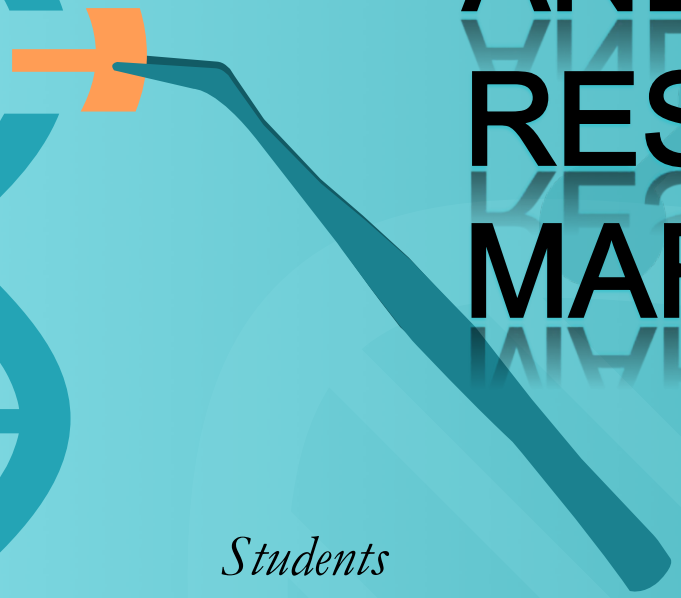
UNIVERSITÀ DEGLI STUDI
DI PERUGIA

GMOs AND ANTIBIOTIC RESISTANCE GENE MARKERS



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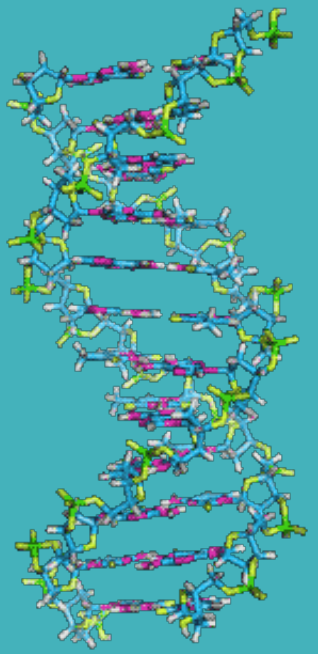
About GMOs…

What is **GMO**?

Genetically

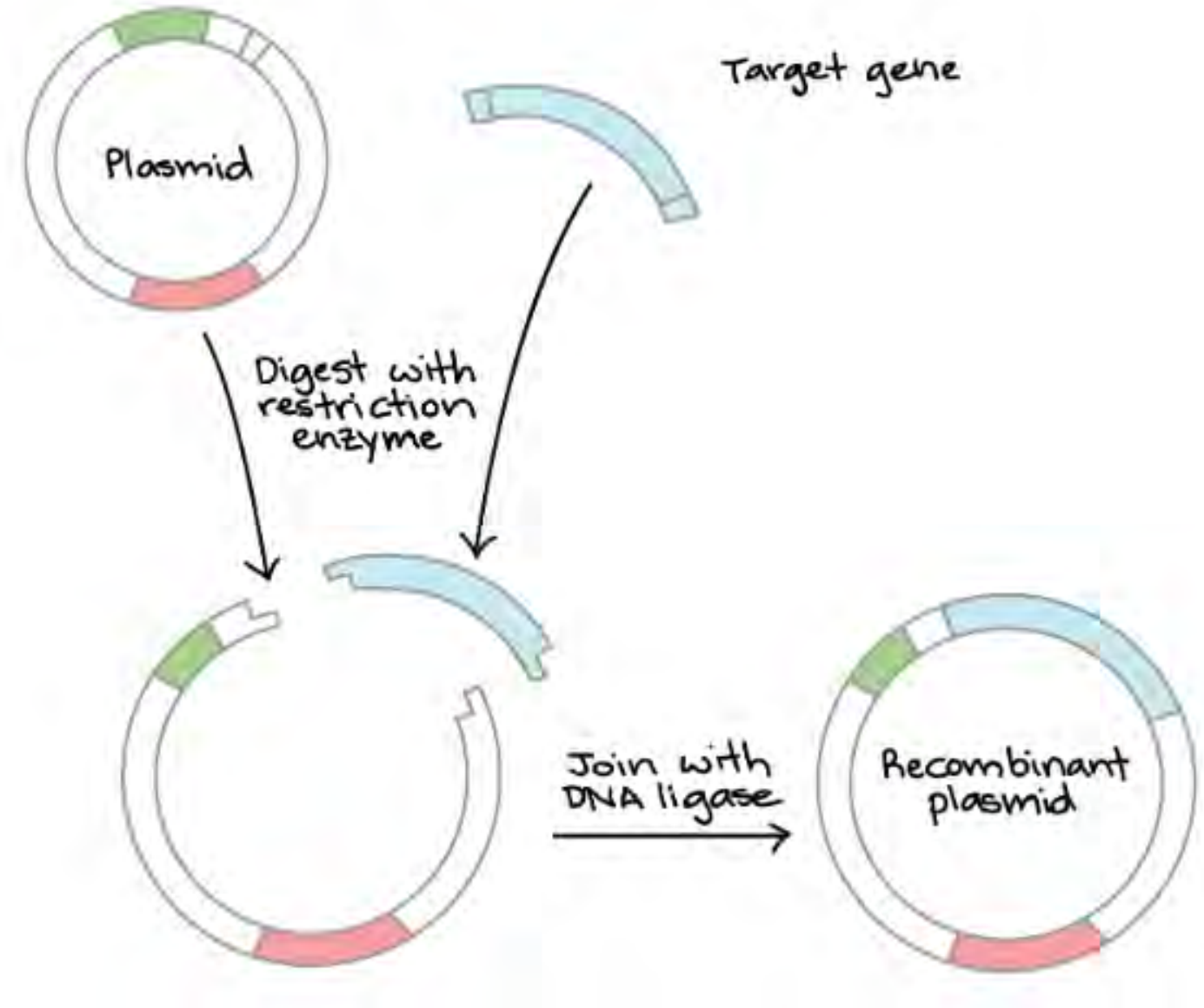
Modified

Organism

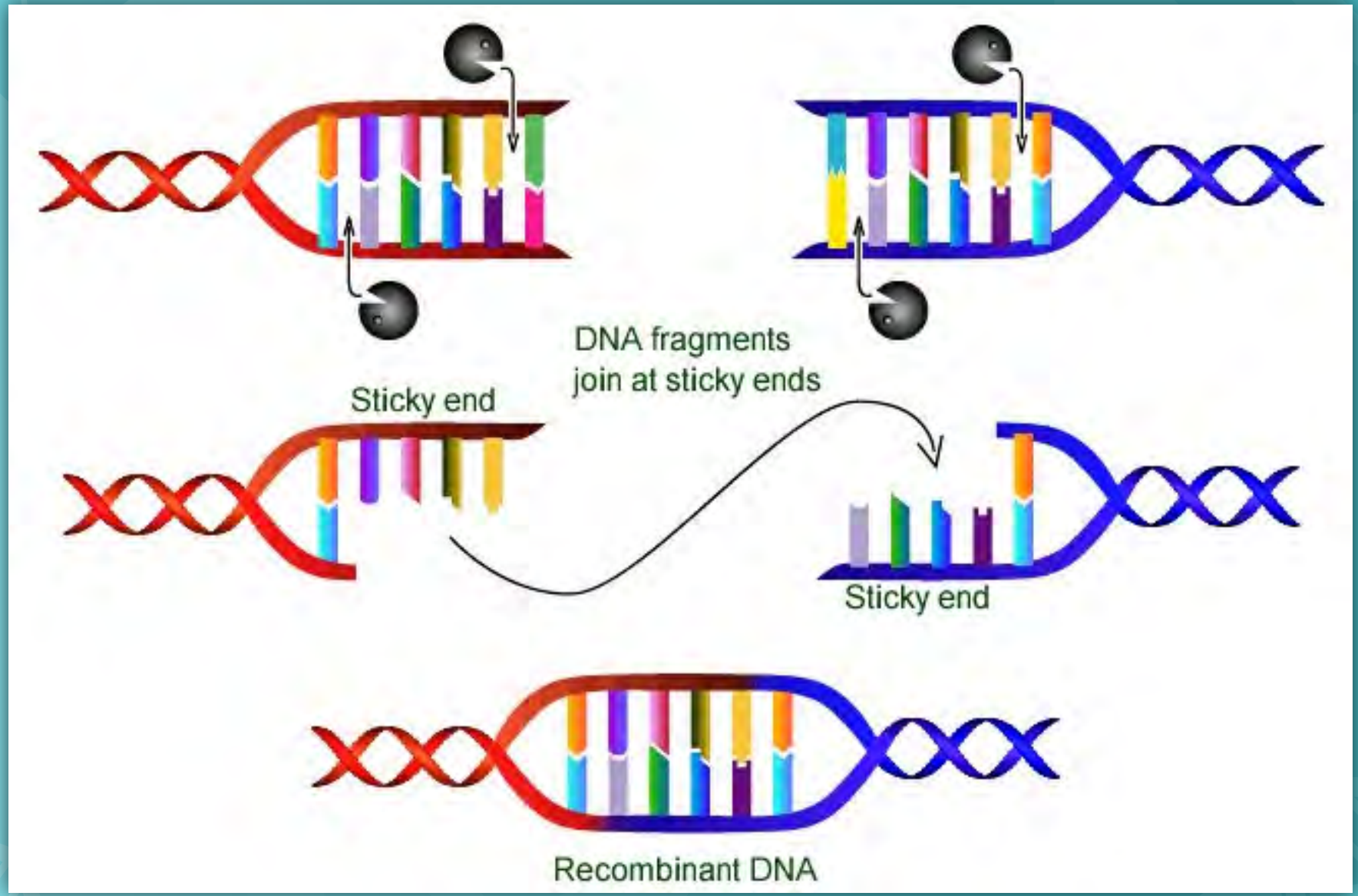


Is any organism whose genetic material has been altered using genetic engineering techniques. DNA is altered artificially in a lab with genes from other plants, animals, viruses or bacteria.

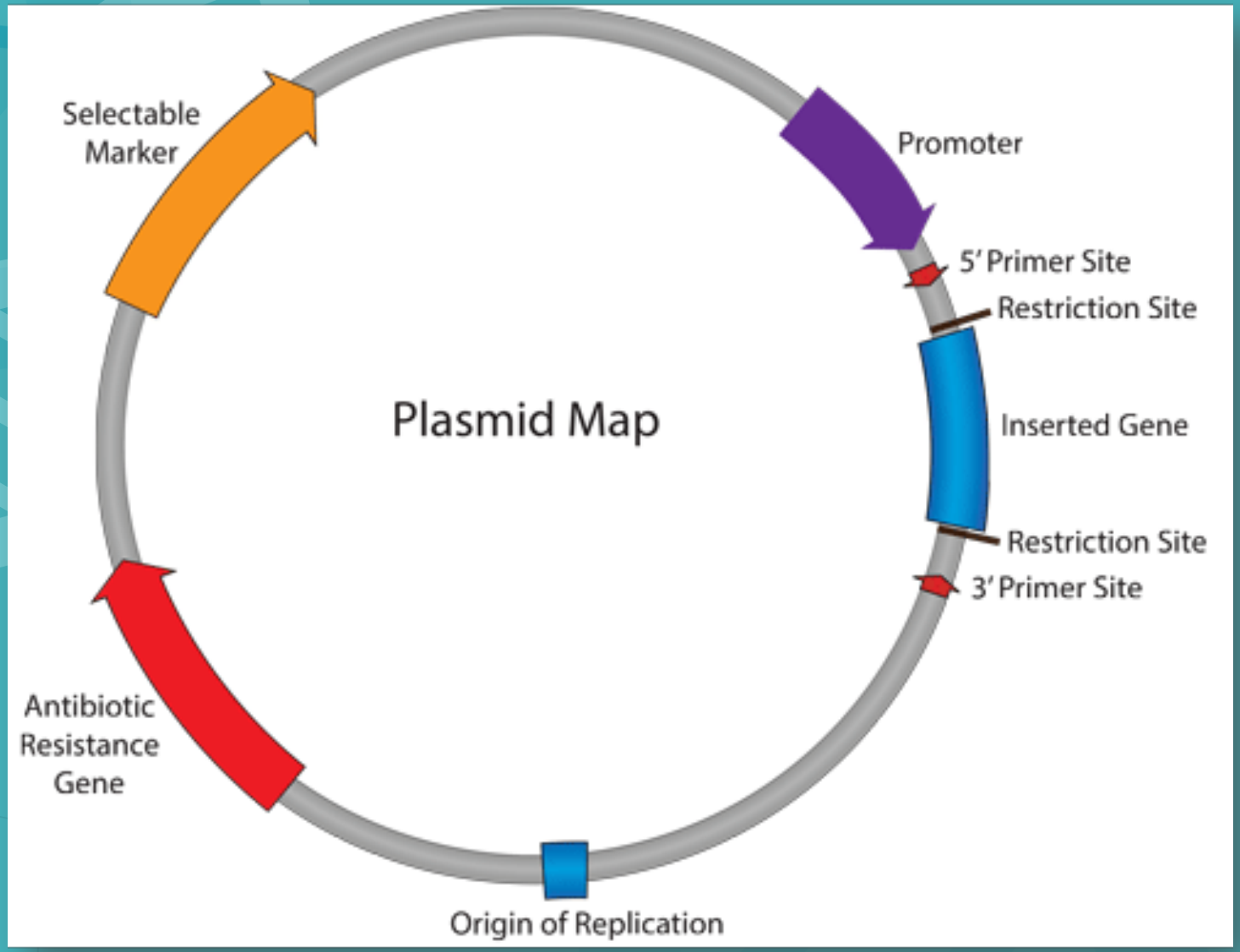
GMOs production



Restriction enzymes



Vector

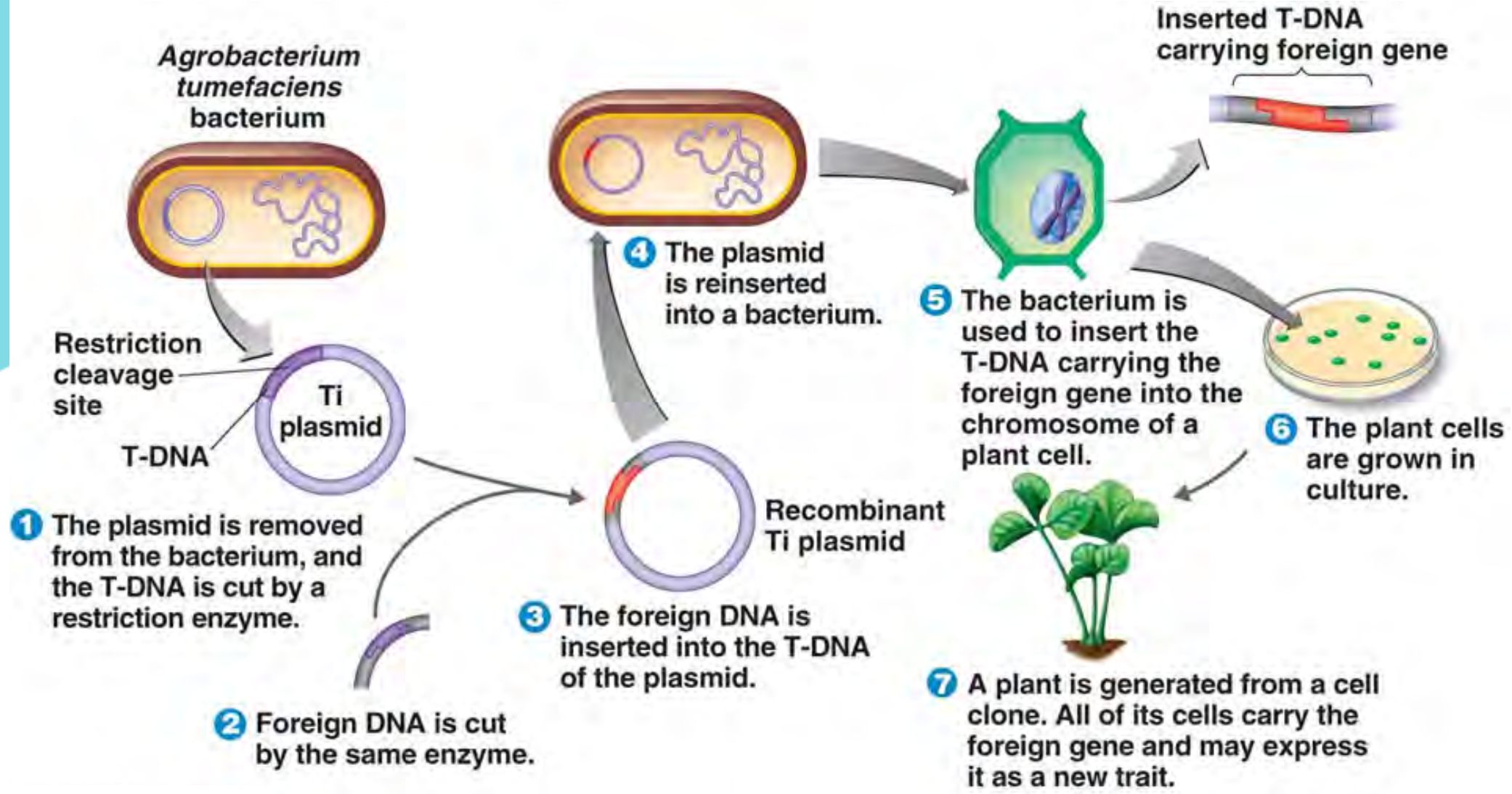

Selectable Marker
Promoter
5' Primer Site
Restriction Site
Plasmid Map
Inserted Gene
Restriction Site
3' Primer Site
Antibiotic Resistance Gene
Origin of Replication

Strategies to create GMOs

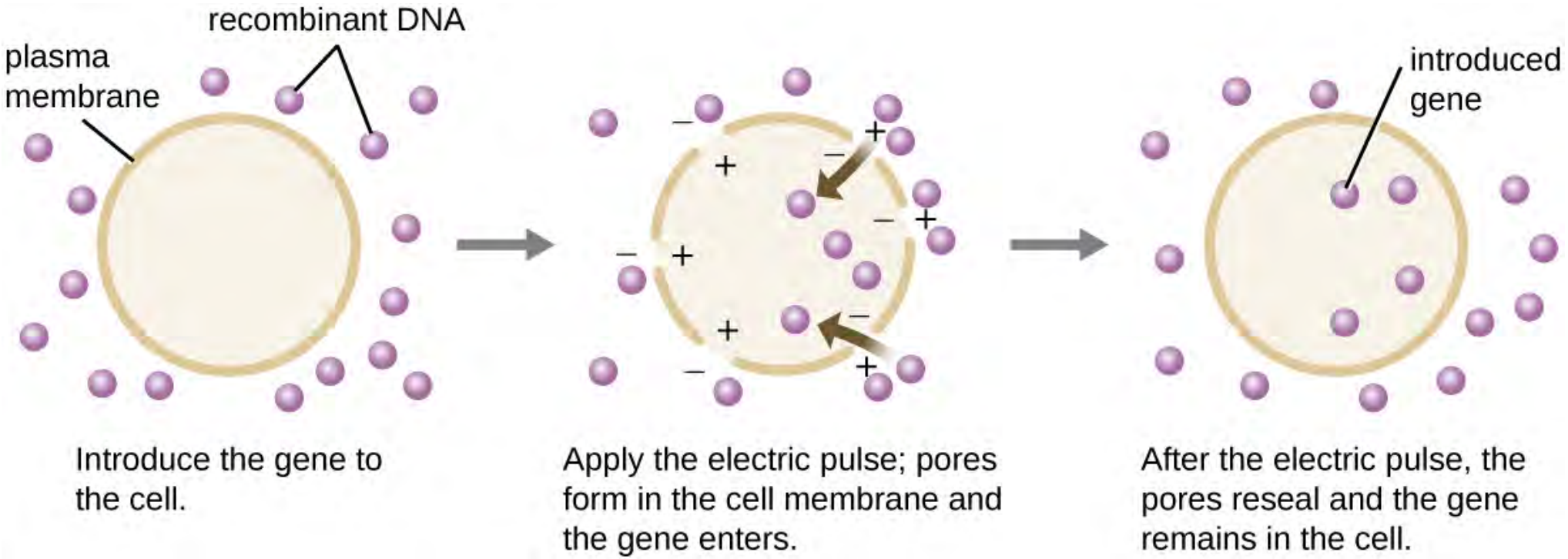
There are three different ways:

- Infection with Agrobacterium tumefaciens;
- Electroporation;
- Gene Gun.

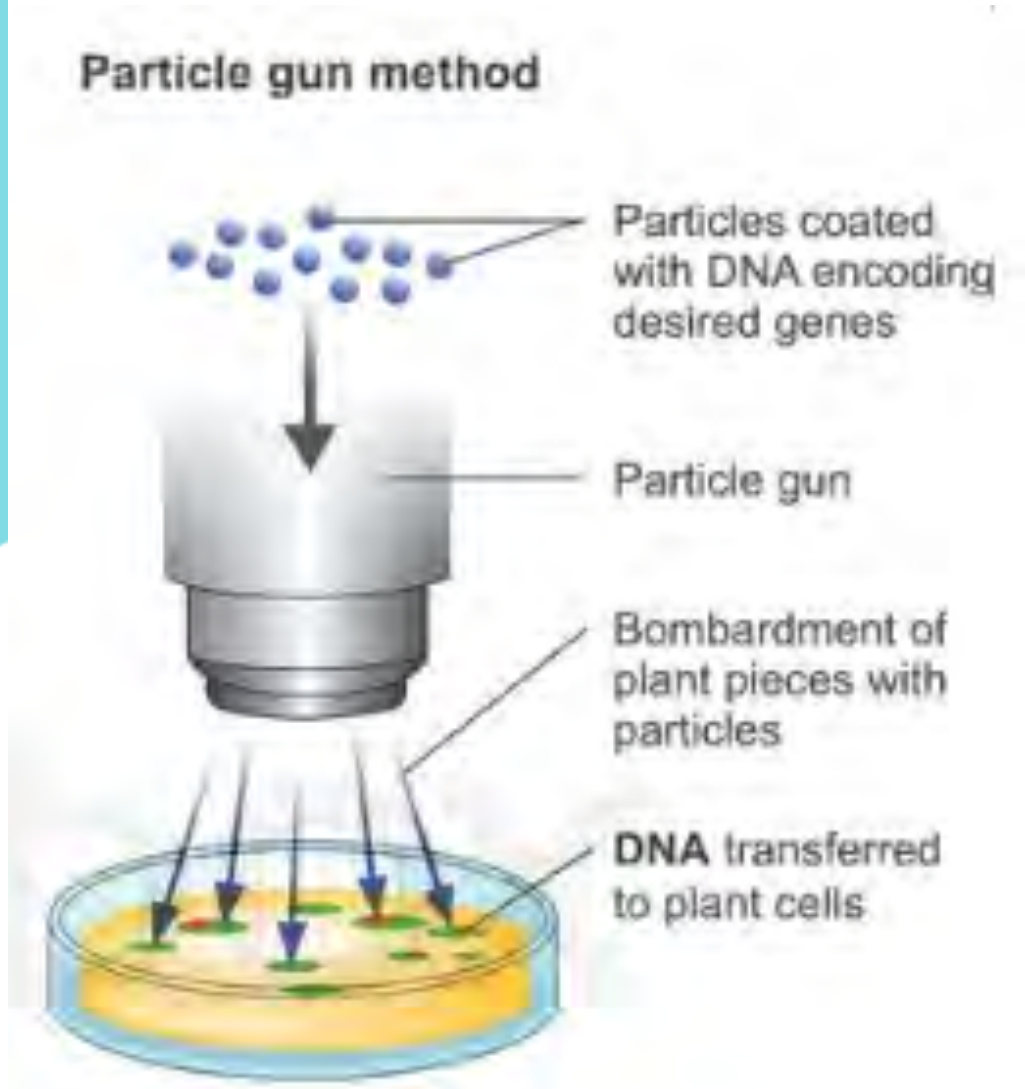
Infection with Agrobacterium tumefaciens



Electroporation



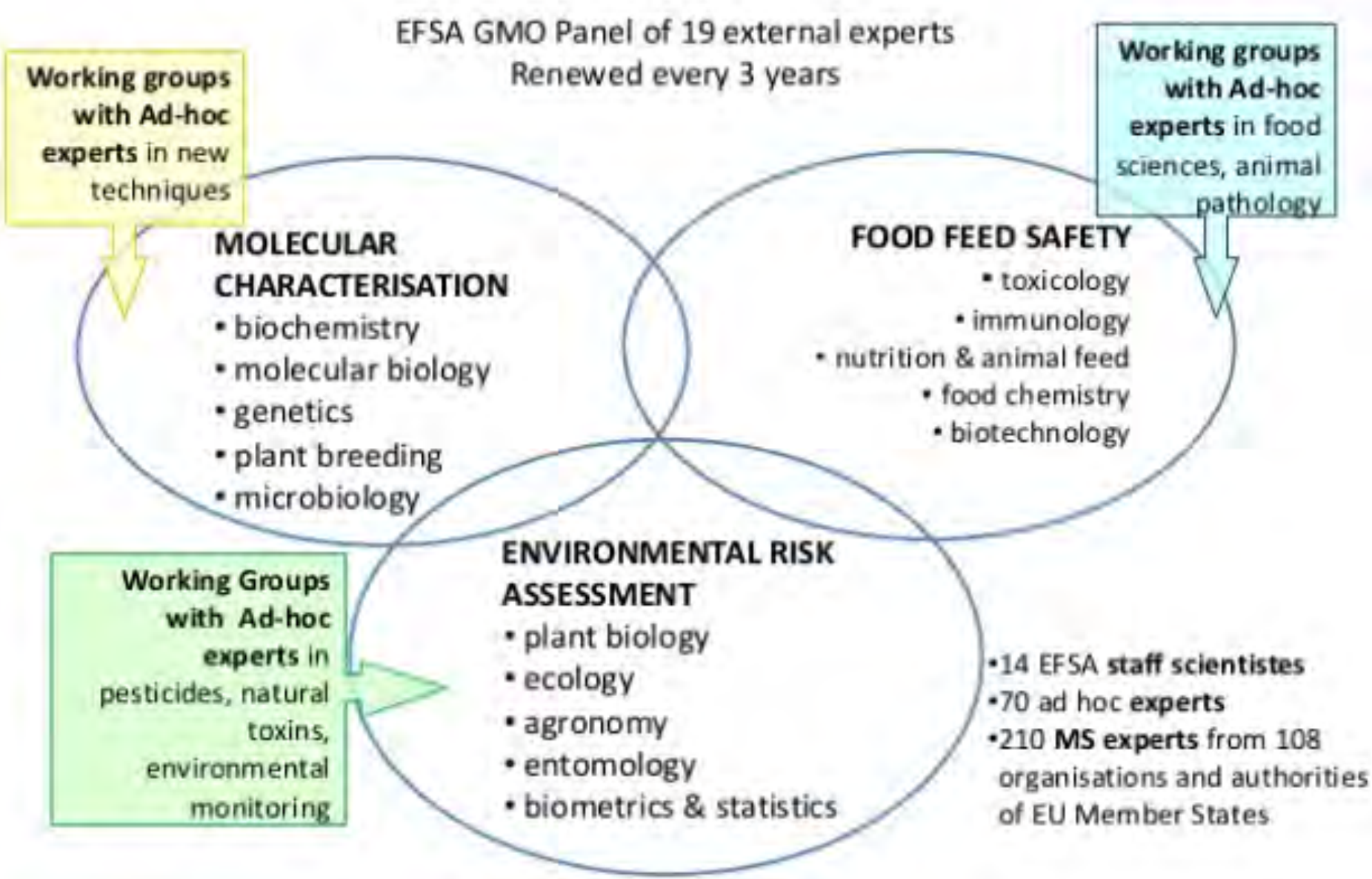
Gene gun


Particle gun method
Particles coated with DNA encoding desired genes
Particle gun
Bombardment of plant pieces with particles
DNA transferred to plant cells

❖ Risk analysis and regolatory approal before they can enter the market in the EU;

❖ Analysis of the possible risks for humans, animals and environment.

EFSA GMO Panel expertise



GMO FREE vs GMO

- Compare the GM plant with its normal counterpart;
- Verify if modified properties alter the level of risk or give rise to additional risks;
- Investigate toxological, allergenic and nutritional properties.

Comparative Analysis

2 different **STEPS**:

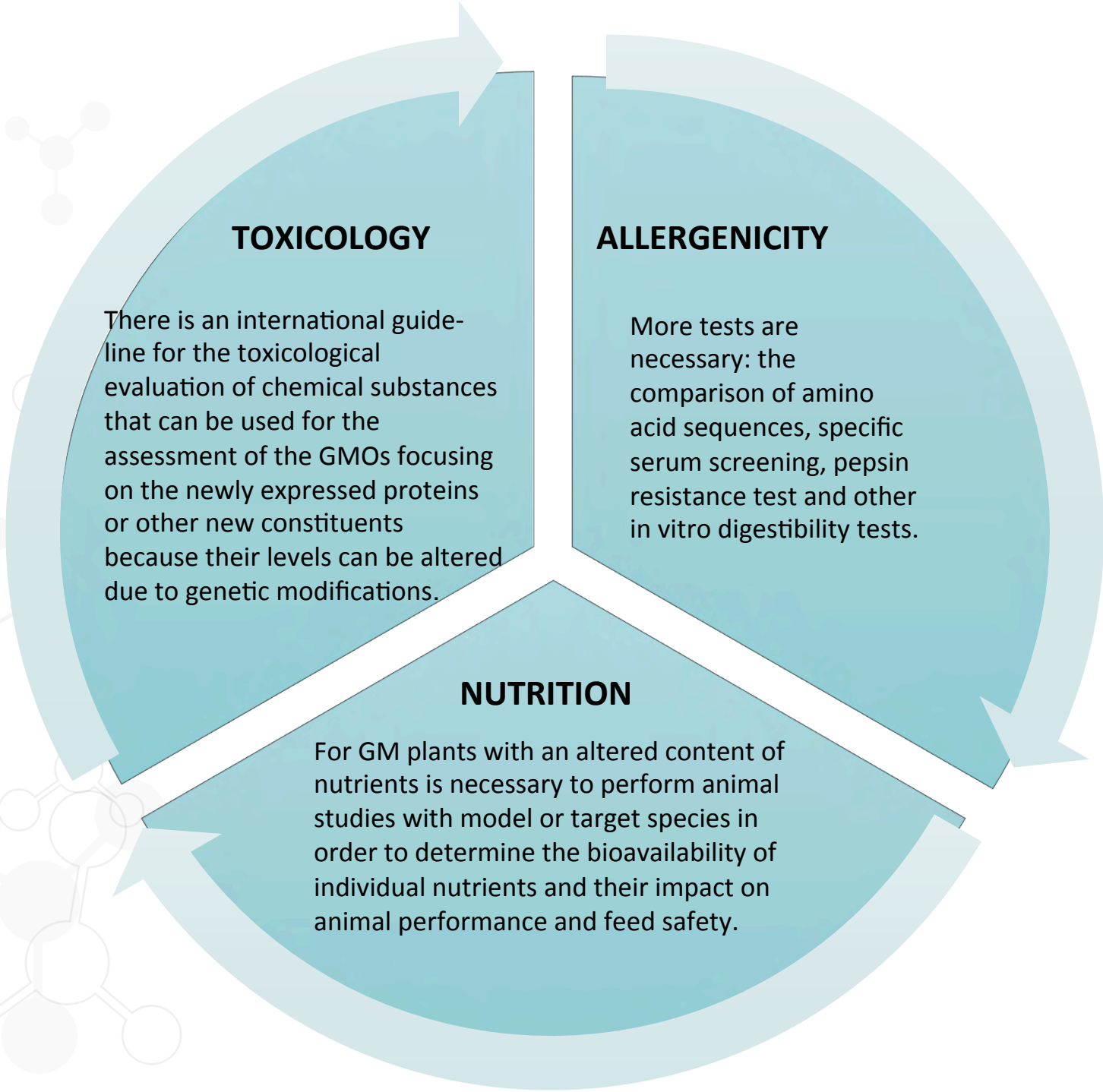
Proof of difference

Identification of possible differences between the GM plant and its appropriately selected comparator

Proof of equivalence

Assessment of the characteristics of the GM plant fall within the range of natural variation estimated from a set of non-GM reference varieties with a history of safe use

Approach of *EFSA*



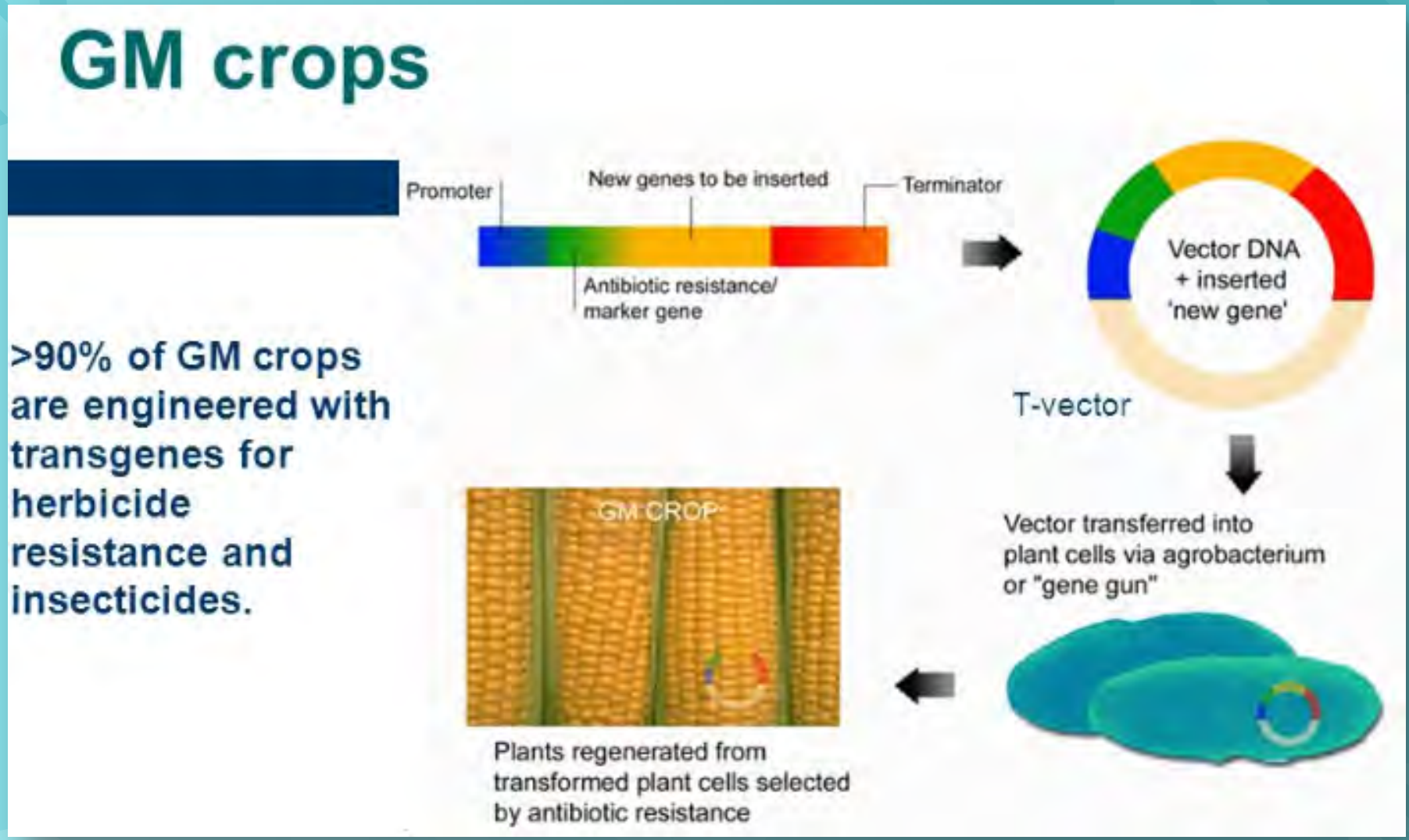
Empiric selection

Phenotypical characteristics:

- Taste;
- Appearance;
- Size of the fruits.

Antibiotic resistance marker genes in GMOs

GM crops

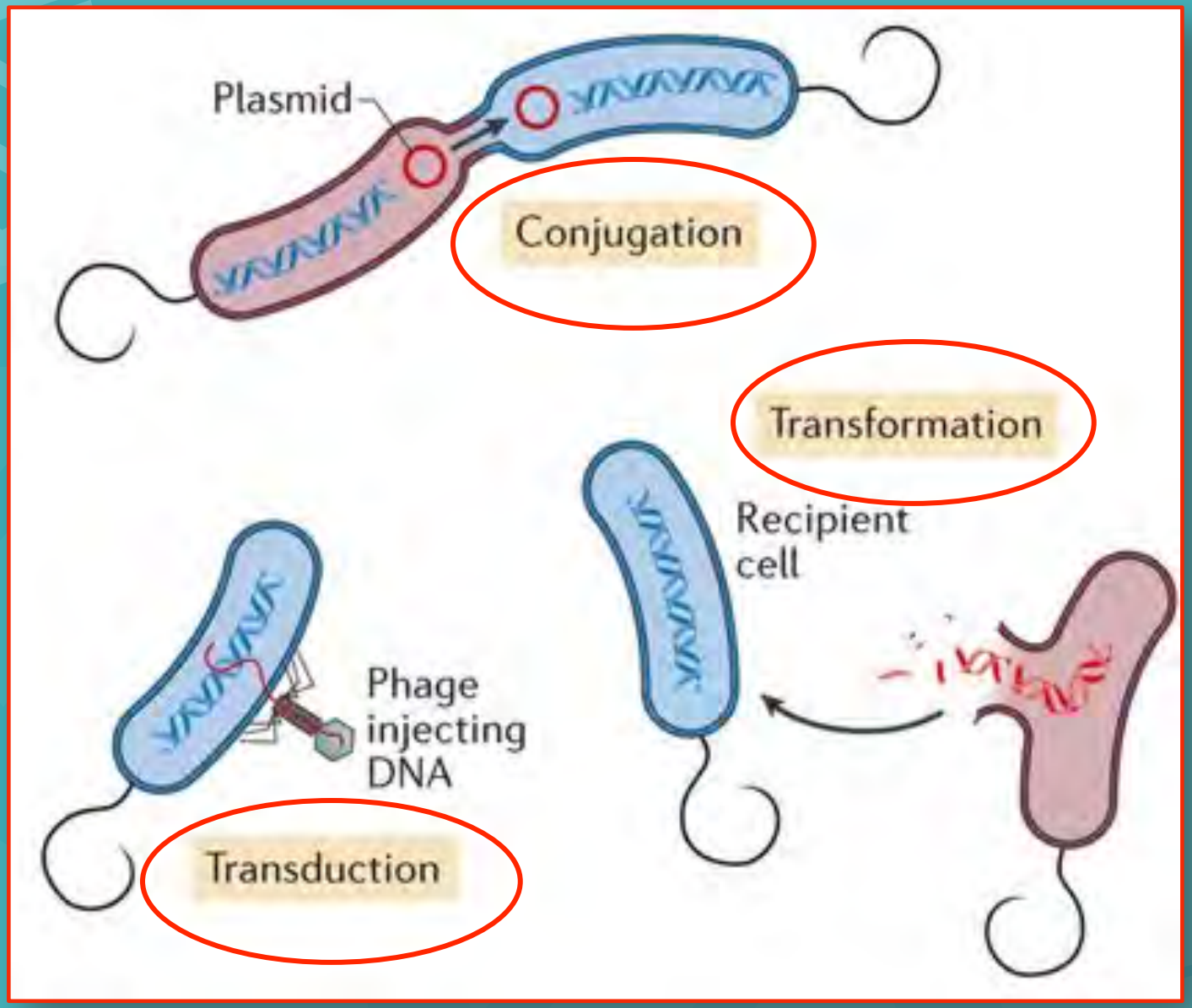


>90% of GM crops are engineered with transgenes for herbicide resistance and insecticides.

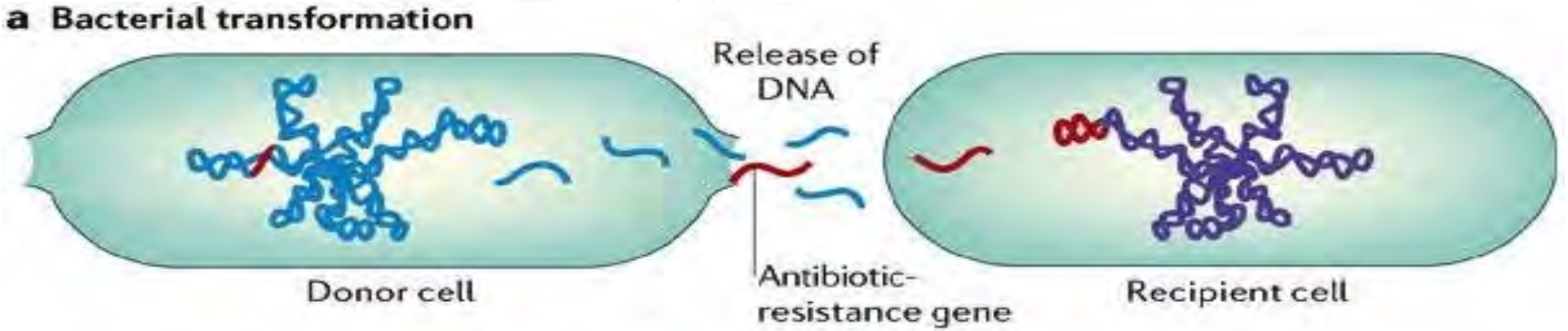
Selection of transformed plant cells

Horizontal spread

❖ Possibility of them to being horizontally spread in the gastrointestinal tract and eventually threatening human health.

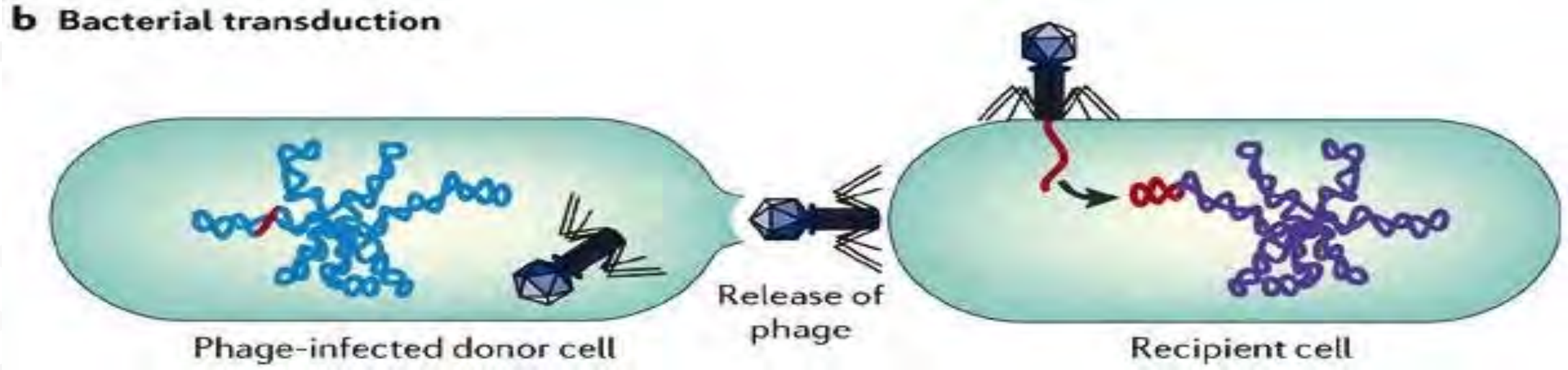


Bacterial transformation



❖ It consists in the incorporation of exogenous genetic material from its surrounding through the cell membrane.

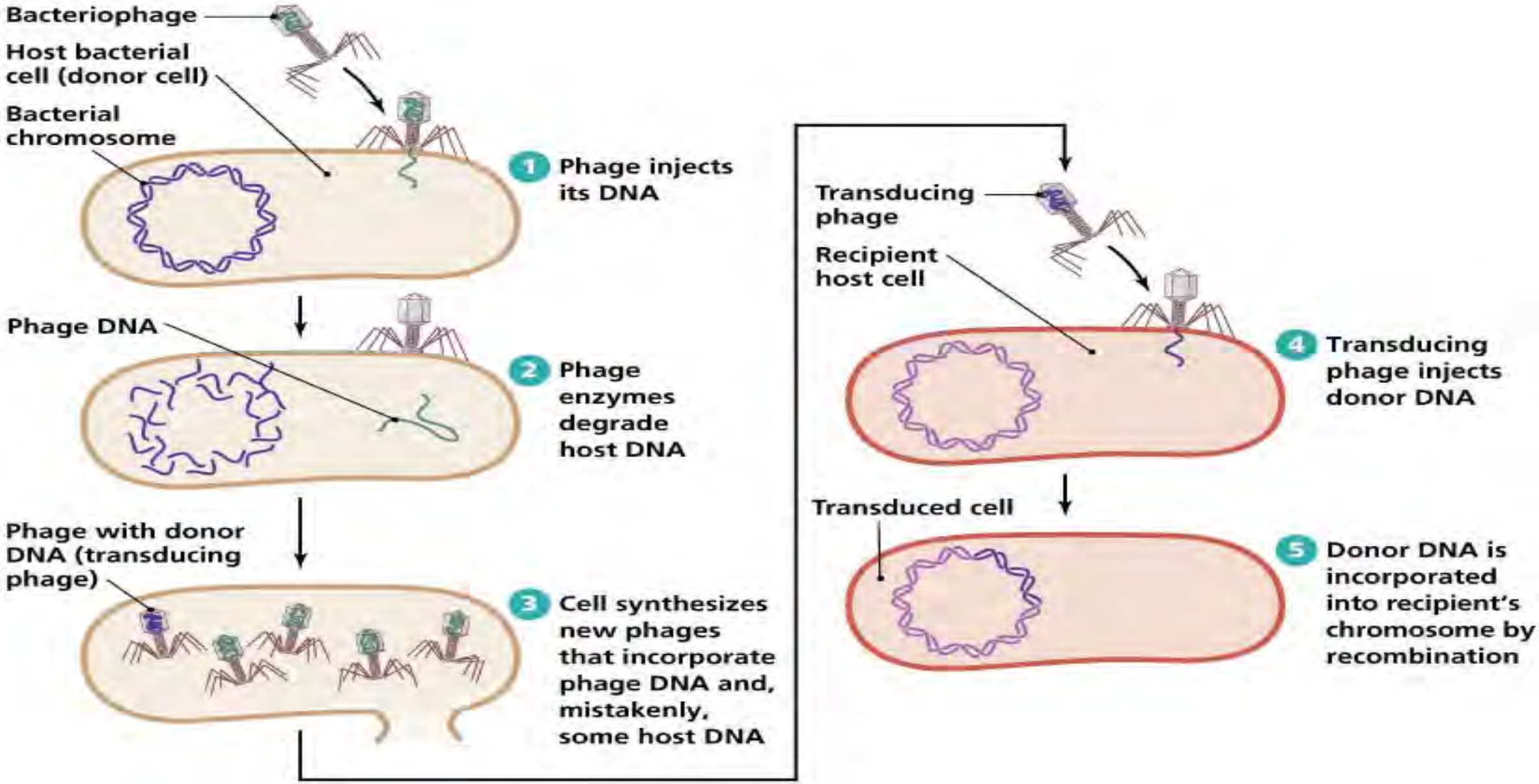
Bacterial transduction



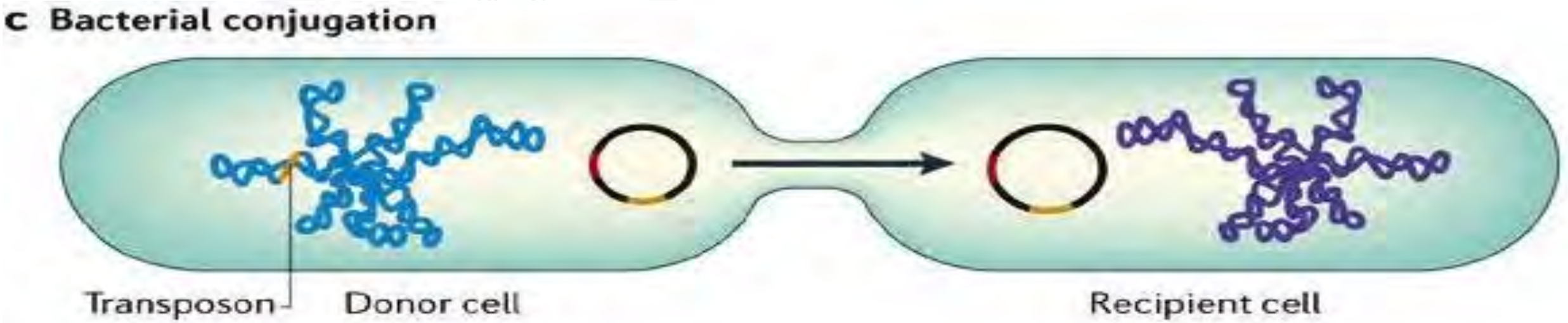
❖ Is the process by which foreign DNA is introduced into a cell by a virus or viral vector.

❖ Is a method used for example by bacteria to stably introduce a foreign gene into their genome.

Bacterial transduction



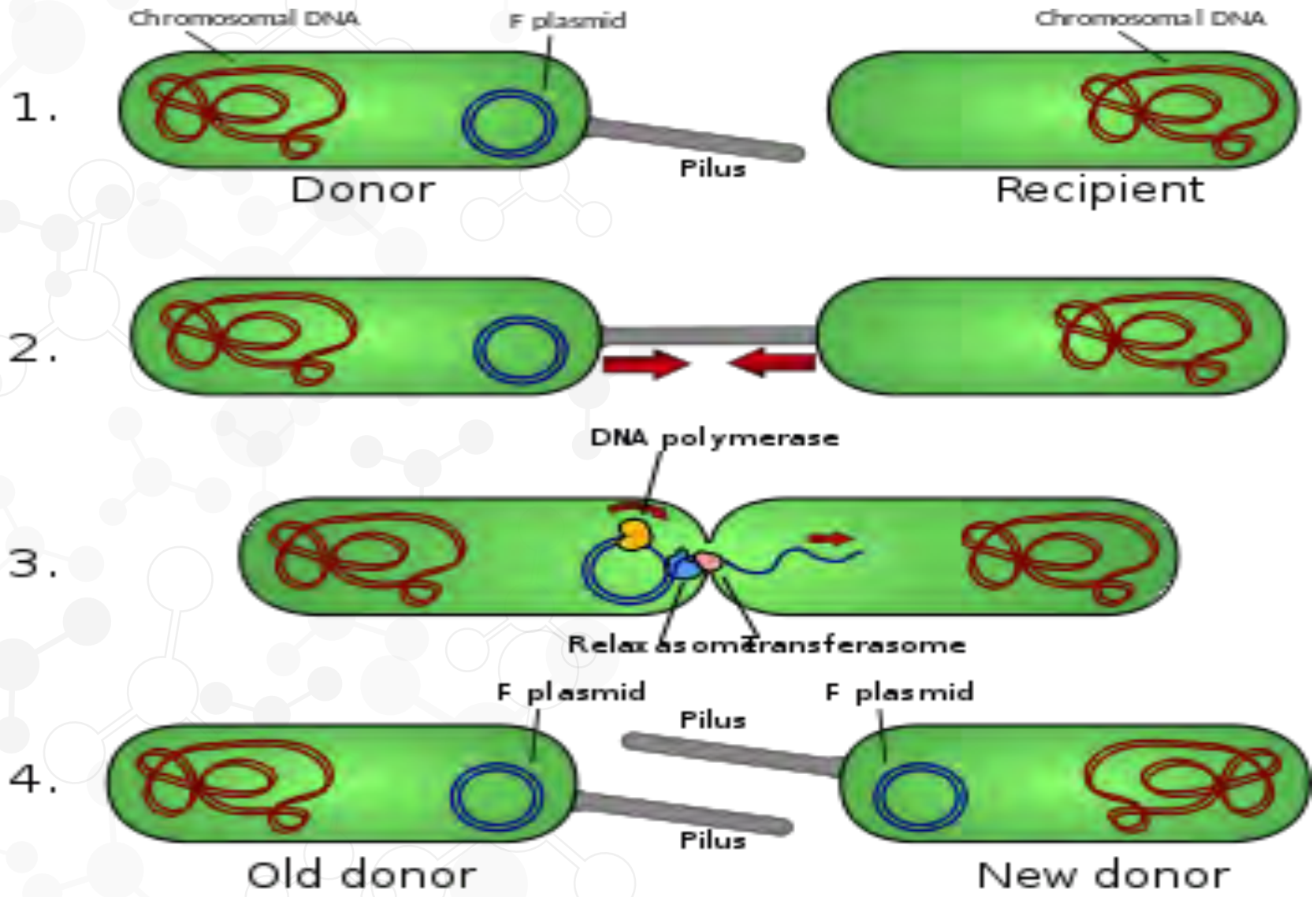
Bacterial conjugation



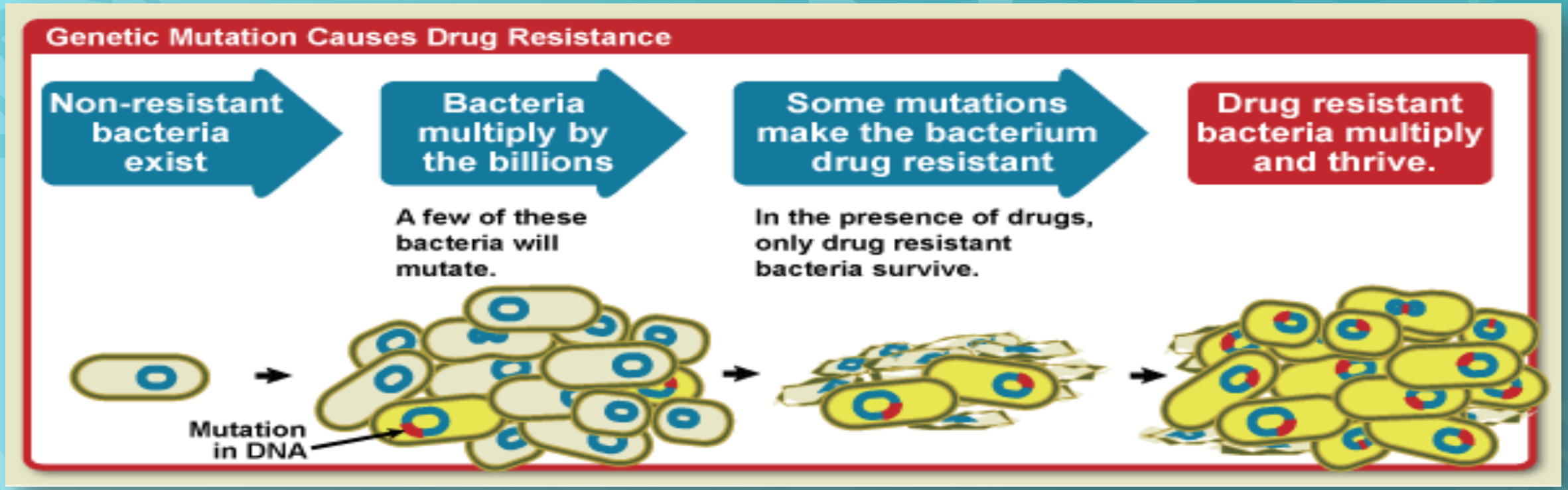
❖ It consists in the transfer of genetic material between bacterial cells by direct cell-to-cell contact (or by a bridge connection between the two cells, the pilus).

❖ The donor cell provides a conjugative element that often is a plasmid or a transposon.

Bacterial conjugation



Vertical transmission



Study conducted in patients with GERD or celiac diseases

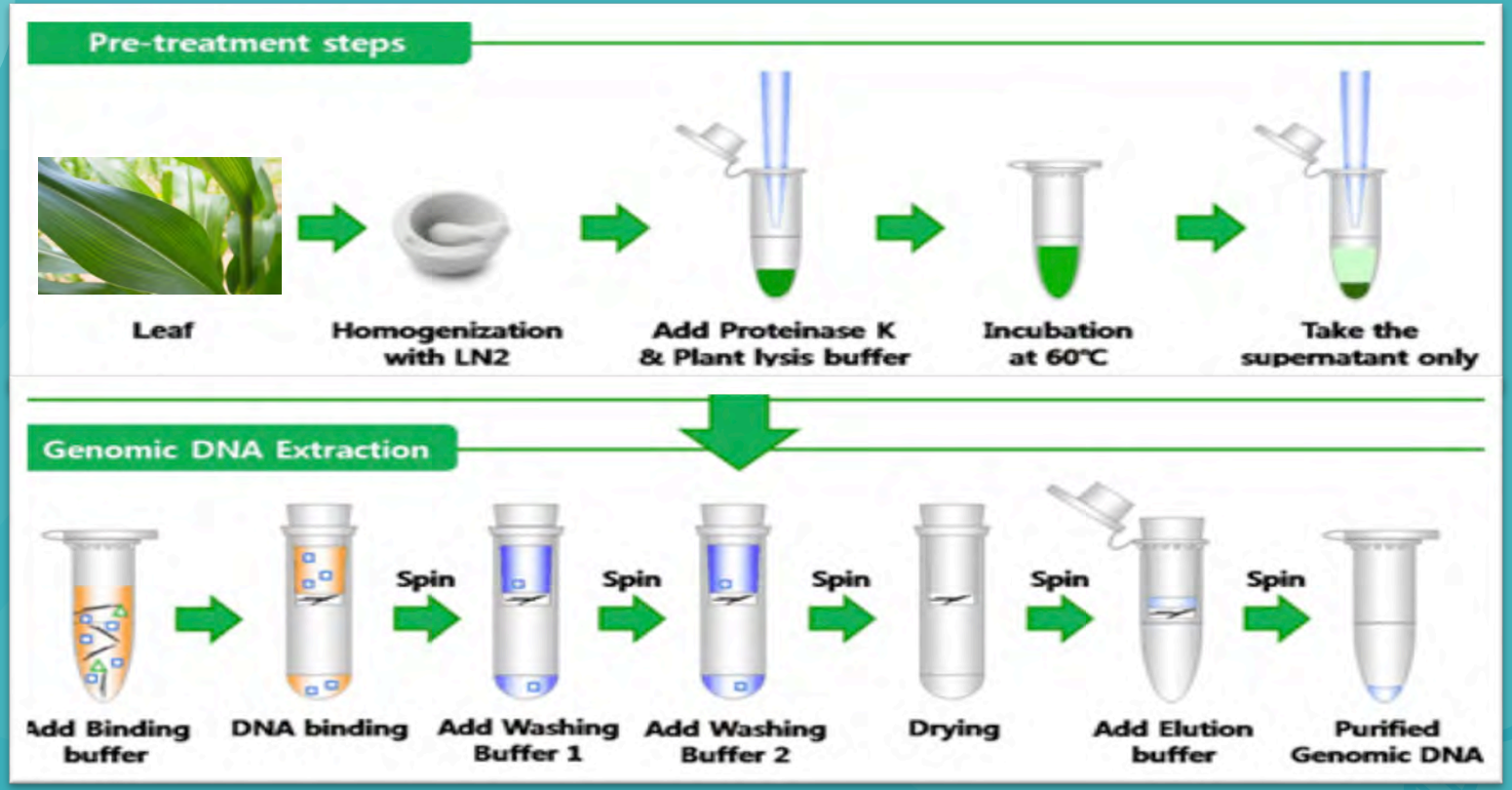
In Bt176 maize it was shown that there is a longer resistance of some DNA traits to gastric juice from gastrointestinal affected patients.

In particular the resistance regarding two genes:

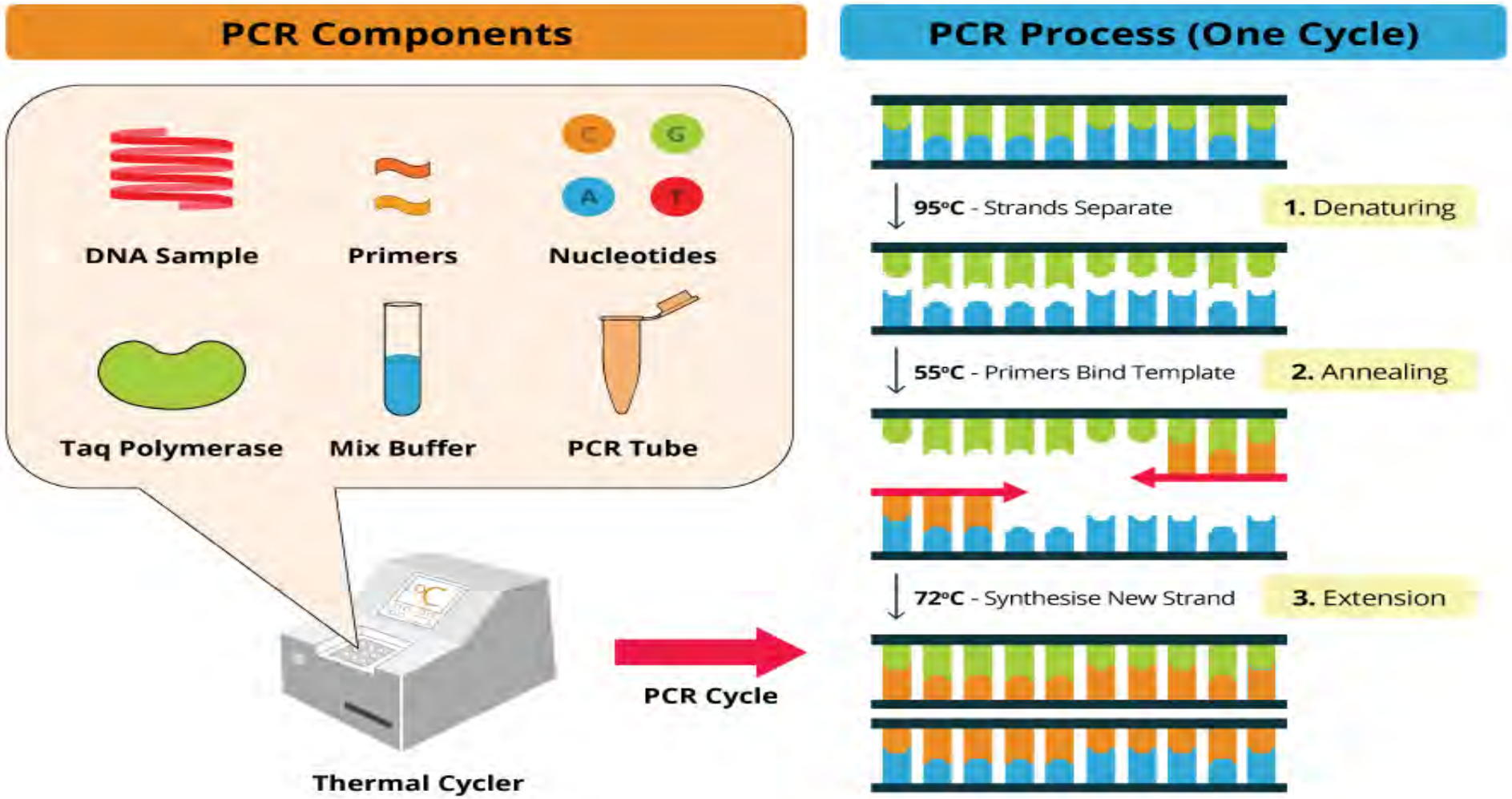
- **bla**
- **cryIA(b)**

DNA extraction

❖DNA was extracted from Bt176 maize and incubated with gastric juice samples.



PCR amplification



PCR amplification

Investigates the survival of the genes incubated in human gastric juice at:

- Increasing time;
- Different values of pH.

Electophoresis

Sample number	Type of sample	pH	Length of the gene/fragment		
			cry1A(b) 1914 bp	*bla* 999 bp	Sub- *cry1A(b)* 211 b
A					
1*	nt	1.59	0	30	120
6	m	3.04	60	120	240
7	m	5.38	60	120	240
2**	nt	2.33	60	120	120
8	m	4.62	120	180	240
B					
3	t	2.47	60	240	240
4	t	4.50	60	180	240
5	t	6.93	30	60	120

A= celiac patients without any drug treatment

B= patients with GERD subjected to an appropriate anti-acid treatment

*=sample 1 and its derivatives 6-7
**=sample 2 and its derivative 8

nt= not treated
t= treated
m= modified pH

Results

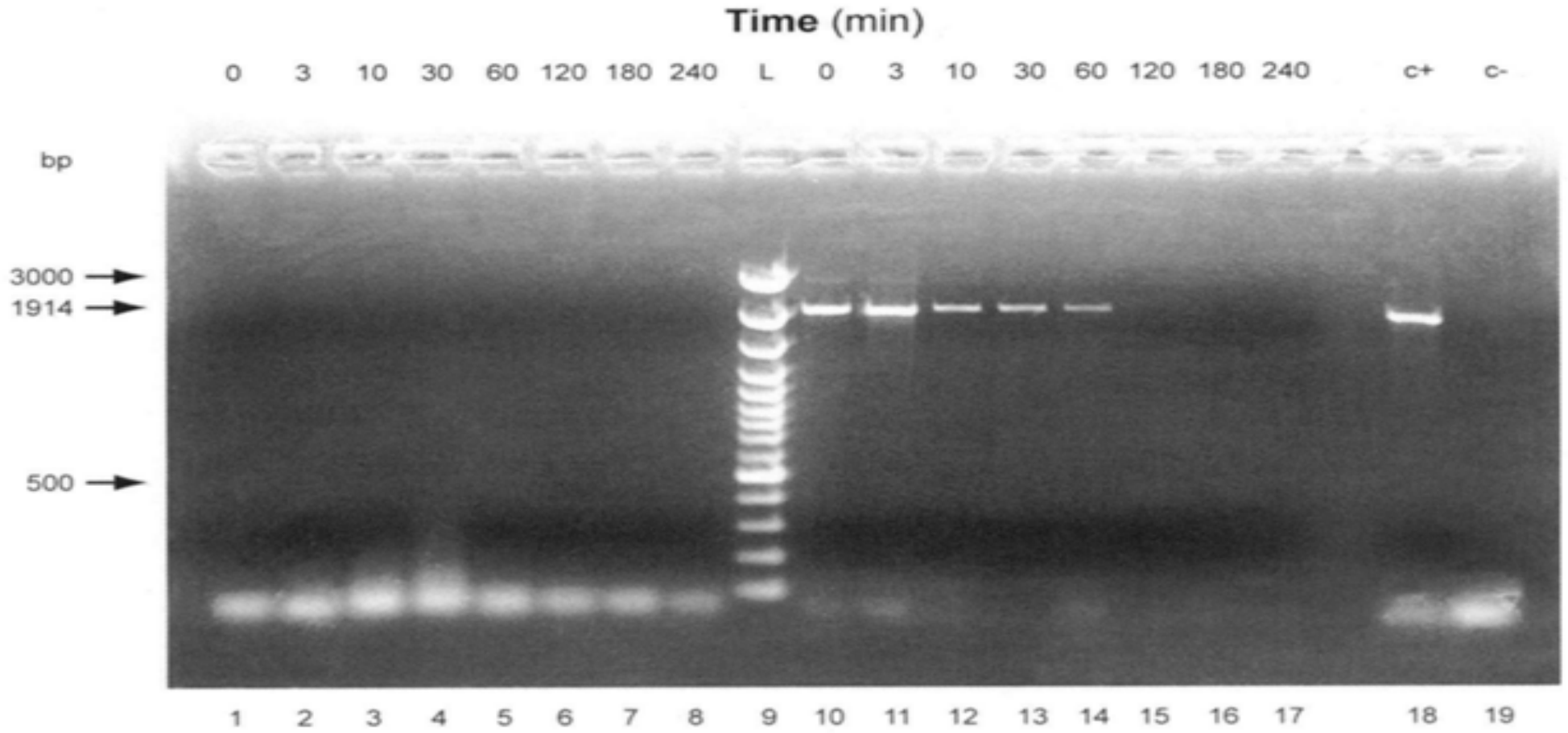


Fig. 1. *Cry01/02-PCR: amplification of 1914 bp fragment Cry I A(b) after incubation with samples 1 and 6 of gastric juice (times: 0 to 240 min). Lanes 1-8: sample 1 (nt sample - pH 1.59); L 9: 100 bp ladder; L 10-17: sample 6 (m sample- pH adjusted to 3.04 with $NaHCO_3$ 0.1 M); 18: positive control; 19: negative control.*

Results

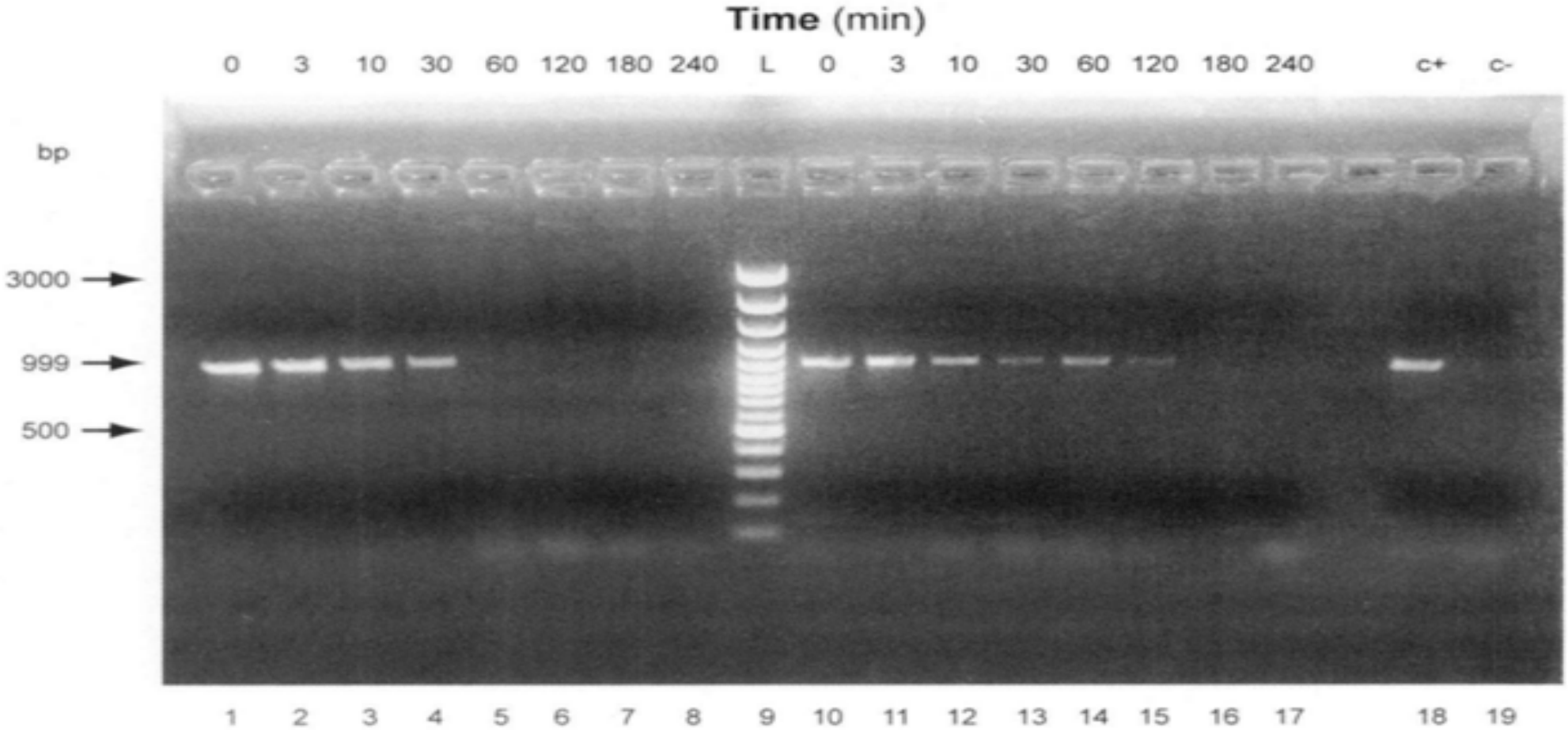


Fig. 3. *BlaTEM1-PCR: survival of bla gene after incubation with samples 1 and 6 of gastric juice (times:0 to 240 min). 1-8: sample 1 (no treated sample- pH 1.59); L 9:100 bp ladder; L 10-17: sample 6 (sample 1 pH adjusted to 5.58 with $NaHCO_3$ 0.1 M); L 18: positive control; L 19: negative control.*

Results

❖ The survival of the considered fragments to gastric juice seemed to be dependent on their lenght;

❖ Bigger fragments showed a lowest resistance respect than smallers;

❖ It confirmed the importance of the length of the PCR amplicon for the survival of target traits after exposure to human gastric juice;

❖ The longer the gene fragment, the shorter was the survival.

Conclusion

❖ The DNA survival could strongly depend on the physiological status of the patient and on the consumption of some substances that can be introduced by food or drugs;

❖It is therefore clear the need for a strict regulation for the introduction of GMOs;

❖An important aspect that have to be considered is the presence of antibiotic resistance gene markers because nowadays is not yet delineated their potential harmful effect on human health.


Thank You