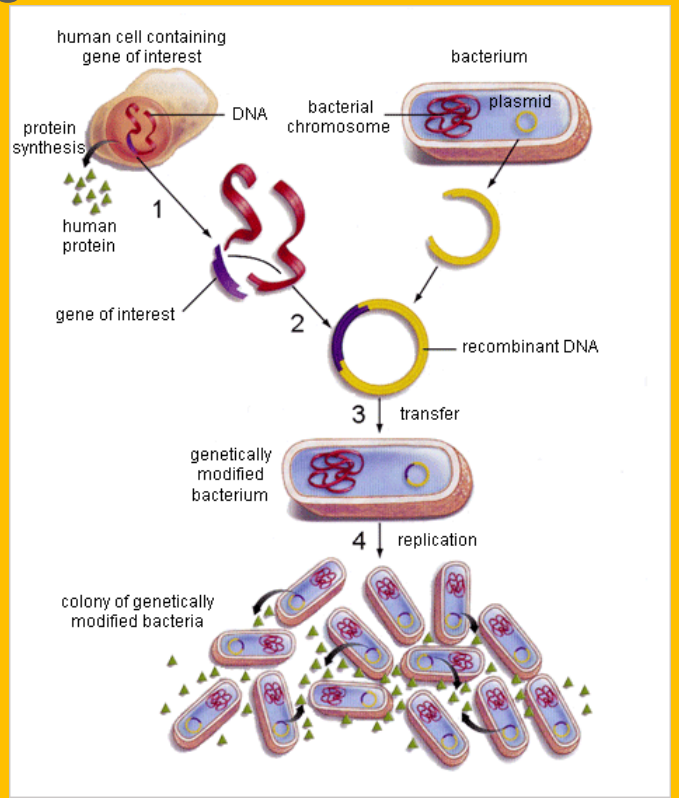


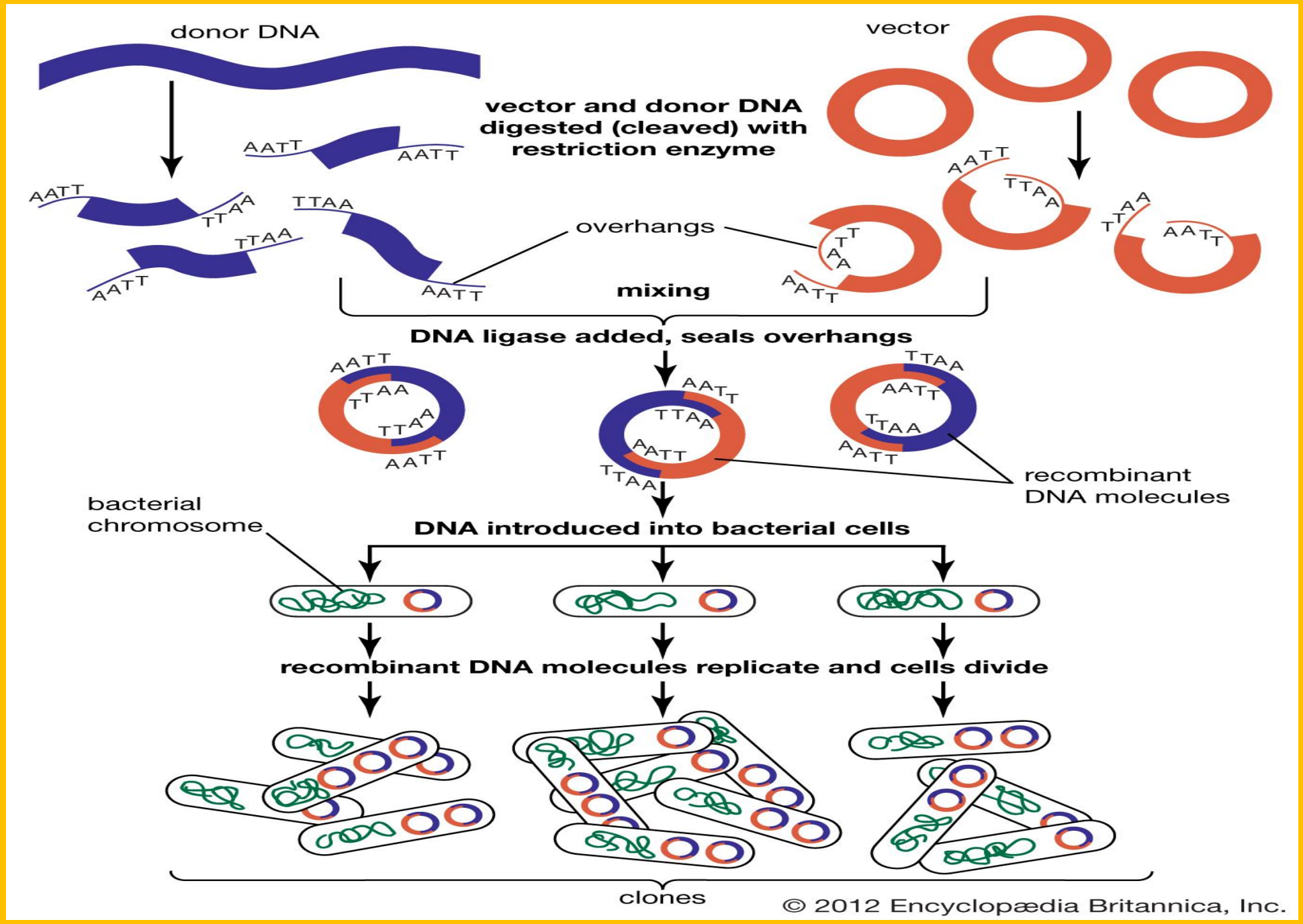
- BACTERIA
- Are prokaryotic unicellular organism, used in molecular biology and genetics to clone genes of interest. Possess a cell wall which can be coated by a capsule composed of polypeptides of D-glutamic acid. The cytoplasm contains a bigger chromosomal DNA and smaller circular DNA molecules called plasmids. Organs of locomotion are: pili or one or more flagella.





- BACTERIA AND BIOTECHNOLOGY
- Biotechnology uses bacteria to make antibiotics, insulin, human growth hormone, vitamins, and other drugs.
- A way to get genes into bacteria easily:
- insert new gene from other organism into plasmid
- ➢ insert plasmid into bacteria
- ➢ bacteria now expresses new gene
- ➢ bacteria make new protein




donor DNA
vector
vector and donor DNA digested (cleaved) with restriction enzyme
overhangs
mixing
DNA ligase added, seals overhangs
recombinant DNA molecules
bacterial chromosome
DNA introduced into bacterial cells
recombinant DNA molecules replicate and cells divide
clones
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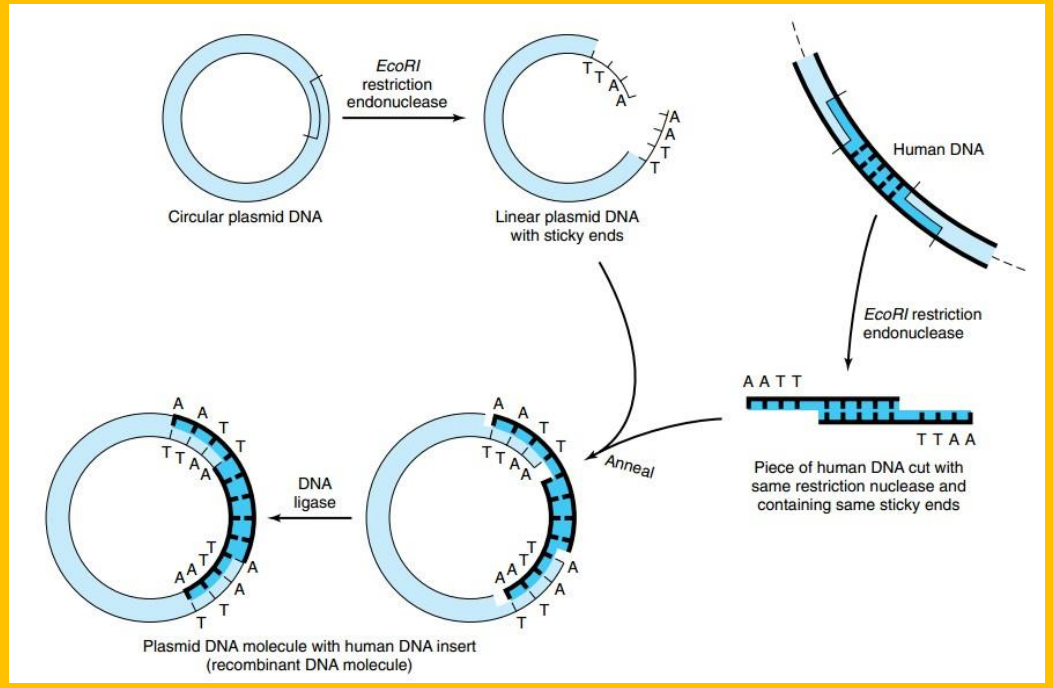
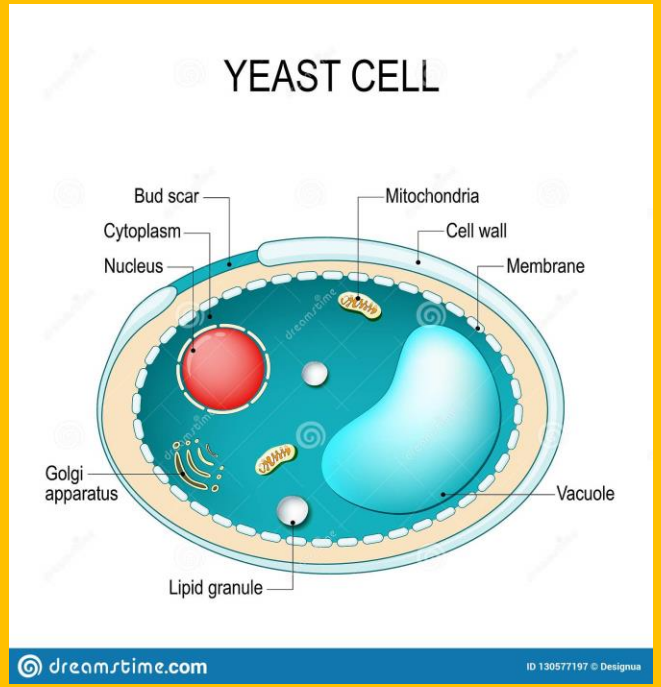
- The bacterial DNA is protected from the cleavage by methylation (process by which methyl groups are added to DNA segments). This modification blocks cleavage, since it makes the restriction sites unrecognizable to the restriction enzymes. These restriction enzymes:
- ➢ cut DNA at specific sequences (restriction site);
- ➢ recognize symmetrical "palindrome" (sequences of four to eight bases that are read in the same way in both directions);
- ➢ produces sticky ends that will bind to any complementary DNA.
- Bacterial restriction enzymes and plasmids can be easily used in recombinant DNA technology to manipulate DNA of an organism.



YEAST CELLS THEY ARE EUKARYOTIC UNICELLULAR ORGANIS

- Yeasts reproducing by budding
- A bud on the parent cell originates a new yeast cell, as a consequence of a division of the cell on a particular site

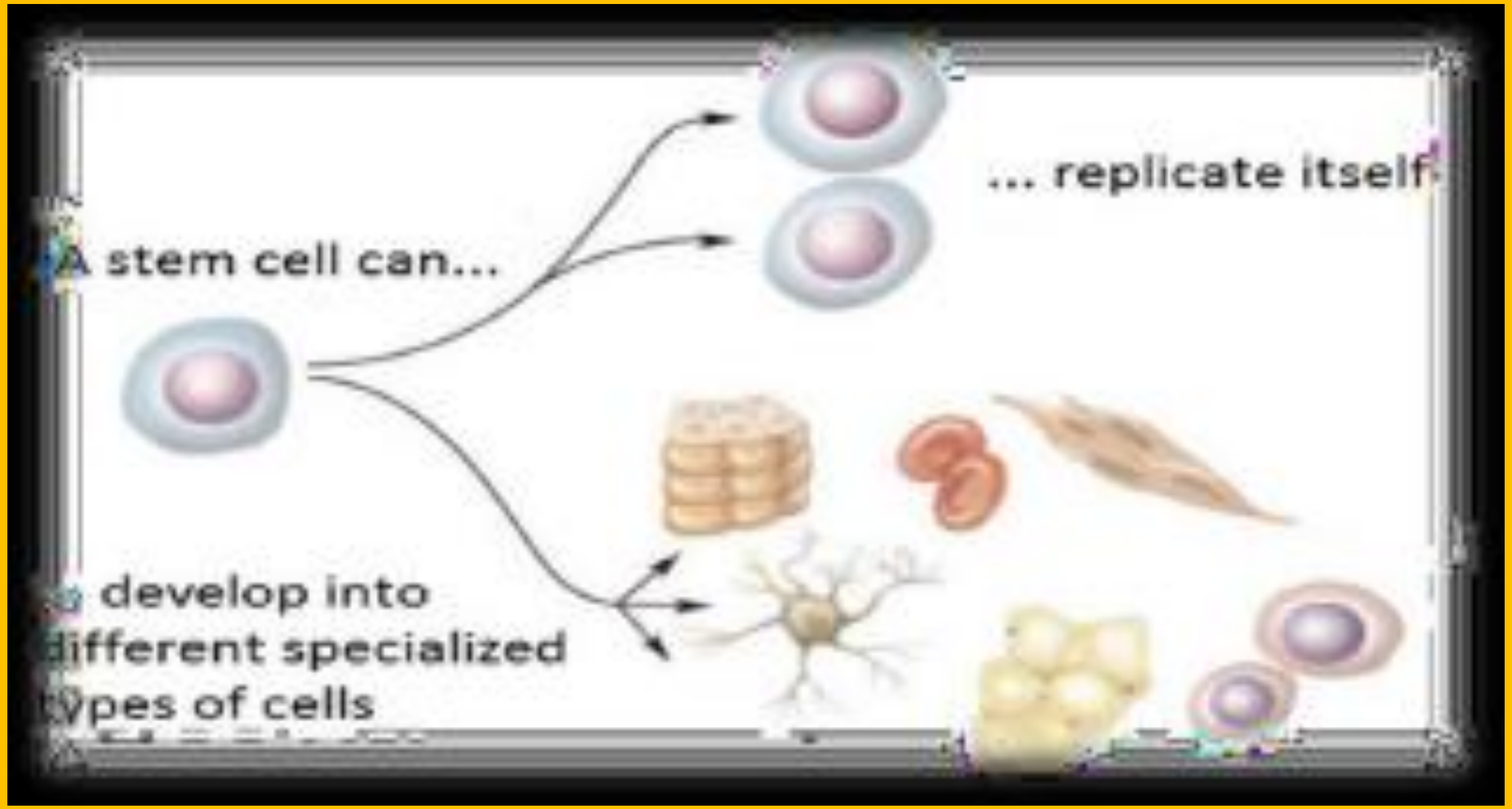


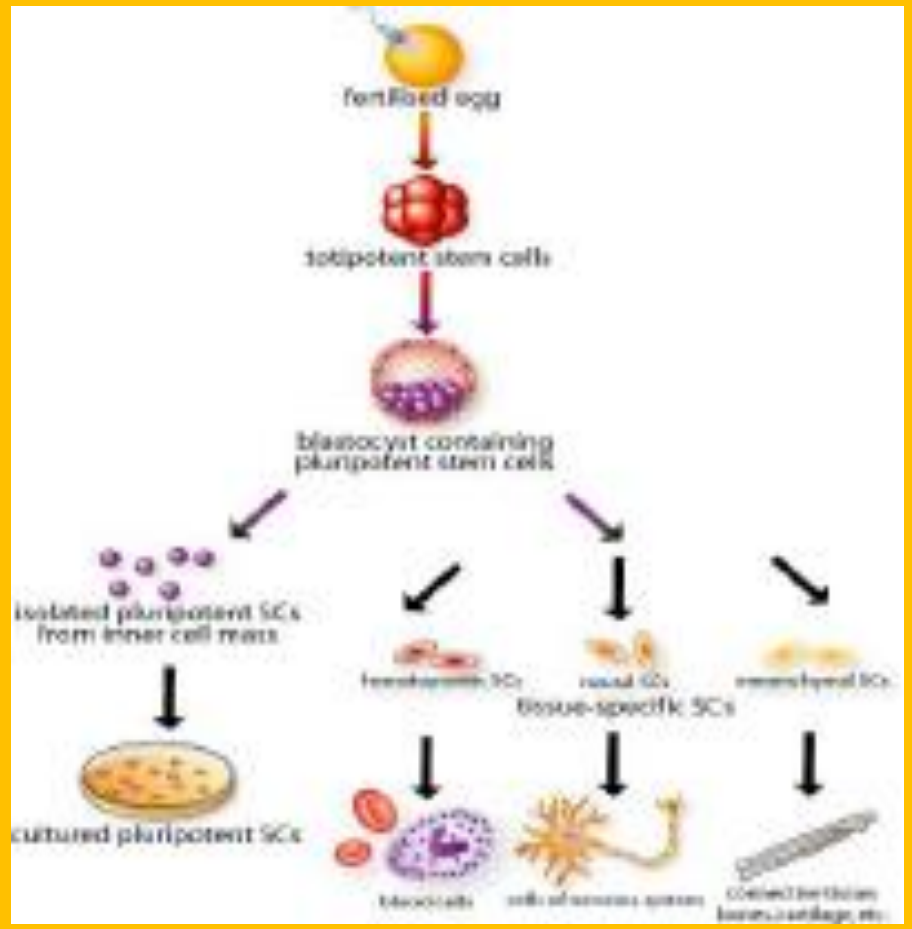
- They have been used for thousands of years in baking and in alcoholic beverage production

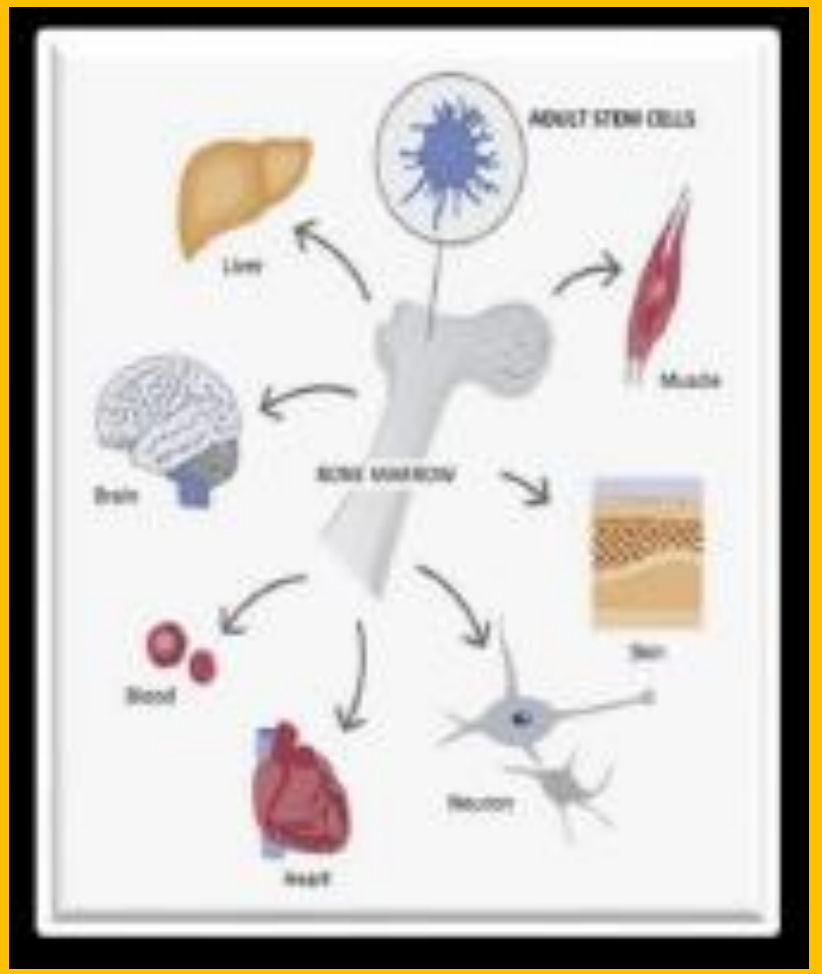
- YEAST CELL AND BIOTECHNOLOGY
- Since the early 1980s the yeast cells have been widely employed in genetics and molecular biology since they can be easily manipulated and cultured in the laboratory. The yeast cells are able to perform different eukaryote-specific post-translational modifications, necessary for proteins to achieve a functional shape. Yeasts are used in research: as protein factories, to express large quantities of eucaryotic proteins; for therapeutic applications; in research to determine their functional and regulatory properties.

STEM CELLS

- They are undifferentiated cells, having the remarkable potential to develop into different specialized cell types in the body. They play important roles in living organisms. For example, the stem cells present during the earliest stage of development of a human embryo, called blastocysts, give rise to all the specialized cell types present in an organism. Stem cells are also found in adult tissues and organs, such as bone marrow, muscle and skin.


A stem cell can...
... replicate itself
develop into
different specialized
types of cells


fertilized egg
totipotent stem cells
blastocyst containing
pluripotent stem cells
isolated pluripotent SCs
from inner cell mass
cultured pluripotent SCs
tissue-specific SCs


ADULT STEM CELLS
Liver
Muscle
Brain
BONE MARROW
Blood
Heart

WHAT DO THEY DO?

- These special cells have the remarkable role of replacing worn out or damaged cell, or act as a repair system, dividing without limits, and replacing the old cells with the new ones, to regenerate the damaged tissue.
- Researchers use human stem cells in various ways:
- ➢ To study the cellular differentiation: the process where a cell changes from one cell type to another. Most commonly the cell changes to a more specialized type.
- ➢ To create tissues that could be used in therapy: tissue culture allows biologists to recreate articular cartilage, nostrils or ears. Thanks to bioreactors, researchers have even managed to create organs with cells from the same organism.
- ➢ To test new drugs: human embryonic stem cells are being used to determinate the toxicity of potential pharmaceuticals.

- Despite their potential, human stem cells are at center of a fierce debate. Research on embryonic stem cells raises an ethical dilemma. We must choose between two moral principles:
- 1) The duty to prevent or relieve suffering
- 2) The duty to respect the value of human life

2 **Mark True (T) or False (F) and then correct the false statements. Check your choices with your partner.**

	T	F
a. Viruses are living organisms.	☐	☐
b. Some viral nucleic acids are able to integrate into the cell chromosome.	☐	☐
c. Plasmids can be used as cloning vectors.	☐	☐
d. Restriction endonucleases cut the bacterial DNA into small fragments.	☐	☐
e. Yeasts are eukaryotic organisms.	☐	☐
f. Yeasts are less suitable than bacteria to express eukaryotic genes.	☐	☐
g. Human embryos contain stem cells.	☐	☐

What is biotechnology?

a. The use of chromosomes to enjoy our lives
b. The use of cells to make our lives better
c. A very old technology

What do they take in?

a. Nutrients from food
b. Nutrients from bacteria
c. Nutrients from drinks

What do they contain?

he blood's material
b. The body's hereditary material
c. The body's material

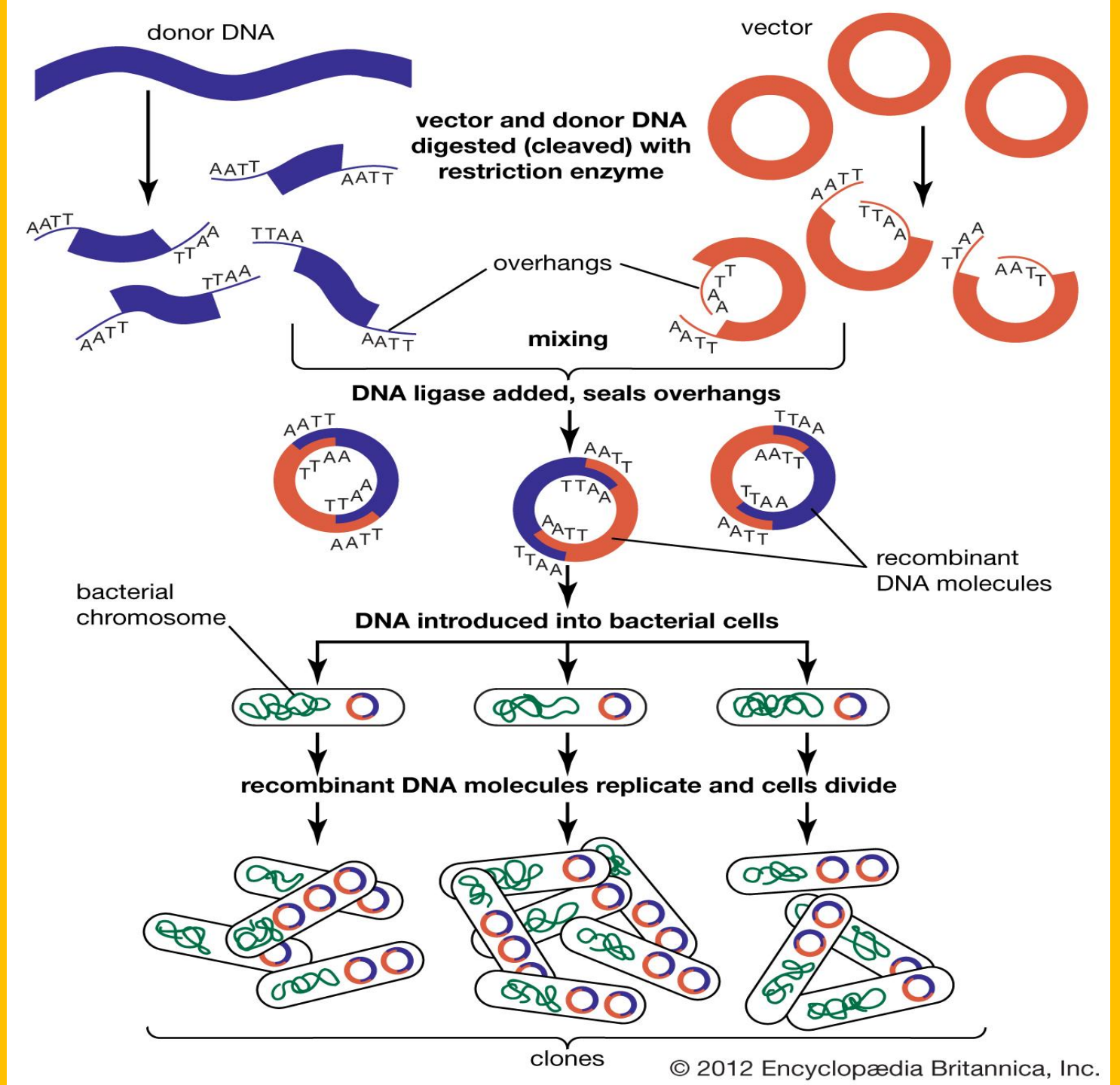
What do almost all cells have?

a. A focus that contains DNA
b. A nucleus that contains DNA
c. The meaning of life

What is recombinant DNA?

A recombinant DNA molecule is created in a laboratory by combining sequences derived from different genetic sources, so as to generate new combinations of genes. Tecnology of recombinant DNA is based on the following criteria:

1. identify the gene which will be transferred;
2. cut (by restriction enzymes) DNA of the donor in fragments to isolate the gene of interest;
3. divide the fragments of restriction and sequence them;
4. product many identical copies of the gene (genetic cloning or PCR, Polymerasis Chain Reaction);
5. introduce the fragments of DNA in a vector (virus or plasmid);
6. cultivate colonies of bacteria or viruses containing, in each colony, only one fragment of DNA;
7. select the colonies which contain the gene target of our study.


donor DNA
vector
vector and donor DNA digested (cleaved) with restriction enzyme
overhangs
mixing
DNA ligase added, seals overhangs
recombinant DNA molecules
bacterial chromosome
DNA introduced into bacterial cells
recombinant DNA molecules replicate and cells divide
clones
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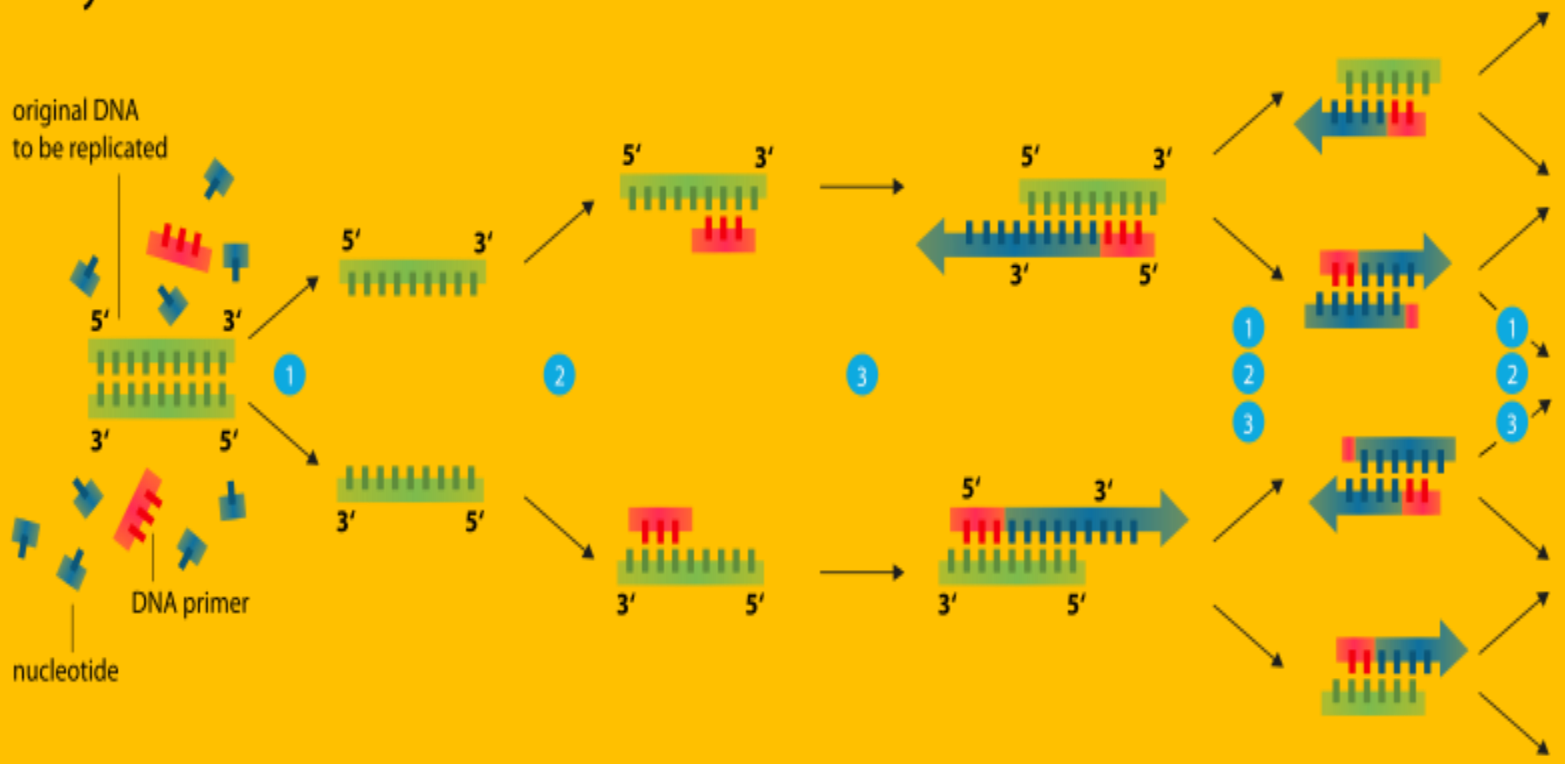
Polymerase chain reaction - PCR



1 **Denaturation** at 94-96°C

2 **Annealing** at ~68°C

3 **Elongation** at ca. 72 °C

Biotechnology and its applications Biotechonlogy refers to the application of technology to modify and improve the biological function of an organism by modifying its genome. For centuries, people have been using microorganisms, in order to make bread, wine, cheese, and to preserve dairy products. Today biotechnology industry relies on recombinant DNA technology and genetic engineering techniques. Biotechnology embraces different fields: medicine, research, agriculture and environment. Biotechnology in Medicine and Research Today, recombinant DNA technology makes it possible to synthesize vaccines or therapeutic products. Vaccines and medical drugs are produced using transgenic organisms that have been genetically engineered to produce a specific pharmaceutical of interest. These kinds of drugs named biopharmaceuticals: the recombinant Growth hormone and the recombinant Factor VIII and Factor IX are only three examples.

Growth hormone GH is a protein hormone secreted by the pituitary gland, which stimulates growth and cell reproduction. Unlike in the past, GH is produced through biotechnology and used in children and adult who suffer from GH deficiency .

Factor VIII and Factor IX Haemophilia is a genetically inherited disorder caused by the deficiency in two proteins **required for blood clotting Factor VIII (haemophilia A) Factor IX** (haemophilia B) People affected by haemophilia need intravenous infusions of these clotting factors, to prevent bleeding episodes In the past these proteins were obtained from fresh frozen plasma.
In this way, deseases like hepatitis B and AIDS could be transmitted. The problem were solved when biologists were able to cloning and product recombinant coagulation factors. To understand how a certain gene works, scientists started to study mice that have been genetically engineered by the inactivation of that gene. In fact, in this way, physical and biochemical characteristics are altered Comparing the obtained data with the normal data from normal mice, it's possible to understand how the gene normally works.
Monoclonal antibodies Engineered molecules, produced by transgenic rats, that recognize cancer cells mimicking the

Depending on the kind of tumor to be treated they can be synthesized in several ways and are used to:

- to mark cancer cells in particular, to be more easily recognized and eliminated by the immune system;
- be combined with a radioactive particle, to provide radiation to the tumor cell;
- to recognize and block the extra copies of the growth factor receptors synthesized by cancer cells.

Specific antigens are synthesized thanks to genes cloned from genomic DNA, used to prepare vaccines. Hepatitis B vaccine, prepared using one of the viral envelope proteins HbsAg (hepatitis B surface antigen), copied by recombinant yeast cells where the gene for HBsAg is inserted.

Biotechnology in Agricolture-GMO Plants

GMO plants can be obtained by manipulating an existing gene, to create a new allele, or by introducing a new gene in a cell or an organism to form a transgenic organism. Genetically modified crops (GMCs, GM crops, or biotech crops) are plants used agricolture, the DNA of which has been modified using genetic engineering techniques. In most cases, the aim is to introduce a new trait to the plant which does not occur naturally in the species. Examples in food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to a herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation.

Why create gmo plants?

1) They are created to make them more resistant to viral desease, insects, and herbicedes
2) To make them more resistant to etreme conditions of temperature, pH or salinity
3) To improve their taste and nutritional values

Colorado Potato Beetle Resistant Potato This variety of potato is an instance of a genetically engineered potato plant that is able to synthesize its own insecticide, naturally produced by the bacterium Bacillus thuringiensis

How to produce Beetle Resistant Potato They have been produced by introducing the gene encoding the CryIIIA protein from the bacterium into the potato genome, this technique is called Agrobacterium tumefaciens-mediated transformation. The analysis of nutrients did not reveal any signifcant differences between the transgenic lines and the normal potatoes, they are extremely unlike to be allergenic and devoid of any potential toxicity.

Golden rice. Golden rice is an example of a biofortified crop, having the capacity to produce β-carotene, a precursor of vitamin A, in its grains Rice plants produce provitamin A only in the green tissue, whereas the grains, which are the only edible part, do not. Thanks to promoter, the gene responsible for beta-carotene synthesis was expressed in the endosperm
Golden rice 2 The anti-GMO criticized that the Golden rice did not have sufficient vitamin A. This problem was solved in 2005 with the production of GOLDEN RICE 2. This variety was able to synthesize obout 20 times more carotenoids than the original Golden rice

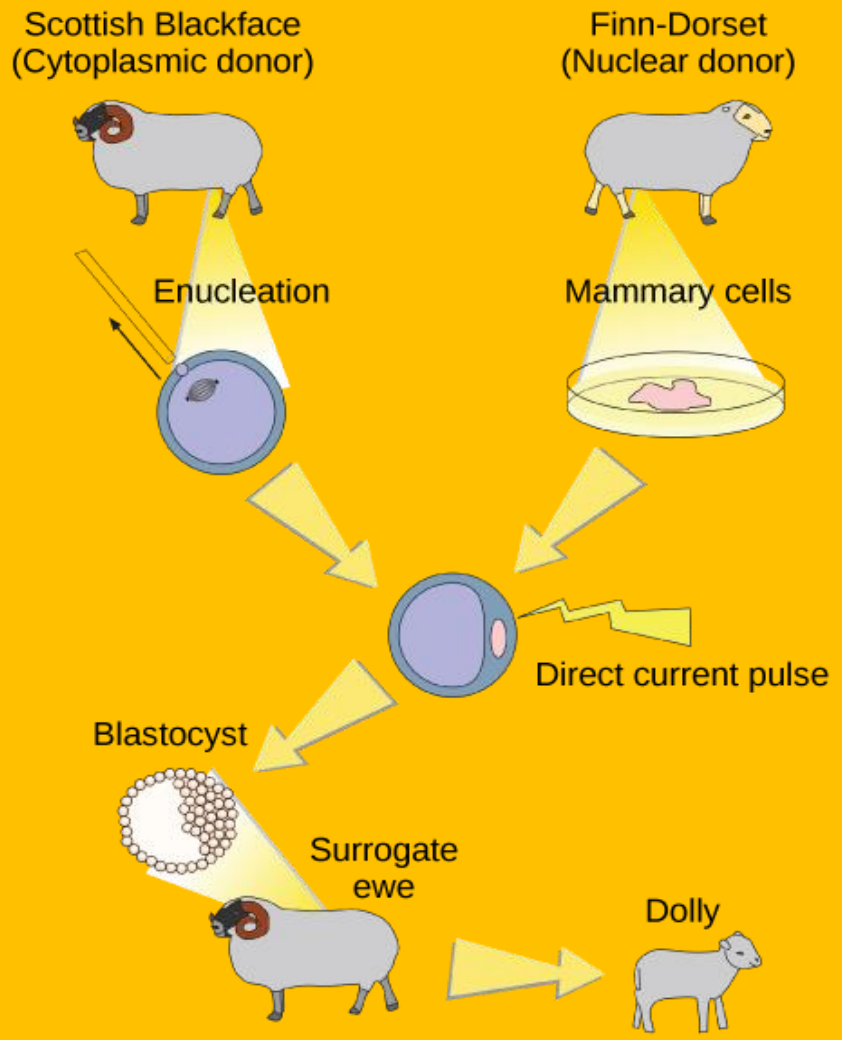
A clone has the same DNA sequence as its parent and so they are genetically identical

Dolly the sheep, as the first mammal to be cloned from an adult cell, is by far the world's most famous clone. However, cloning has existed in nature since the dawn of life. The plants have been cloned: a cutting is capable of producing a clone of the original plant; identical human twins are they clones. Several clones had been produced in the lab before Dolly, including frogs, mice, and cows, which had all been cloned from the DNA from embryos. Dolly was remarkable in being the first mammal to be cloned from an adult cell. This was a major scientific achievement as it demonstrated that the DNA from adult cells, despite having specialised as one particular type of cell, can be used to create an entire organism

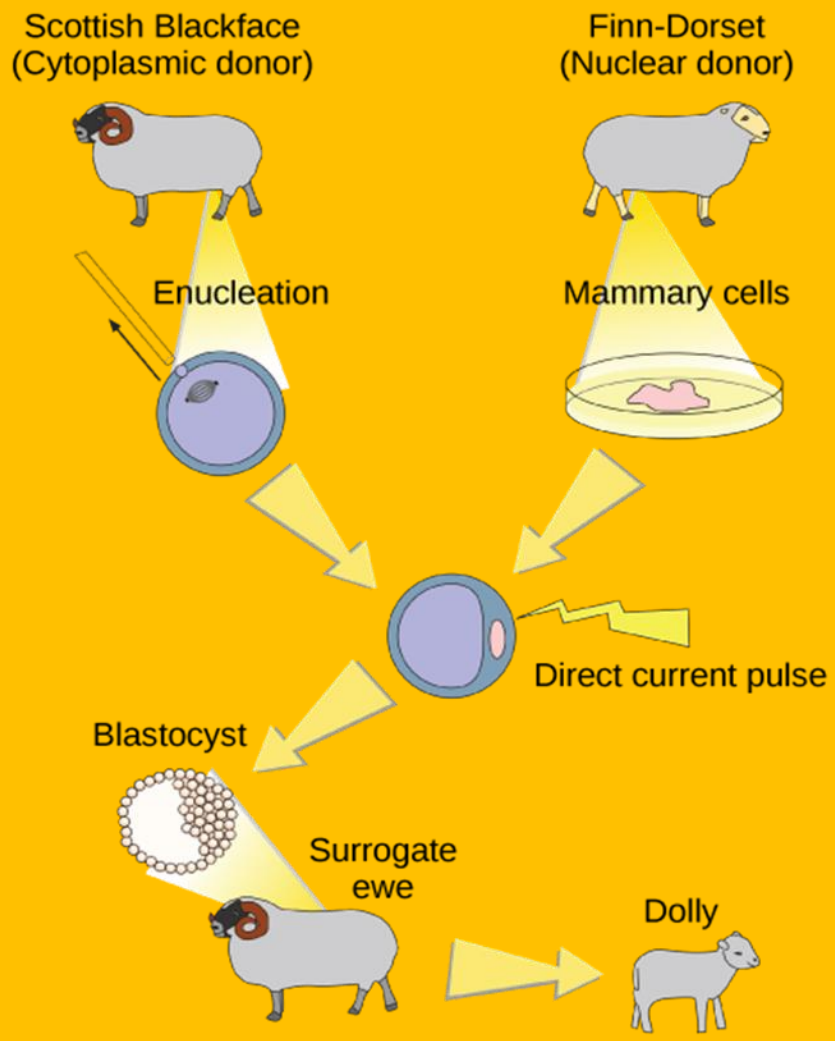
How Dolly was cloned

Animal cloning from an adult cell is much more difficult than from an embryonic cell. So when scientists working at the Roslin Institute in Scotland produced Dolly, the only lamb born from 277 attempts, it was a major news story around the world.

n 1996, University of Edinburgh scientists celebrated the birth of Dolly the Sheep. The Edinburgh team's success followed its improvements to the single cell nuclear transfer (SCNT) technique used in the cloning process. Dolly became a global scientific icon and SCNT technology has spread around the world and has been used to clone multiple farm animals. Professor Sir Ian Wilmut and colleagues worked on methods to create genetically improved livestock by manipulation of stem cells using nuclear transfer



Then they injected the cell into an unfertilised egg cell came from a Scottish Blackface ewe which had its nucleus removed, and made the cells fuse by using electrical pulses. When the research team had managed to fuse the nucleus from the adult white sheep cell with the egg cell from the black-faced sheep, they needed to make sure that the resulting cell wold develop into an embryo. They cultured it for six or seven days to see if it divided and developed normally, before implanting it into a surrogate mother, another Scottish Blackface ewe. Dolly had a white face. T



To produce Dolly, scientists used an udder cell from a six-year-old Finn Dorset white sheep. They had to find a way to 'reprogram' the udder cells - to keep them alive but stop them growing – which they achieved by altering the growth medium (the 'soup' in which the cells were kept alive).

Dolly lived a pampered existence at the Roslin Institute. She mated and produced normal offspring in the normal way, showing that such cloned animals can reproduce. Born on 5 July 1996, she was euthanased on 14 February 2003, aged six and a half. Sheep can live to age 11 or 12, but Dolly suffered from artrithis in a hind leg joint and from sheep pulmonary adenomatosis, a virus-induced lung tumour that is common among sheep which are raised indoors

Dolly the sheep was produced at the Roslin Institute as part of research into producing medicines in the milk of farm animals. Researchers have managed to transfer human genes that produce useful proteins into sheep and cows, so that they can produce, for instance, the blood clotting agent factor IX to treat haemophilia or alpha-1-antitrypsin to treat cystic fibrosis and other lung conditions.Inserting these genes into animals is a difficult and laborious process; cloning allows researchers to only do this once and clone the resulting transgenic animal to build up a breeding stock. The development of cloning technology has led to new ways to produce medicines and is improving our understanding of development and genetics.Since 1996, when Dolly was born, other sheep have been cloned from adult cells, as have cats, rabbits, horses and donkeys, pigs, goats and cattle.In 2004 a mouse was cloned using a nucleus from an olfactory neuron, showing that the donor nucleus can come from a tissue of the body that does not normally divide.

What are “Transgenic Organisms”?

Transgenic organisms are organisms whose genetic material has been changed by the addition of foreign genes. This foreign material can come from other organisms of the same species, from a whole different species, or synthetic sources.

Already today thousands of products come from Transgenic organisms. Everything from medicines, foods, feeds, and fibers. One of the biggest applications (and largest debates) of transgenics is in Agriculture. There are currently four nations involved in growing transgenic crops. They are the United States (68%), Argentina (23%), Canada (7%), and China (1%)

This is a genetically modified strain of malaria-resistant mosquito which has been created successfully by a scientist.

The creation of mosquitoes with green fluorescent testicles will help curb the spread of malaria carrying mosquitoes.

Uses for Transgenics

Crops

-Enhances taste and quality
-Increases nutrients, yields, and stress tolerances
-Improves resistance to disease, pests, and herbicides
-Allows for new products and growing techniques

Animals

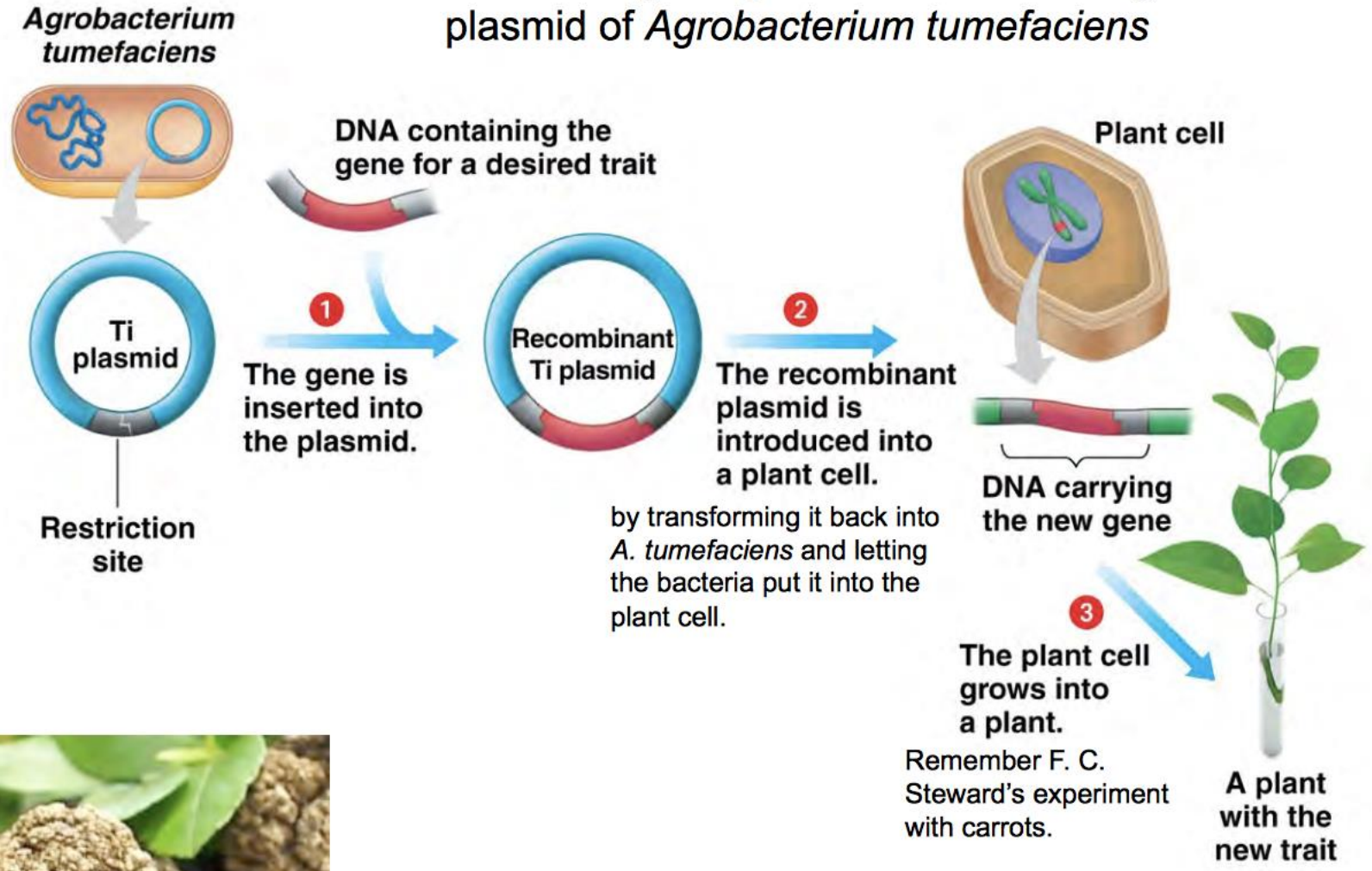
-Increases resistance, productivity, hardiness, and feed efficiency
-Allows for better yields of meat, eggs, and milk
Improves animal health and diagnostic methods

More uses. . .

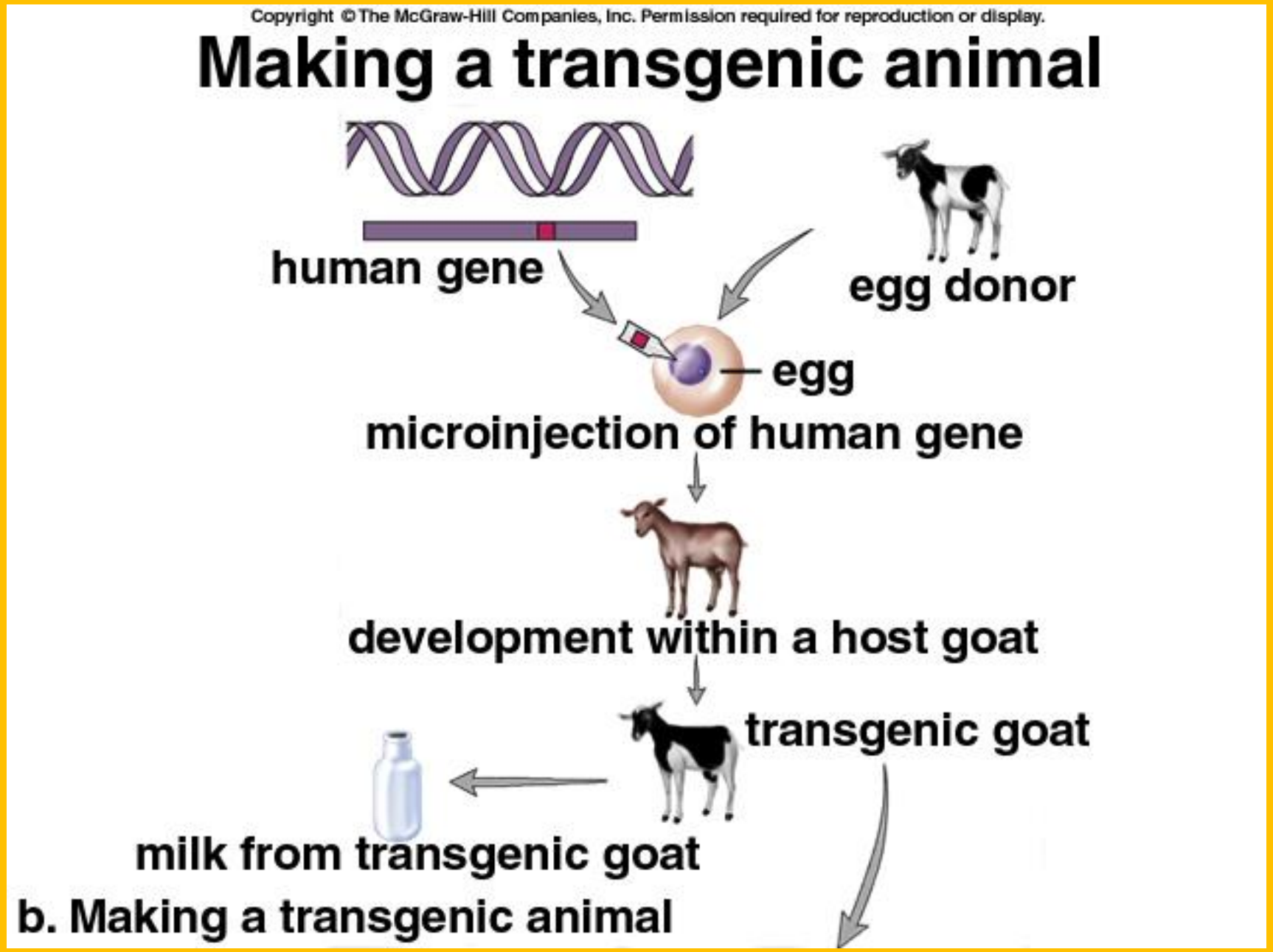
Transgenic organisms can be used to produce proteins for people or animals that cannot produce such proteins on their own. For example, insulin is a protein produced by humans to break down sugars in the bloodstream. However, some people are born without the ability to produce their own insulin thus making it hard for them to live. Since the advent of transgenic organisms, scientists have been able to modify animals so that they produce insulin in large quantities. This insulin can then be harvested, processed, and made available to diabetics who need it.

Another use of transgenics is to discover what certain genes do. By taking an unknown gene from one organism and inserting it into another organism, scientists can observe that changes that the gene produces in the new organism thus gaining insight into what exactly the gene does phenotypically.

Most transgenic plants are made using the Ti plasmid of *Agrobacterium tumefaciens*



A crown gall tumor. The tumor-inducing genes have been deleted from the Ti plasmid for use as a recombinant DNA vector .



ADVANTAGES

- Agriculture
  - Breeding
    - Quickly produce animals with desired traits
  - Quality
    - Cows that produce more milk, pigs and cattle that have more meat, sheep that grow more wool, etc.
  - Disease Resistant
- Medicine
  - Xenotransplantation
    - Animals produce organs that can be used in transplants
  - Nutritional Supplements
  - Pharmaceuticals
  - Human Gene Therapy
- Industry
  - Production of useful materials
    - Ex] Spider Goat ~ makes silk

DISADVANTAGES

- Animal Welfare
- Mutants can form from the DNA placed in the organism
- Success rate of making a transgenic organism is very low
  - Ex] Gene transfer studies revealed that only 0.6% of transgenic pigs were born with a desired gene after 7,000 eggs were injected with a specific gene.

Glossary

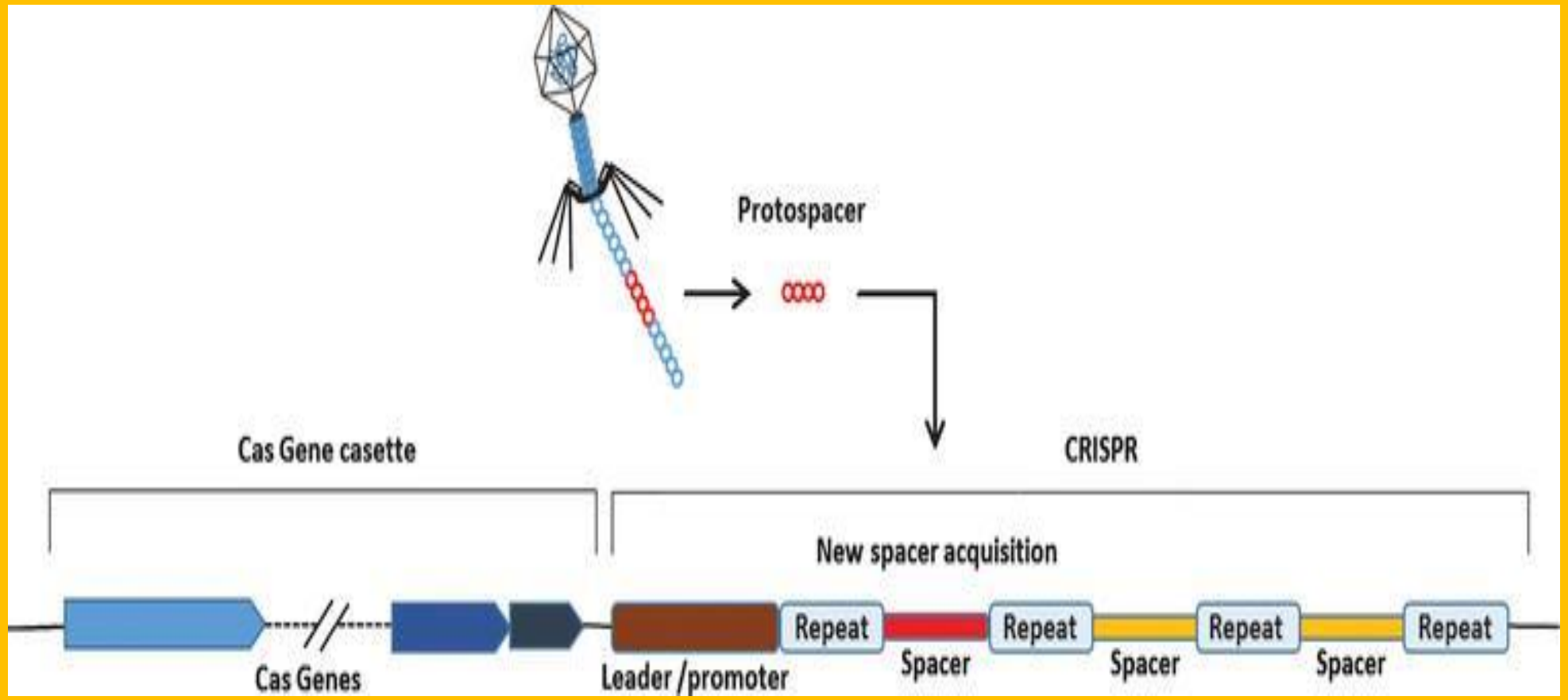
Donor= Donatore
Cloning vector=Vettore di clonazione
Host cell= Cellula ospite
Yeast cell= Lievito
Stem cell= Cellula staminale Inner
core= Nucleo interno
Protein coat= Copertura proteica
Capsid= Involucro(capside)
Enveloped virus=Virus avvolto-coperto
Autonomously= Autonomamente
Target cell= Cellula bersaglio
Plasmid= Plasmide
Metabolic pathway=Percorso metabolico
Foreign DNA= DNA estraneo
Restriction endonuclease= Nucleasi di restrizione
Palindromic=Palindromo
Cleavage=Sfaldamento
Embryo=Embrione
Blastocyst=Blastocisti
Bone marrow= Midollo osseo

What is CRISPR?

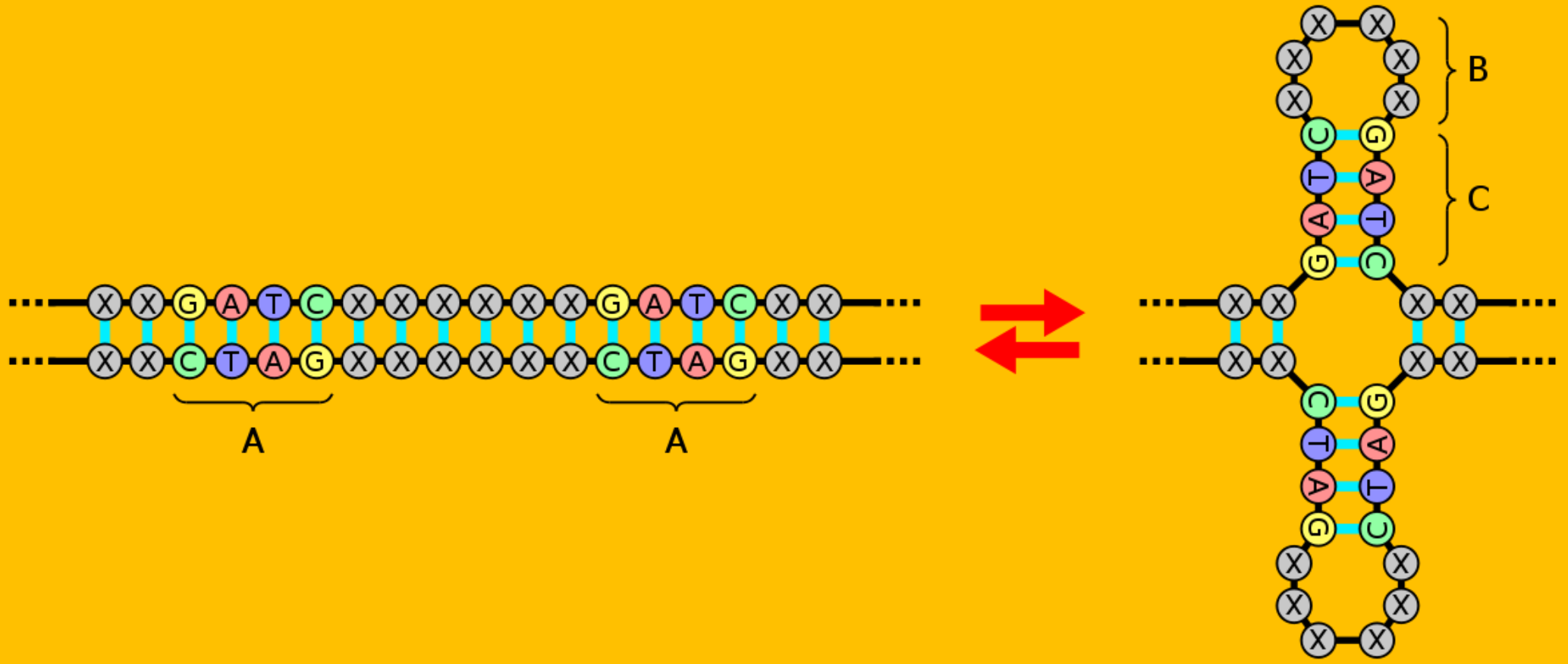
- Clustered
- Regularly
- Interspaced
- Short
- Palindromic
- Repeats

1. Virus attacks bacteria cell, injecting its DNA to wreak havoc and spread
2. Bacteria counters this with CRISPR/Cas9 immune system response
   - CRISPR/Cas9 is a nuclease (cuts DNA) with guide RNAs
3. CRISPRs can match with viral DNA sequences
4. Cas9 can literally cut at those specific sequences
5. Once cut, viral DNA is no longer a threat
6. CRISPR/Cas9 can do this with any DNA, not just viral DNA (more on this later!)
7. This advance has led to a gene-editing revolution

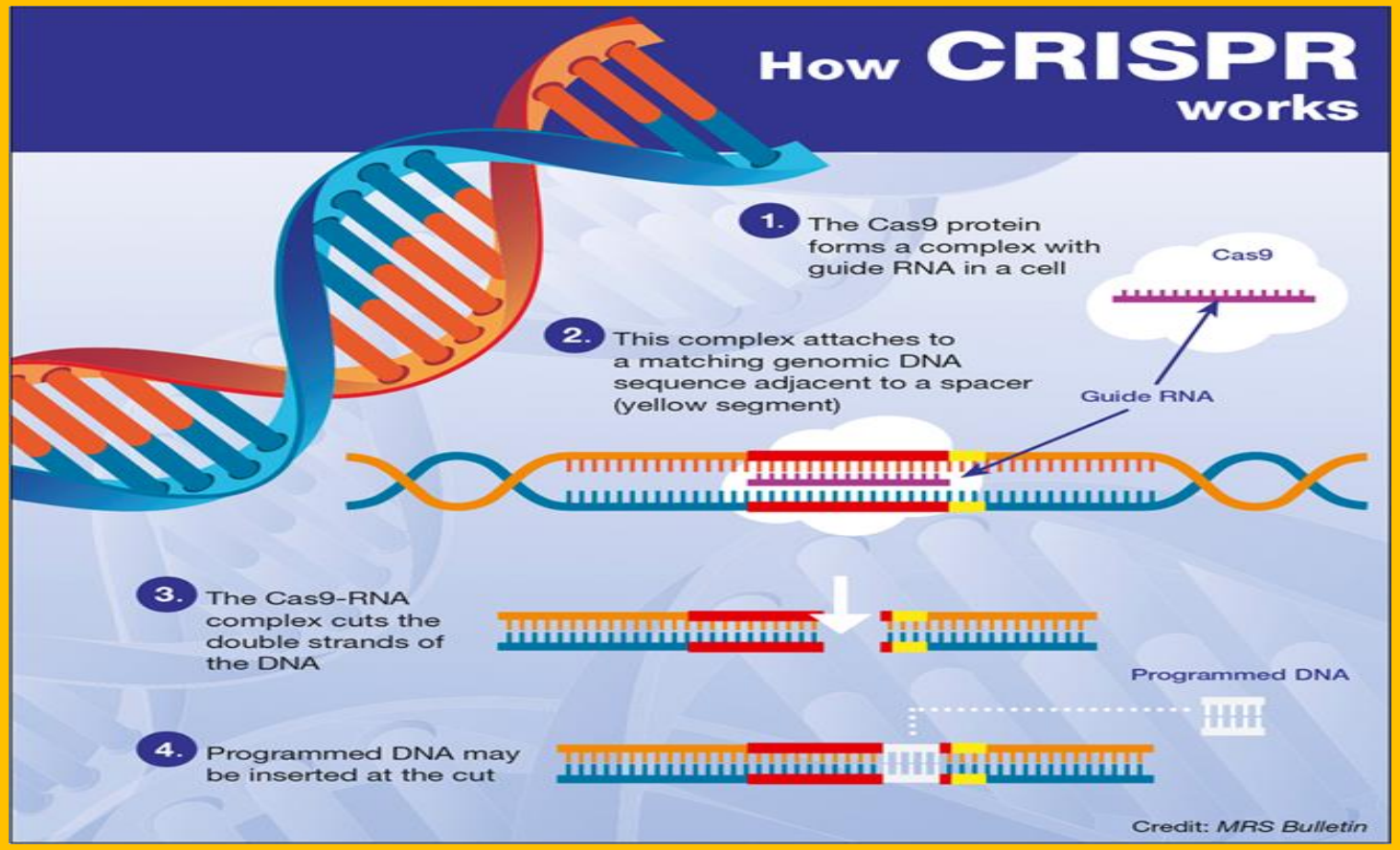
RESEARCH AMERICA


Protospacer
Cas Gene casette
CRISPR
New spacer acquisition
Repeat
Repeat
Repeat
Repeat
Spacer
Spacer
Spacer
Cas Genes
Leader /promoter

Palindrome

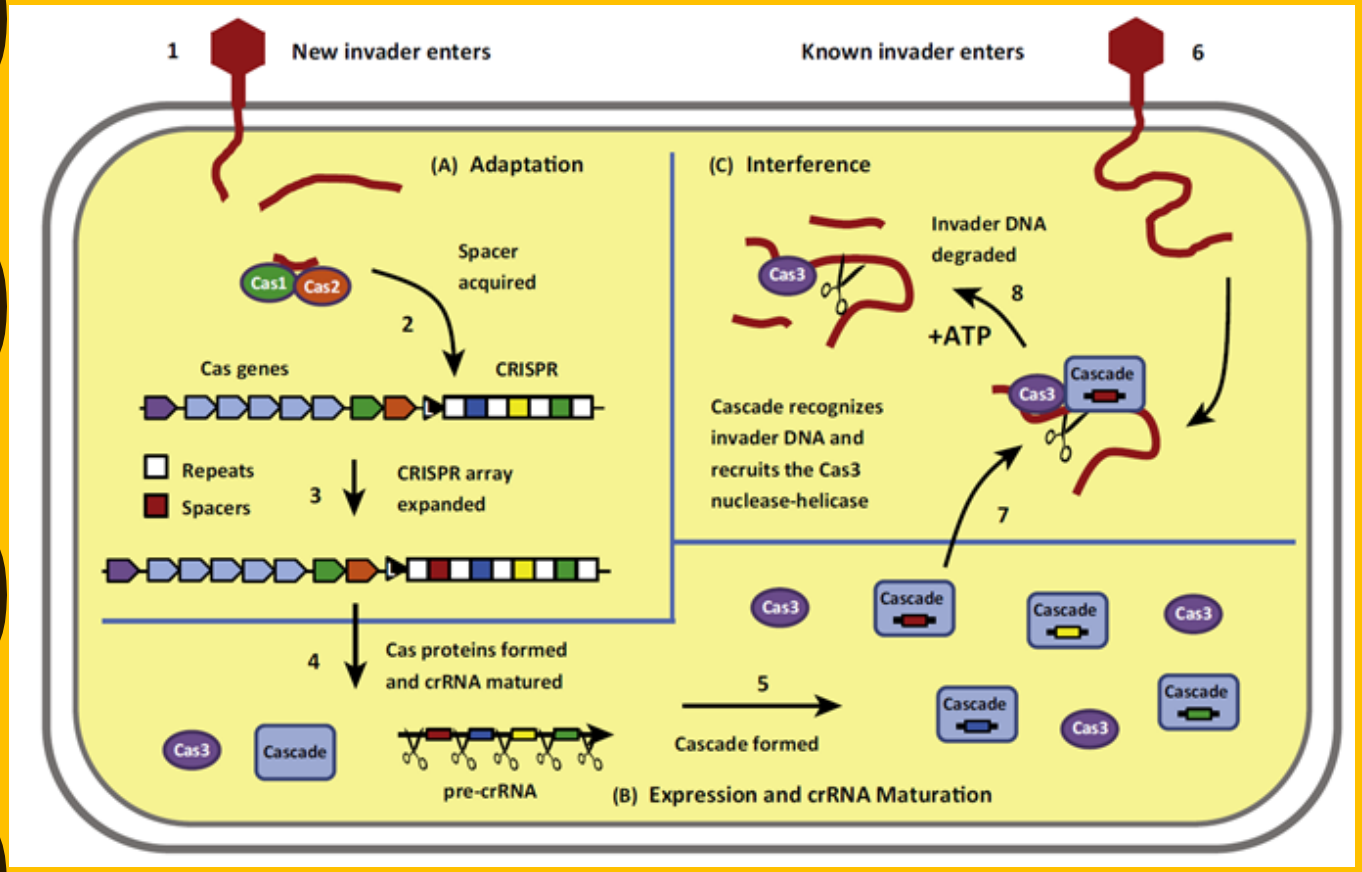


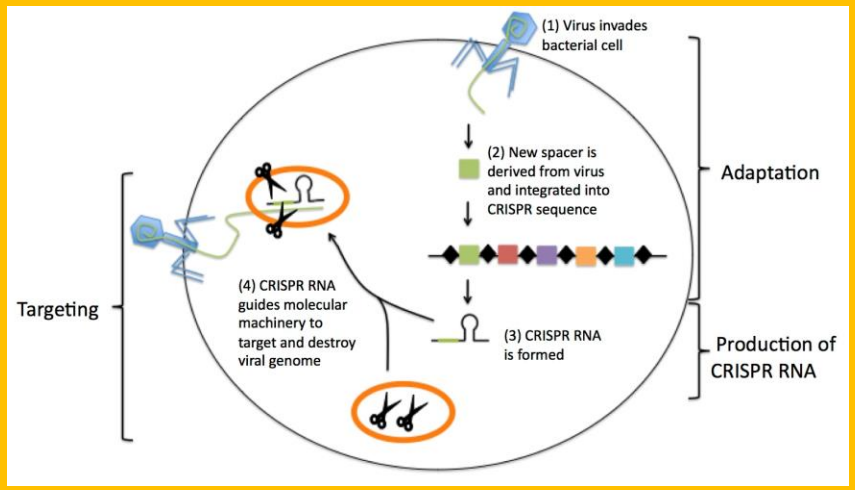
Ci sono parecchie tecniche per modificare il DNA. Fino a un paio di anni fa la più diffusa, anche nell'industria biotech, era **l'enzima di restrizione** sintetico "nucleasi a dita di zinco". Come una forbice, taglia via solo gli errori tipografici che devono essere specificati uno per uno. L'ultima, molto più efficiente, si chiama **CRISPR/Cas9**. L'acronimo sta per l'enzima prodotto dal gene Cas9 e i *Clustered Regularly Interspaced Short Palindromic Repeats*, le ripetizioni palindromiche di gruppi di DNA estraneo disposti a intervalli regolari.



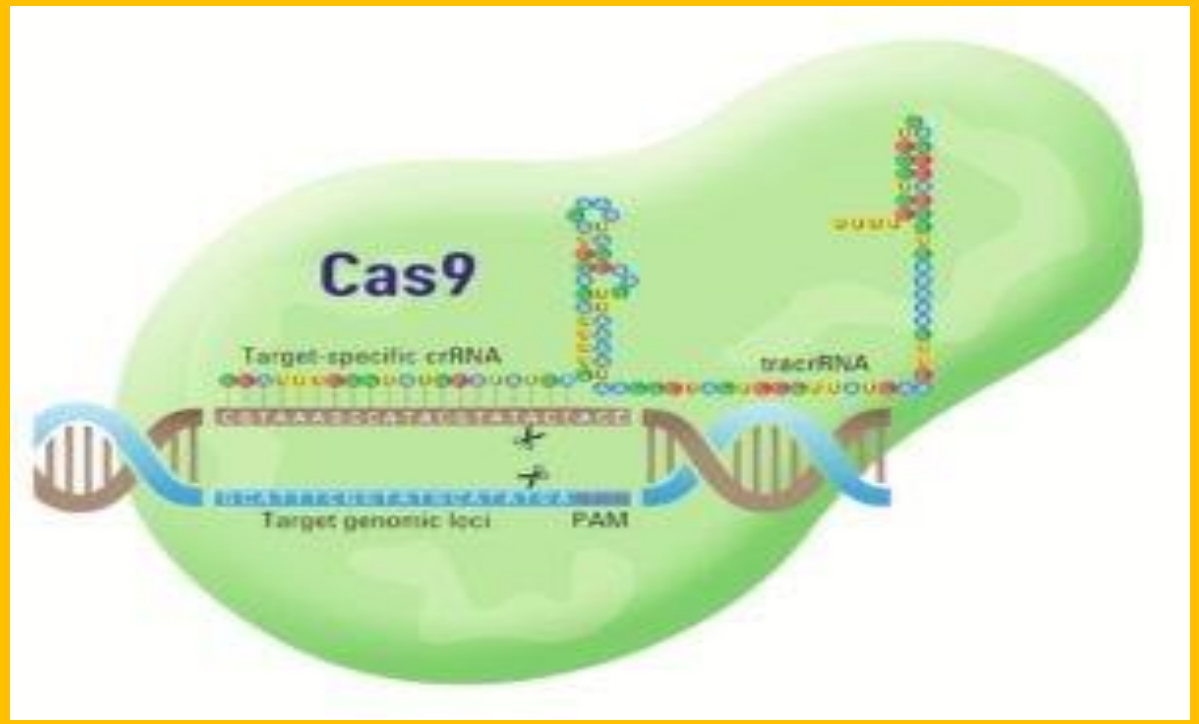
Così la grossa molecola diventa uno strumento preciso e potente. Nei laboratori è stato subito provato su cellule umane, staminali soprattutto, e su animali che fanno da modello per tumori, malattie virali, neurodegenerative e altre patologie.

I CRISPR fanno parte del **sistema immunitario** dei batteri, si era scoperto dodici anni fa in quelli dello yogurt. Sono anche dei "redattori genetici" grazie all'endonucleasi Cas che riconosce l'RNA nel quale il DNA virale si traduce per replicarsi. L'enzima Cas si appropria di quell'RNA, così riconosce esattamente i pezzi di DNA virale e li elimina tutti. La correzione resta nel genoma del batterio ed è ereditato dalle cellule figlie.





A differenza dei batteri, noi **eucarioti** abbiamo due copie di ciascun gene. Se una è mal funzionante, la cellula usa l'altra come matrice per riparare il gene e se nemmeno l'altra copia è integra, siamo da capo. Il sistema CRISPR + Cas9 + RNA-guida da solo non basta a modificare geneticamente piante e animali: occorre aggiungere la sequenza di DNA corretta da inserire nel gene al posto di quella tagliata via.



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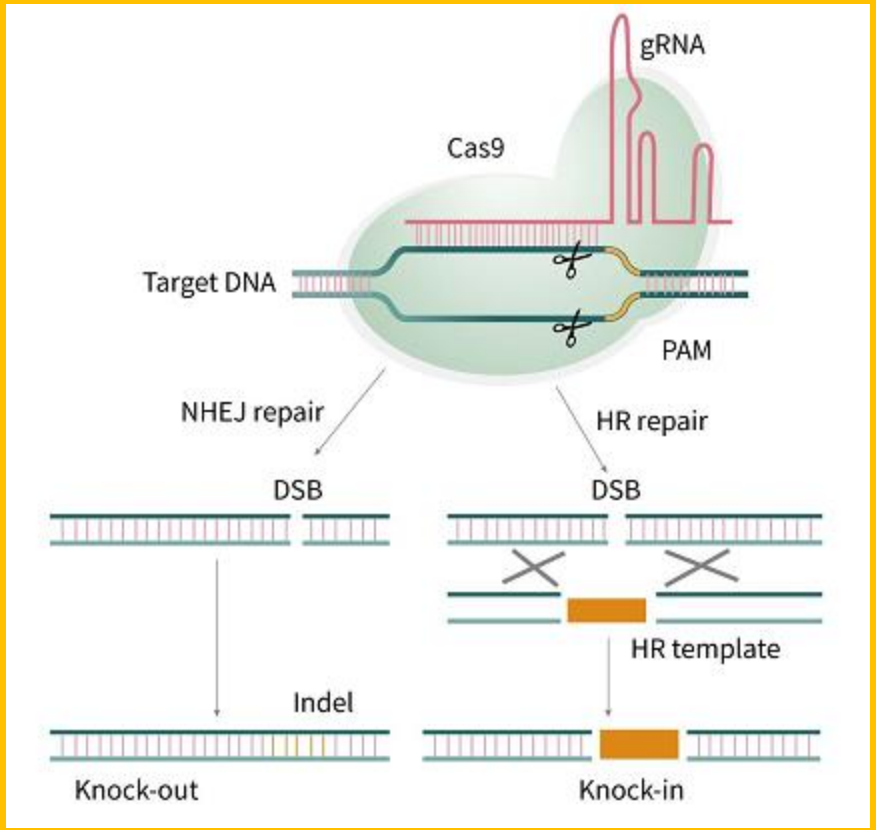
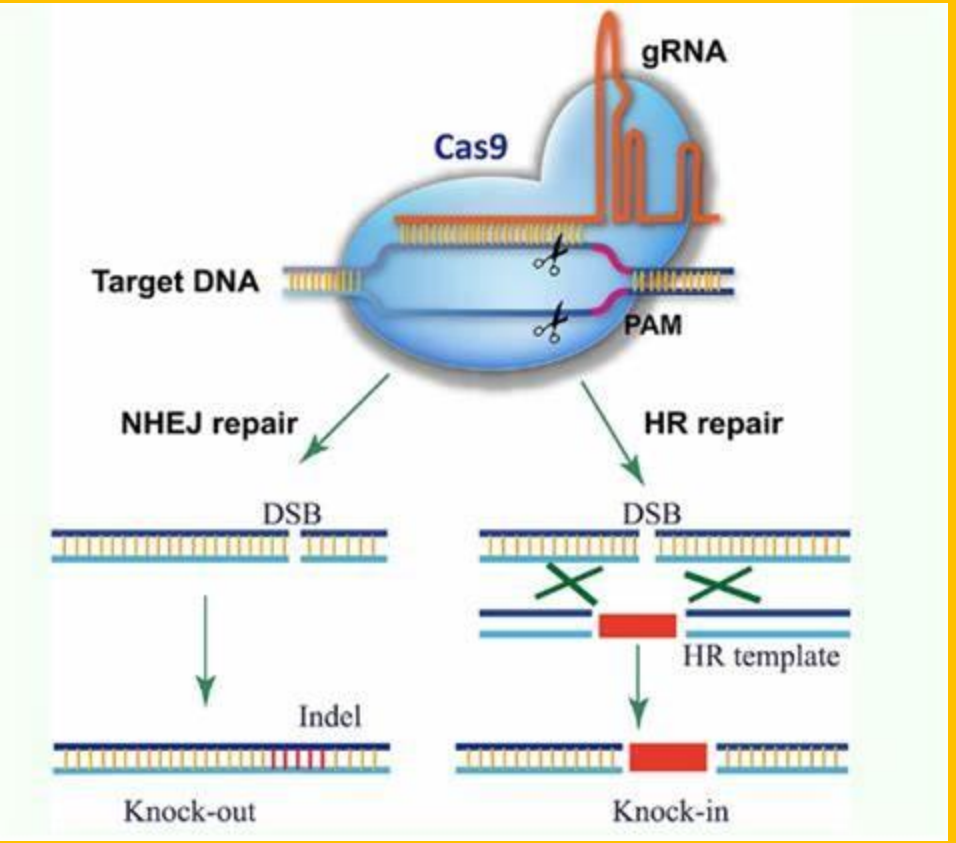
applicazioni

In generale, il "taglia e incolla genetico" consente di fare tre cose:

Produrre mutazioni in tutto e per tutti simili a caratteristiche o cambiamenti naturali, che possono anche funzionare come interruttore per spegnere un gene dannoso.
Correggere i difetti di un gene per permettergli di tornare a funzionare come dovrebbe, servendosi di uno stampo che corregga la sequenza sbagliata di nucleotidi.
Inserire un nuovo segmento di genoma che aggiunga una caratteristica utile a tutto il Dna.
Queste possibilità possono essere applicate a qualunque essere vivente, almeno nella teoria, e aprono orizzonti fino ad ora mai nemmeno immaginati. Per capire meglio cosa significhi nella pratica questa scoperta, proviamo a capire insieme come vengano utilizzate in agricoltura e nell'industria e come potrebbero essere utilizzare anche in medicina.


gRNA
Cas9
Target DNA
PAM
NHEJ repair
HR repair
DSB
DSB
HR template
Indel
Knock-out
Knock-in


gRNA
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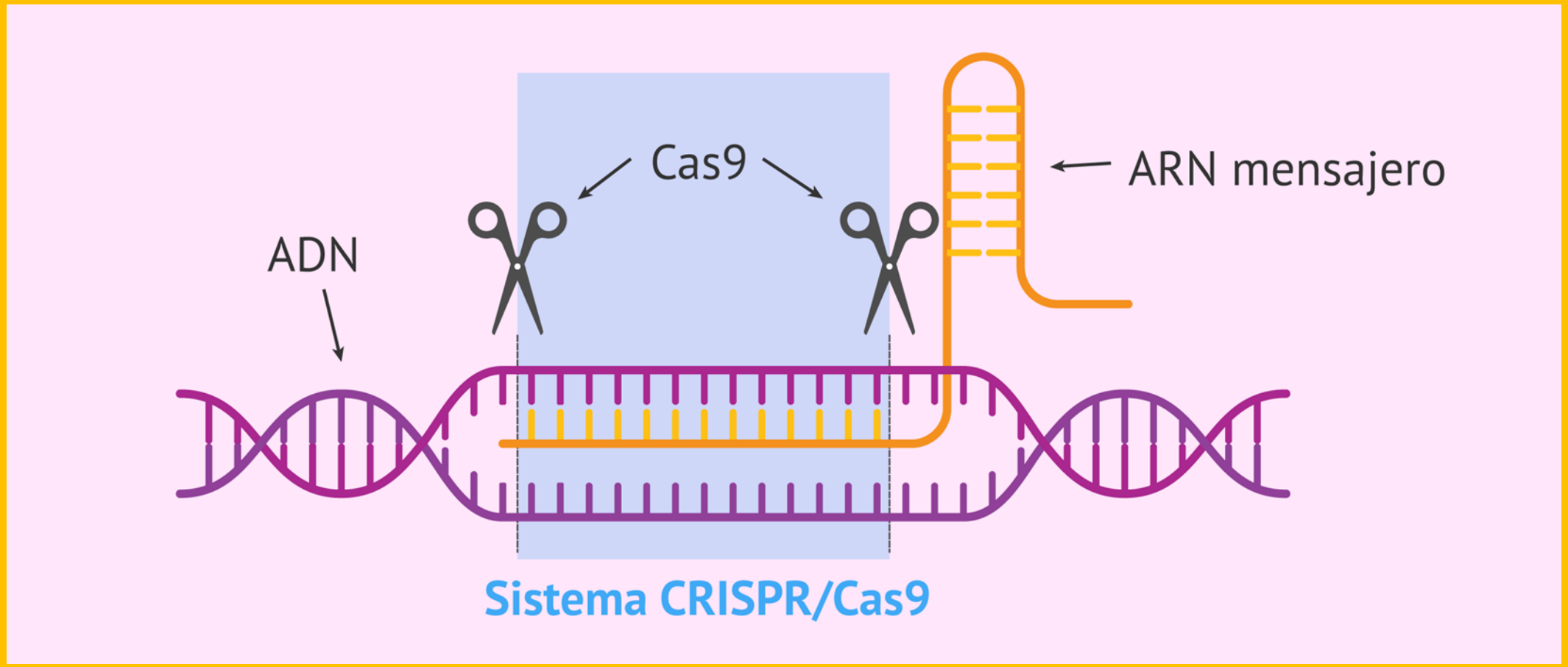
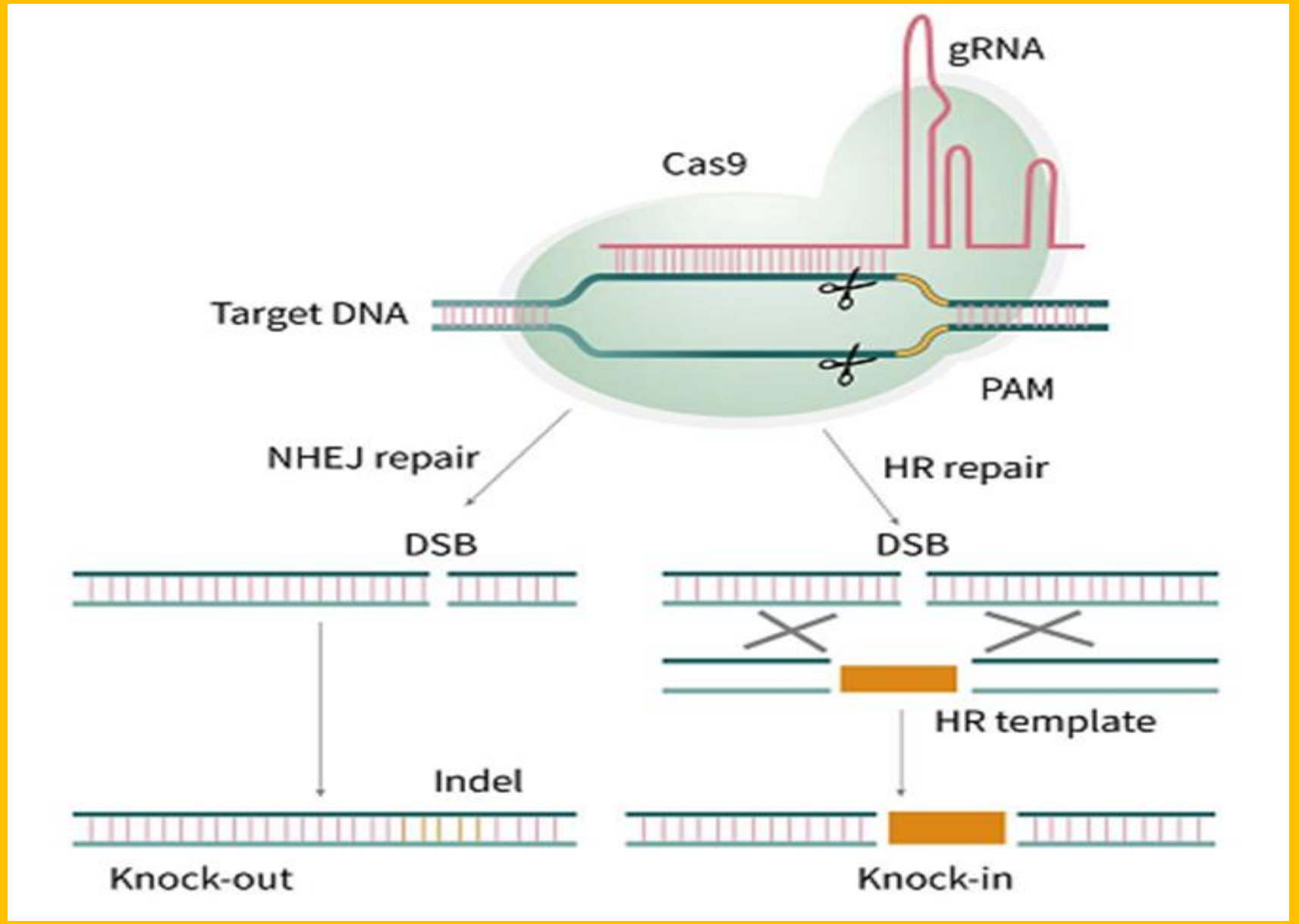
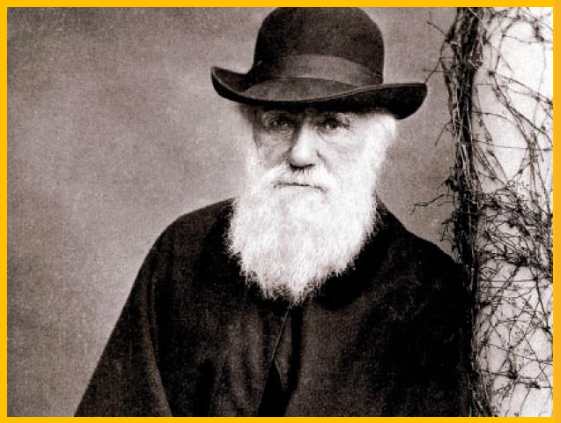

Cas9
ARN mensajero
ADN
Sistema CRISPR/Cas9


gRNA
Cas9
Target DNA
PAM
NHEJ repair
HR repair
DSB
DSB
HR template
Indel
Knock-out
Knock-in

In agricoltura
L'agricoltura è uno dei primi ambiti in cui il metodo Cripr/Cas9 ha trovato applicazione. Al momento, è già una realtà negli Stati Uniti, in Gran Bretagna e Germania, mentre potrebbe presto mettere radici anche in Francia. L'Italia è ancora un po' indietro nella sua messa a punto. Ma cosa permette e di fare nella pratica? Bè, saprai già che la selezione genetica esiste più o meno da quando l'essere umano ha scoperto la coltivazione delle piante. Attraverso gli incroci, si cercava di ottenere culture più resistenti o addirittura altri tipi di ortaggi. Nascono così, ad esempio, il limone, le carote come le conosci oggi e le clementine. Un metodo che continua a venire utilizzato ancora oggi, ma che richiede tanto tempo e diversi passaggi. Si tende infatti a favorire la selezione naturale, potenziando le varietà che si desiderano, perché sono più resistenti o perché producono più frutti.
Con il metodo CRISPER/CAS9 è possibile ottenere in modo sostenibile specie vegetali resistenti



La modifica del Dna delle specie vegetali può trovare applicazioni anche nel settore industriale, e di nuovo possono rappresentare una strategia contro il cambiamento climatico e il riscaldamento globale. Si tratta di biocarburanti, che già esistono, certo, ma che potrebbero diventare molto più semplici da produrre e soprattutto garantire migliori performance.

Merito di Crispr, ovviamente, e della Nannochloropsis gaditana, una microalga che non necessita di acqua dolce per vivere. I ricercatori della Synthetic Genomics sono infatti riusciti a raddoppiare il contenuto lipidico dell'alga, rendendola conveniente e sostenibile come possibile fonte di biocarburanti. E se è presto, avvertono, per pensare allo sfruttamento commerciale della scoperta, anche in questo caso la strada sembra ormai tracciata.

Ricapitolando

Il sistema CRISPR/Cas9 è stato identificato originariamente studiando i batteri, dove la proteina Cas9 svolge la sua funzione di forbice molecolare aiutando questi microorganismi a proteggersi da virus patogeni, svolgendo quindi la funzione di una sorta di sistema immunitario dei batteri.

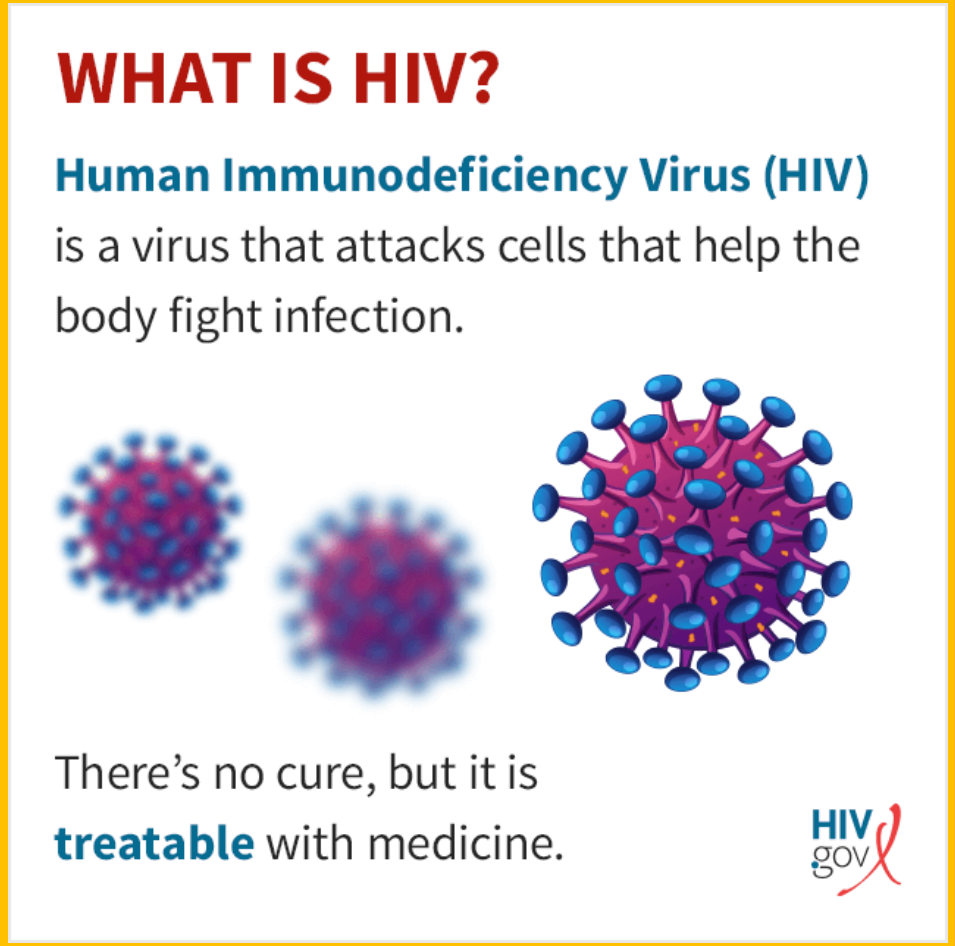
Tra il 2012 e il 2013 due gruppi di ricerca americani (provenienti dall'Università di Berkeley e dall'MIT di Boston) hanno per primi dimostrato che questa tecnologia può essere presa in prestito dai batteri per essere applicata come strumento biotecnologico per tagliare specifiche sequenze di DNA all'interno del genoma di una cellula non batterica.

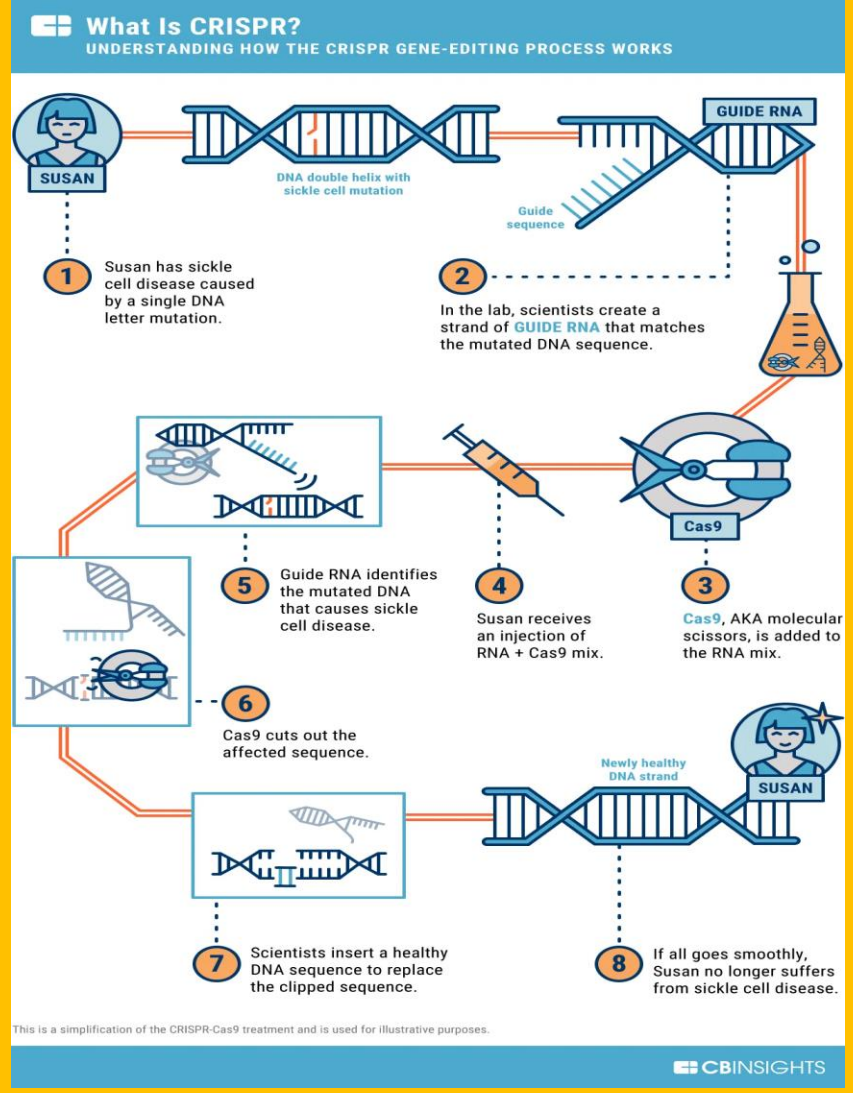
Questa scoperta è stata una vera e propria rivoluzione per la ricerca biomedica, poiché per la prima volta si è riusciti ad introdurre modificazioni desiderate nel genoma in modo semplice, efficace, veloce ed economico. A dimostrazione di ciò CRISPR/Cas9 in pochi anni si è diffuso nei laboratori di tutto il mondo e viene oggi impiegato sia per la ricerca di base che per scopi applicativi. Infatti, pur essendo una tecnologia ancora relativamente nuova e in forte evoluzione, la sua robustezza la sta spingendo rapidamente verso la sperimentazione clinica.

Ricapitolando

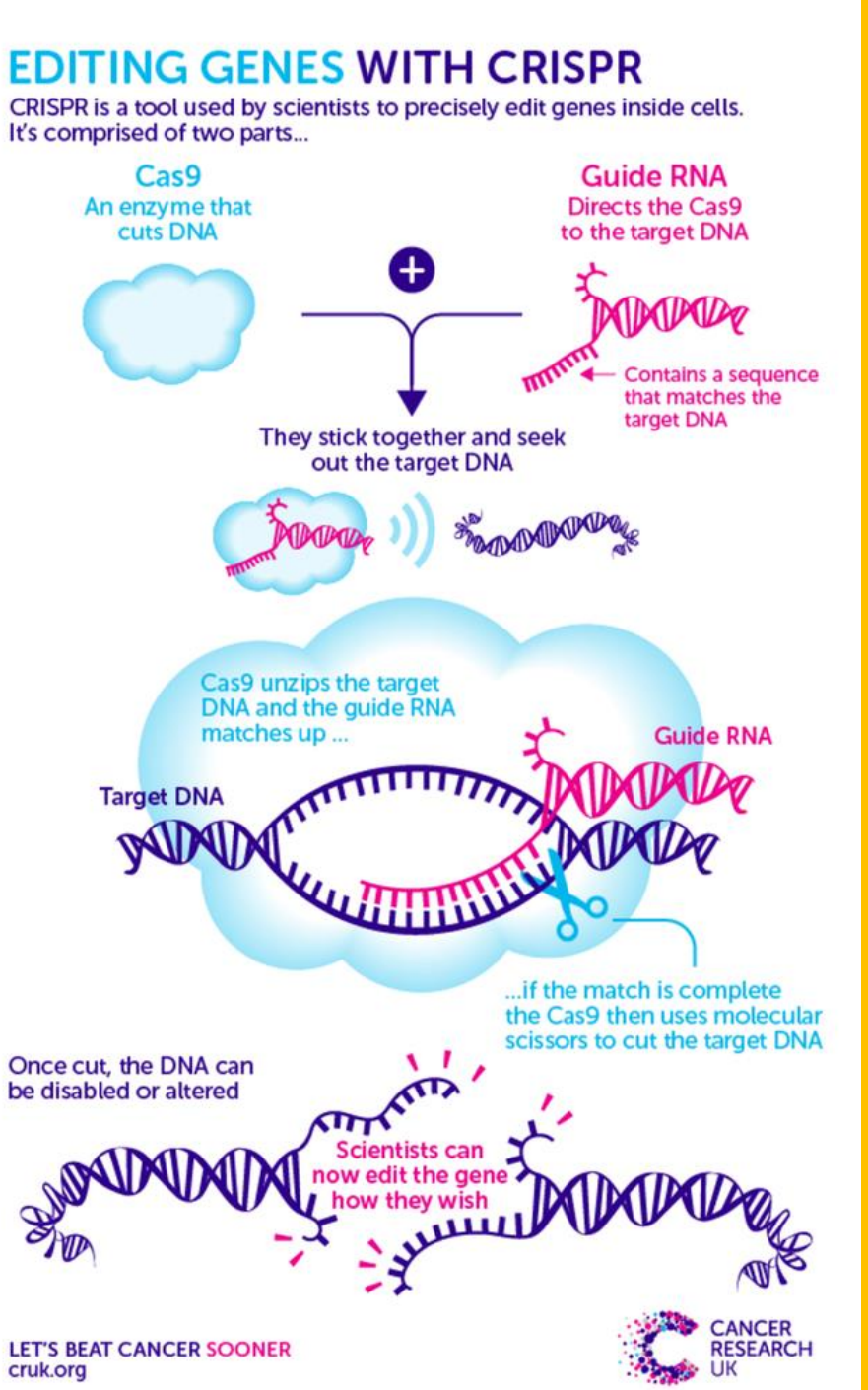
I sistema CRISPR/Cas9 (si pronuncia crisper) si basa sull'impiego della proteina Cas9, una sorta di forbice molecolare in grado di tagliare un DNA bersaglio, che può essere programmata per effettuare specifiche modifiche al genoma di una cellula, sia questa animale, umana o vegetale.

A seguito del taglio introdotto da Cas9, attraverso opportuni accorgimenti, è infatti possibile eliminare sequenze di DNA dannose dal genoma bersaglio oppure è possibile sostituire delle sequenze, andando ad esempio a correggere delle mutazioni causa di malattie
HIV, tagliare il dna virale che si è inserito nel DNA umano
cancro, tagliare i geni mutati
Distrofia muscolare correggere mutazioni



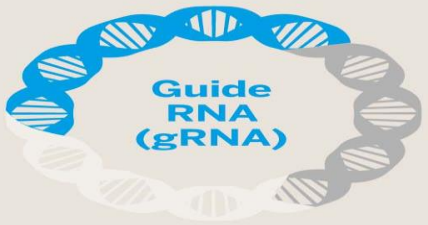


La programmazione del bersaglio di Cas9 avviene attraverso una molecola di RNA, chiamata RNA guida, che può essere facilmente modificata in laboratorio e, una volta associata a Cas9, agisce come una specie di guinzaglio, ancorandola alla sequenza di DNA bersaglio da noi scelta.



CRISPR-Cas9

How the genome editor works



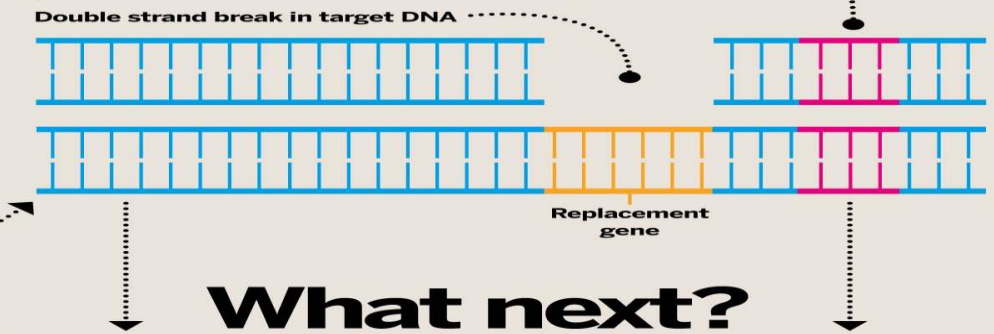
1 A cell is transfected with a DNA plasmid that expresses both the Cas9 protein and a sequence of guide RNA (gRNA), which matches that of the gene of interest.

2 Cas9 identifies the corresponding DNA sequence on the host cell's genome, and cuts both strands of DNA.

3a The cell's attempt to repair the break effectively **silences the targeted gene** by joining the cleaved DNA back together, using a process called non-homologous end joining (NHEJ).

OR

3b A **faulty gene can be 'corrected'** with a replacement segment of DNA, or **a new gene altogether can be introduced**. If a modified piece of DNA whose flanking regions match the target sequence is also supplied, then there is a good chance that it will recombine with the host DNA when the cut is made, thus introducing a new or replacement gene. This pathway is known as homology directed repair (HDR).



Cas9 requires a simple and common sequence of base pairs called a **PAM sequence** to actually bind to target DNA. This feature means bacteria can prevent Cas9 from chopping up important 'memorised' sequences of foreign DNA in their own genome – by ensuring there are no PAM sequences in those regions.

What next?

FOOD AND LIVESTOCK MODIFICATION
Researchers have already created plants and mammals with edited genomes. It is hoped such technology could help boost productivity and improve food security.

GENE DRIVE
Some genes are more likely to be passed on than others. If an 'edit' is linked to these genes, it will quickly spread through a wild population. That sounds alarming, but could help eradicate malaria-carrying mosquitos.

GENE THERAPY
Genetic disease could be treated by introducing gene editing systems into affected cells. Researchers in the USA are trialling this to treat HIV by knocking out the gene for the specific T-cell receptor that the virus targets.

HUMAN GERM LINE
Modifying human embryos, sperm or eggs would introduce changes to the genome of future generations. Some argue that other techniques, such as embryo screening, can just as effectively prevent genetic disease.

DESIGNER ORGANISMS AND MORE...
In future, could babies be 'designed' with a genome of our choosing? Could amateur biologists do their own gene editing outside regulatory systems?