

## Modern Biotechnology and the BCH

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## CEE REGIONAL BCH TRAINING WORKSHOP

**JANUARY 2024**

# RECORDS

Introduced or modified genetic element(s)

Some of these genetic elements may be present as fragments or truncated forms. Please see notes below, where applicable.

- BCH-GENE-SCBD-14972-12** PHOSPHINOTHRICIN N-ACETYLTRANSFERASE GENE |  
Protein coding sequence | Resistance to herbicides (Glufosinate)
- BCH-GENE-SCBD-14985-12** CRY1AB | BACILLUS THURINGIENSIS - BT, BACILLUS, BACTU |  
Protein coding sequence | Resistance to diseases and pests (Insects, Lepidoptera (butterflies and moths))
- BCH-GENE-SCBD-14975-5** BETA-LACTAMASE GENE | (BACTERIA) |  
Protein coding sequence | Resistance to antibiotics (Ampicillin)
- BCH-GENE-SCBD-100287-7** CAMV 5S PROMOTER |  
Promoter
- BCH-GENE-SCBD-100290-6** CAMV 35S TERMINATOR |  
Terminator

Genetic element  
Promoter  
Terminator  
Marker gene  
Agrobacterium  
Coding sequence  
Truncated gene  
Unique identifier  
Transformation cassette  
Gene gun  
Risk Assessment  
Detection and identification

## Description

This LMO contains two copies of a truncated synthetic version of the full length *cry1Ab* gene from *Bacillus thuringiensis* subsp. *kurstaki*. The synthetic truncated *cry1Ab* gene encodes a protein that corresponds to the first 648 amino acids of the N-terminal of the 1155 amino acid full length native Cry1Ab protein and includes the portion of the native protein that is necessary for insect control.

EN

- Also note that the cassette has genetic elements belonging to corn to dupe the plant cell so that it does not recognize that

Additional information concerning the *bla* gene insert in this LMO:

The *bla* gene from *Escherichia coli* is not expressed in plant cells, but was employed as a selectable trait for screening bacterial colonies for the presence of the plasmid vector.

Additional information on the inserted genetic material:

EN

## Basic concepts

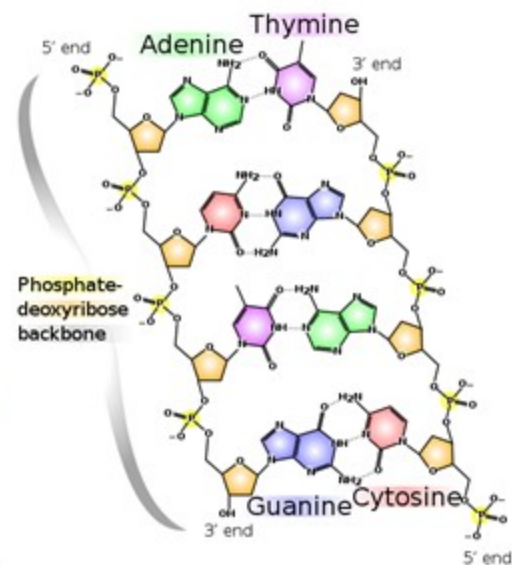
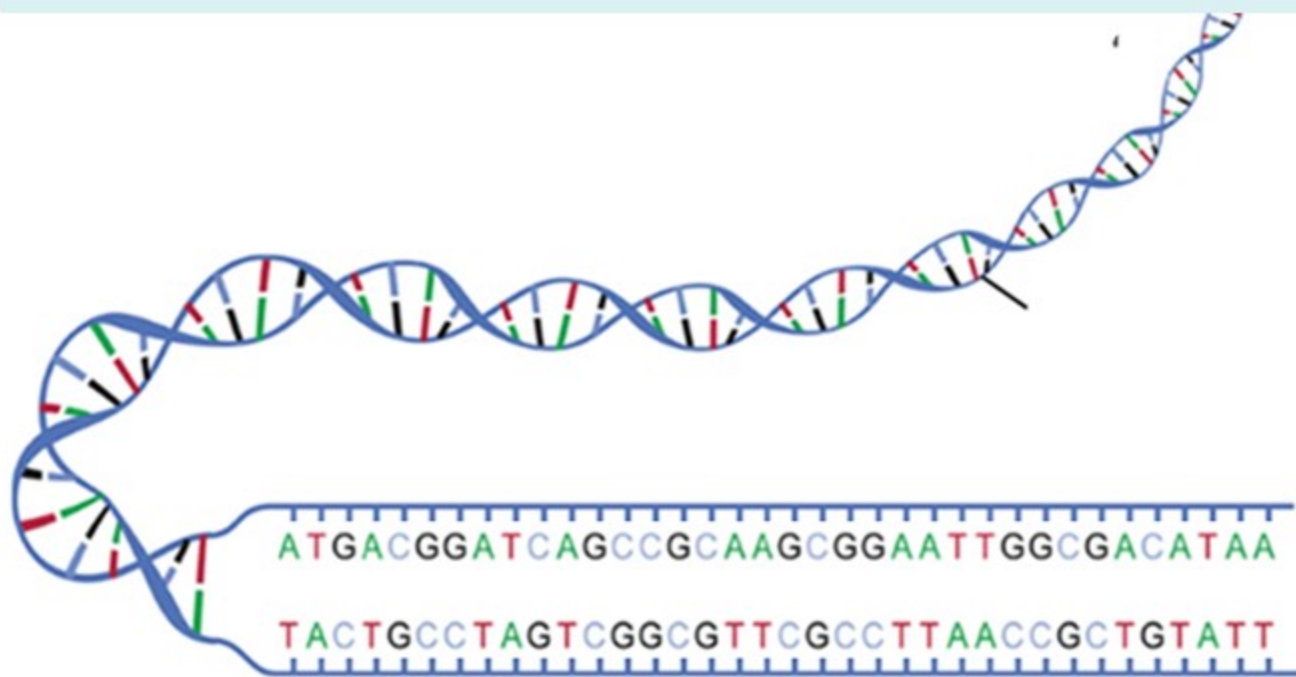
- What is the difference between Biosafety / Biosecurity?
- What is meant by Biosafety in the context of Cartagena Protocol?
- a term used to describe efforts to minimize and avoid the potential environmental and human health risks resulting from modern biotechnology and its products.

# WHAT IS GENETIC MATERIAL?



The medium by which inherited characteristics/traits of a living organism are transmitted from one generation to the next.

# WHAT IS THE GENETIC CODE?

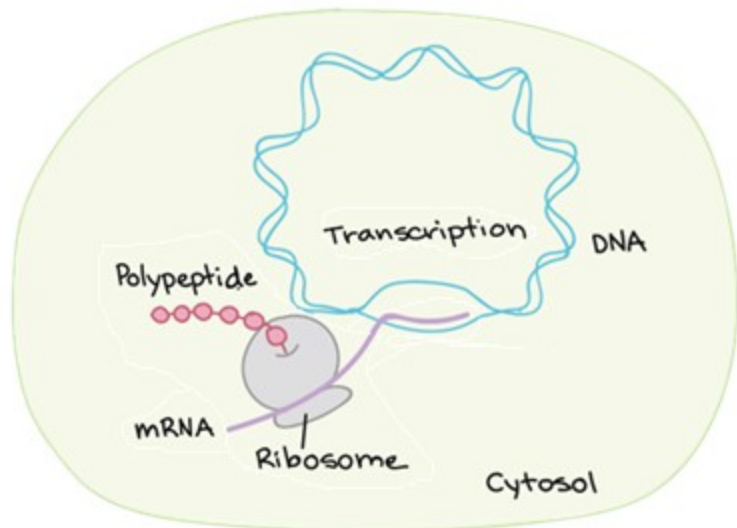


Sequences of nucleic acids that contain instructions for cell development and functions.

# WHAT IS GENE EXPRESSION?

Is the synthesis of a specific protein with a sequence of amino acids encoded in the genetic code.

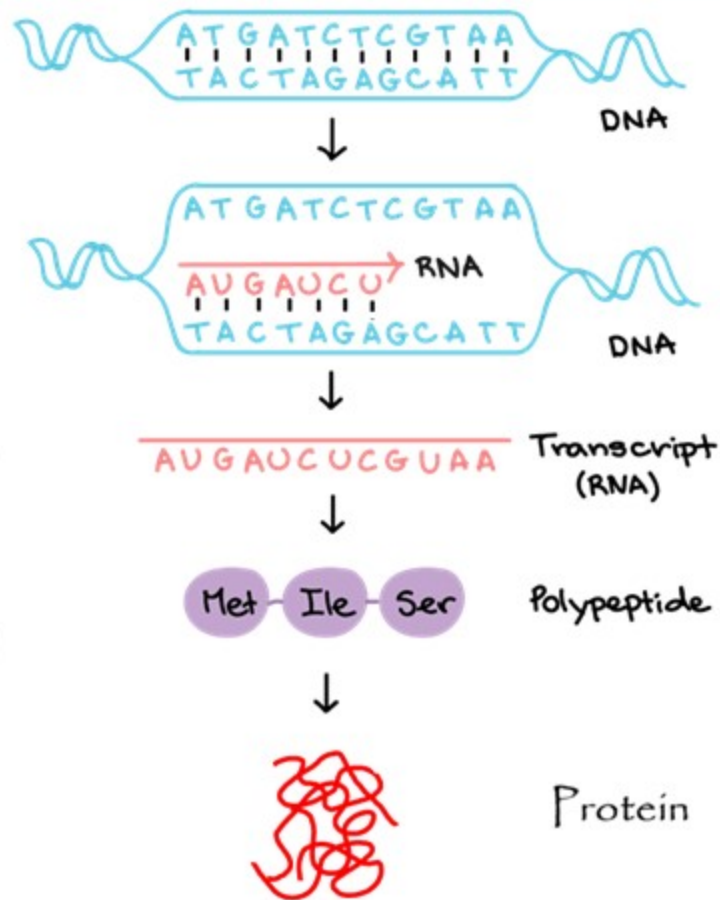
**BACTERIUM**



Transcription

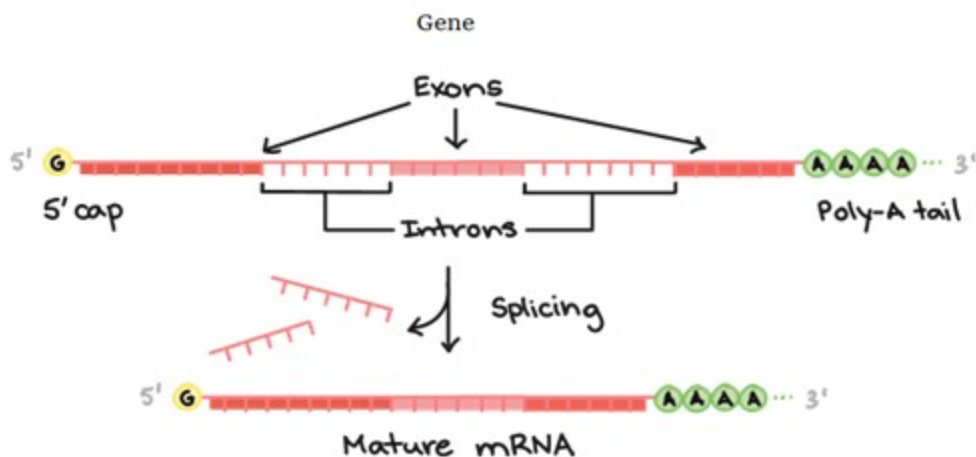
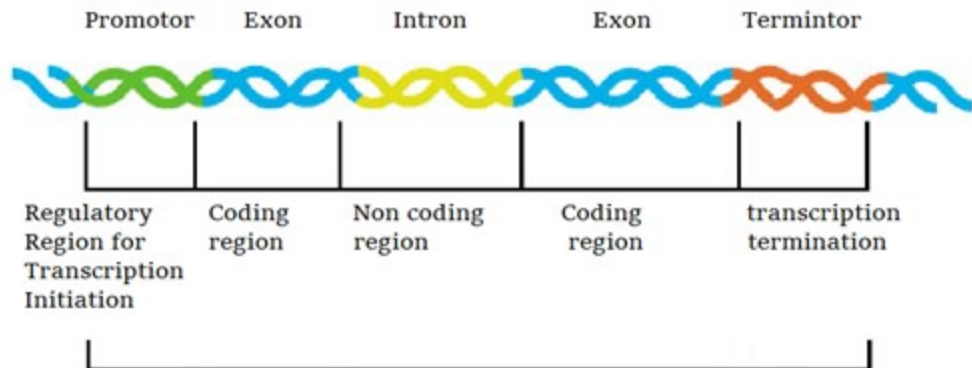
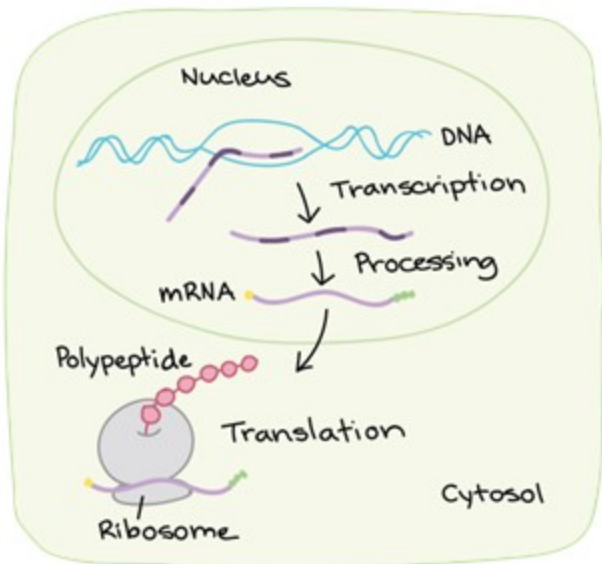
Translation

Folding

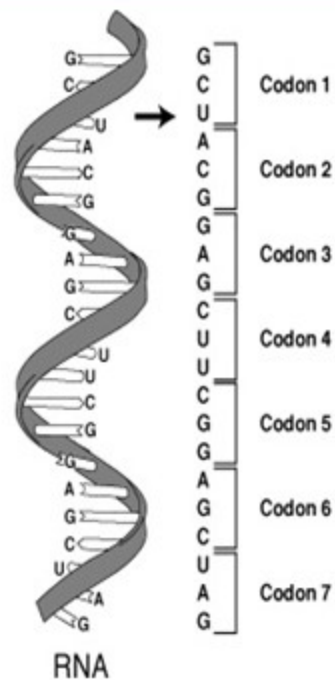


# WHAT IS GENE EXPRESSION?

## EUKARYOTIC CELL



# GENETIC CODE



Ribonucleic acid

|          |                        | 1st base          |                   |                |              |   |  |
|----------|------------------------|-------------------|-------------------|----------------|--------------|---|--|
|          |                        | U                 | C                 | A              | G            |   |  |
| 2nd base | U                      | UUU Phenylalanine | UCU Serine        | UAU Tyrosine   | UGU Cysteine | U |  |
|          | UUC Phenylalanine      | UCC Serine        | UAC Tyrosine      | UGC Cysteine   | C            |   |  |
|          | UUA Leucine            | UCA Serine        | UAA Stop          | UGA Stop       | A            |   |  |
|          | UUG Leucine            | UCG Serine        | UAG Stop          | UGG Tryptophan | G            |   |  |
| C        | CUU Leucine            | CCU Proline       | CAU Histidine     | CGU Arginine   | U            |   |  |
|          | CUC Leucine            | CCC Proline       | CAC Histidine     | CGC Arginine   | C            |   |  |
|          | CUA Leucine            | CCA Proline       | CAA Glutamine     | CGA Arginine   | A            |   |  |
|          | CUG Leucine            | CCG Proline       | CAG Glutamine     | CGG Arginine   | G            |   |  |
| A        | AUU Isoleucine         | ACU Threonine     | AAU Asparagine    | AGU Serine     | U            |   |  |
|          | AUC Isoleucine         | ACC Threonine     | AAC Asparagine    | AGC Serine     | C            |   |  |
|          | AUA Isoleucine         | ACA Threonine     | AAA Lysine        | AGA Arginine   | A            |   |  |
|          | AUG Methionine (Start) | ACG Threonine     | AAG Lysine        | AGG Arginine   | G            |   |  |
| G        | GUU Valine             | GCU Alanine       | GAU Aspartic Acid | GGU Glycine    | U            |   |  |
|          | GUC Valine             | GCC Alanine       | GAC Aspartic Acid | GGC Glycine    | C            |   |  |
|          | GUA Valine             | GCA Alanine       | GAA Glutamic Acid | GGA Glycine    | A            |   |  |
|          | GUG Valine             | GCG Alanine       | GAG Glutamic Acid | GGG Glycine    | G            |   |  |

Nonpolar, aliphatic  
 Polar, uncharged  
 Aromatic  
 Positively charged  
 Negatively charged

It is universal in all living organisms with negligible exceptions. Three consecutive bases (codon) code for one amino acid

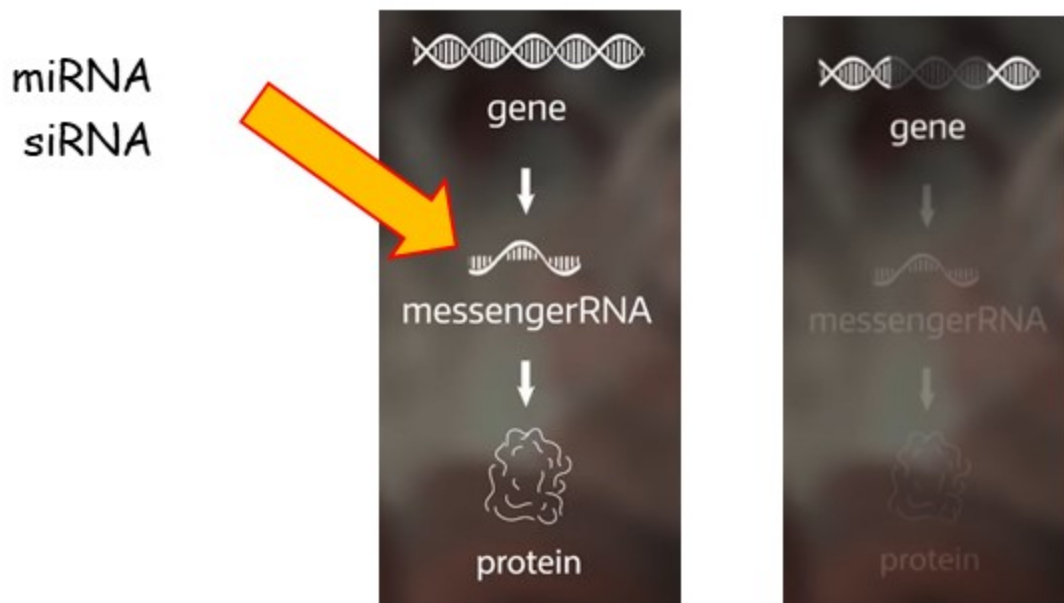


# GENE SILENCING?



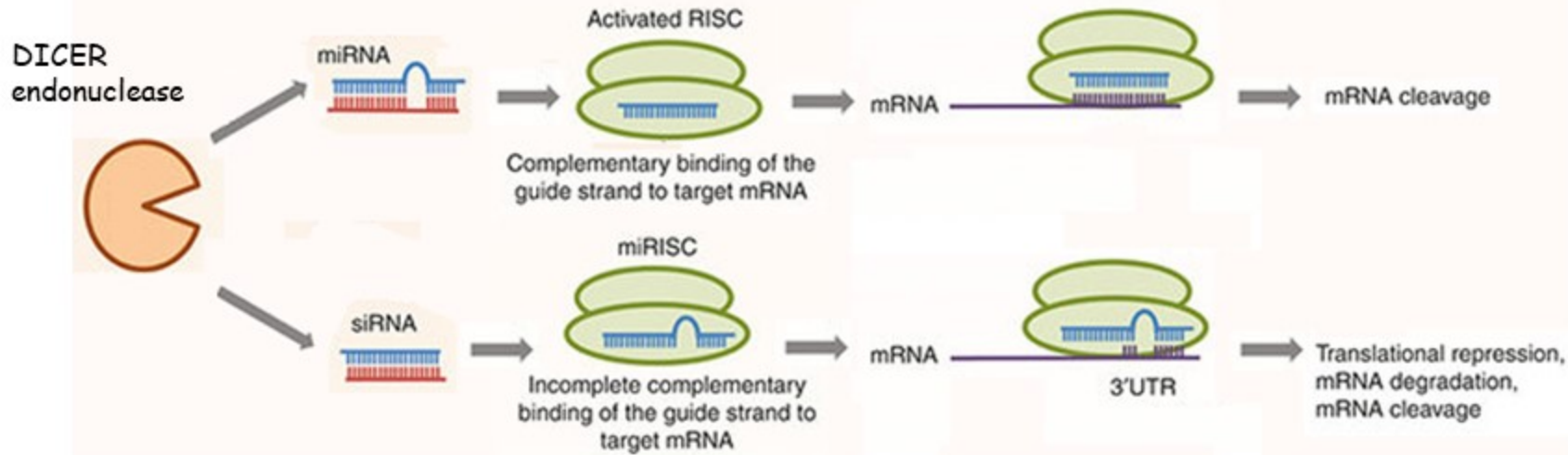
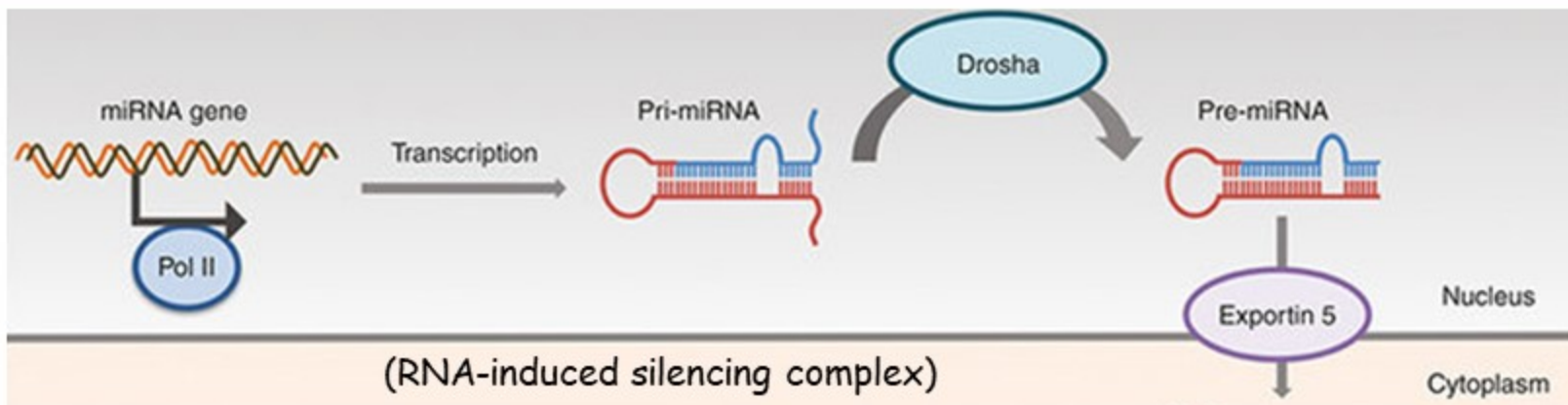
- Our body consists of different **types of cells** (skin, muscles, or bone cells) with **identical genetic materials**.
- Through **gene silencing** genetic information is **switched off** so during development a cell only reads instructions that are necessary for gaining the **characteristics structures and functions**.

# GENE SILENCING?



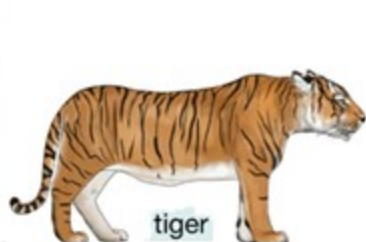
Turn off genes by inactivating mRNA necessary for translating genetic information into proteins. They participate in regulating the cells from their development to their death.

# miRNA BIOGENESIS AND RNA-INDUCED GENE SILENCING



# WHAT IS A SPECIES?

- Is a group of living organisms consisting of similar individuals capable of exchanging genes or interbreeding to produce fertile offspring.

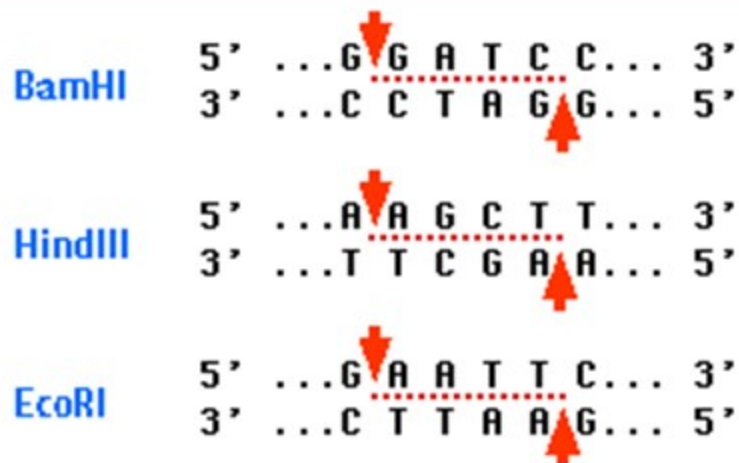
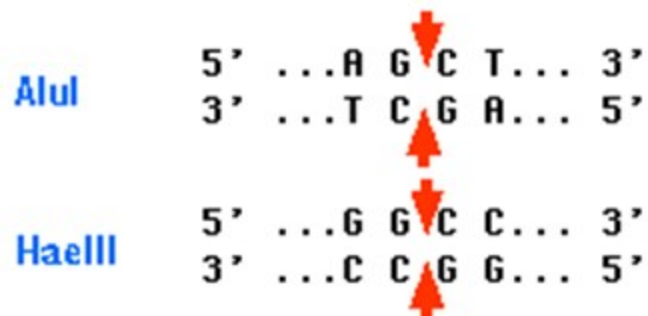


# GENETIC BARRIERS BETWEEN SPECIES



Each living cell can identify foreign genetic materials belonging to other species and will make it unfunctional by destroying it or by repairing its own DNA, creating barriers between species.

# RESTRICTION ENZYMES



- Restriction enzymes are naturally occurring defense mechanisms to digest foreign DNA molecules.
- They recognize specific DNA sequences, mostly 4-6 bp, and cut DNA into fragments by breaking the phosphodiester linkage between two successive nucleotides of DNA.
- Now, if these restriction sites may be present in the organism's DNA, the DNA methylase enzymes carry out methylation of their DNA to protect it from digestion.

# GENETIC ENGINEERING – MODERN BIOTECHNOLOGY



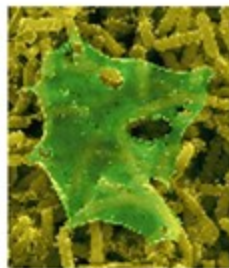
Genetic material is altered or artificially introduced *in vitro* to induce a desirable new trait that does not occur naturally in the species. Inserted genes usually come from a different species.

# OVERVIEW OF THE PROCESS OF GENETIC ENGINEERING

1. Identify and isolate genetic sequence of interest from a donor organism and manipulate it in the laboratory to enhance their expressions in the intended recipient organism



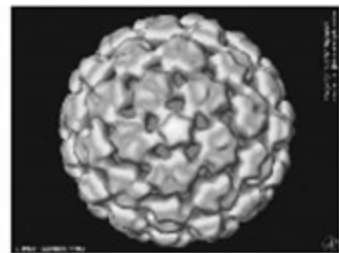
*Bacillus thuringiensis*



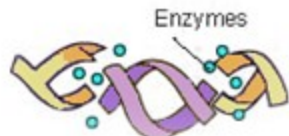
*Streptomyces hygroscopicus*



*Escherichia coli*



Cauliflower mosaic virus

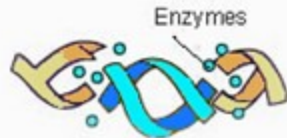


Cry1Ab gene

Resistance to Insects - Lepidoptera  
(butterflies and moths)



Phosphinothricin N-acetyltransferase gene  
Resistance to herbicides - Glufosinate



Beta-lactamase gene  
Resistance to antibiotics - Ampicillin

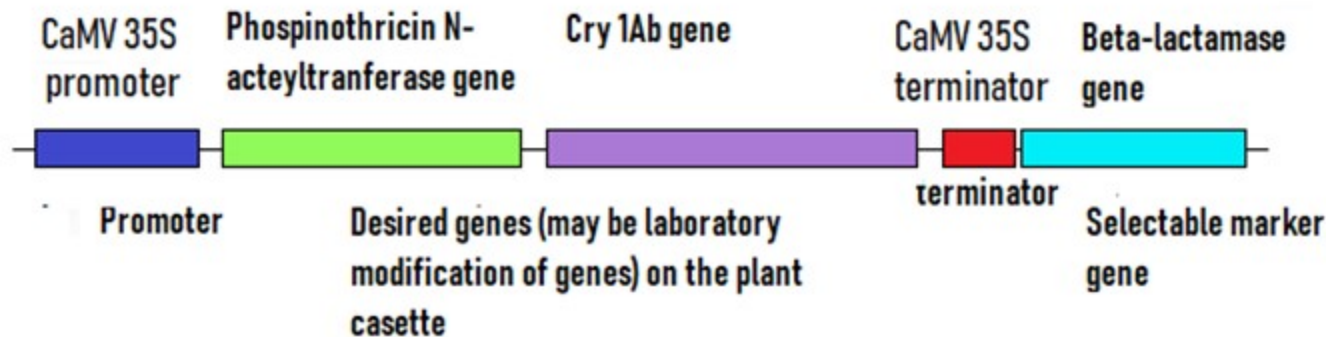


CaMV 35S promoter  
CaMV 35S terminator



# OVERVIEW OF THE PROCESS OF GENETIC ENGINEERING

2. Build the manipulated genes of interest and other nucleotide sequences needed for their proper functioning in an orderly sequence "transformation cassette."



3. Finally, the cassette is integrated into the recipient organism's genome through a process known as 'transformation'.

# OVERVIEW OF THE PROCESS OF GENETIC ENGINEERING

## Techniques used for the modification ✕

Search the list (min 3 chars to begin search)



0 keywords selected.

- Agrobacterium-mediated DNA transfer
- Biolistic / Particle gun
- Cell fusion
- Cross breeding
- 'de novo' synthesis
- Direct DNA transfer
  - Electroporation
  - Heat shock
  - Microinjection
  - Osmotic shock
- Embryonic stem cell-mediated gene transfer
- Gene editing (e.g. CRISPR-Cas, etc.)
- Virus-mediated gene transfer
- Other

Apply

# GENETIC ENGINEERING – MODERN BIOTECHNOLOGY

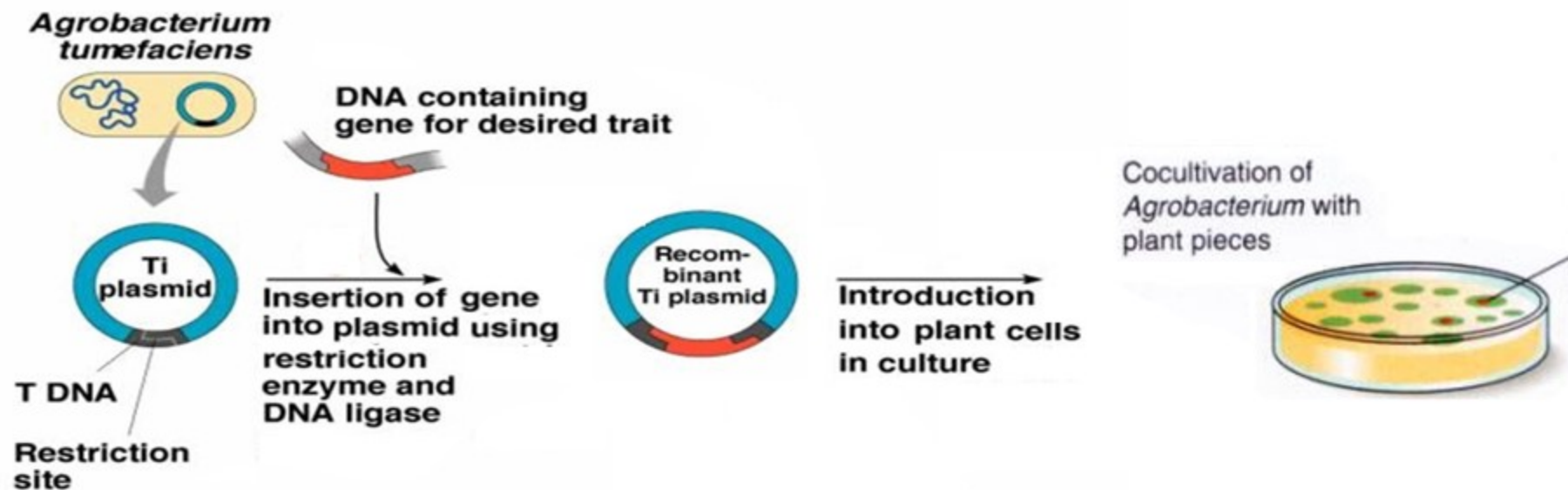
## COMMONLY USED METHODS IN GENETIC ENGINEERING

- *Agrobacterium tumefaciens* is a rod-shaped, Gram-negative soil bacterium.
- is the causal agent of crown gall disease (the formation of tumors) in over 140 species of eudicots.



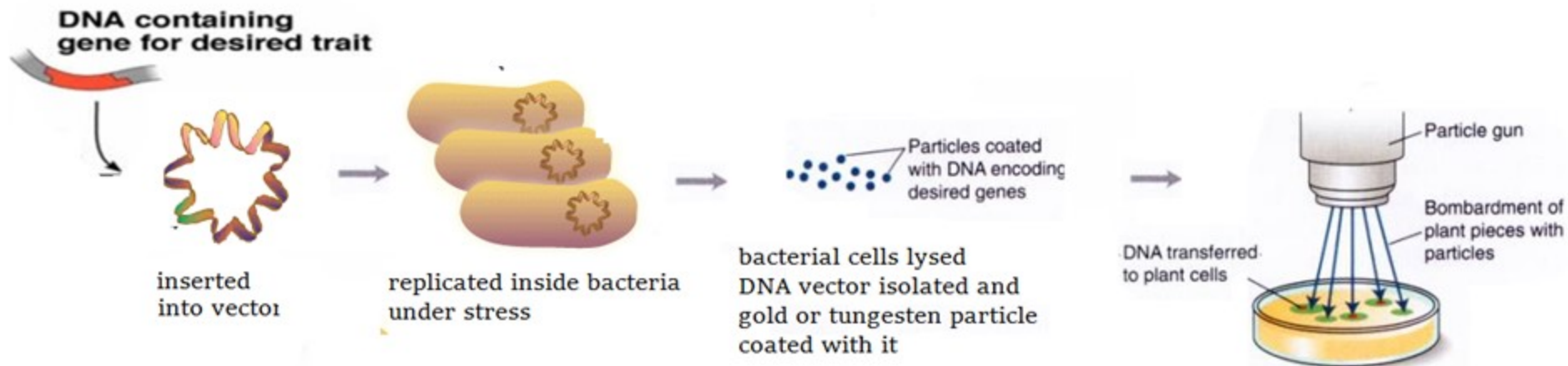
# GENETIC ENGINEERING – MODERN BIOTECHNOLOGY

## TRANSFORMATION USING AGROBACTERIUM



# GENETIC ENGINEERING – MODERN BIOTECHNOLOGY

## TRANSFORMATION USING GENE GUN



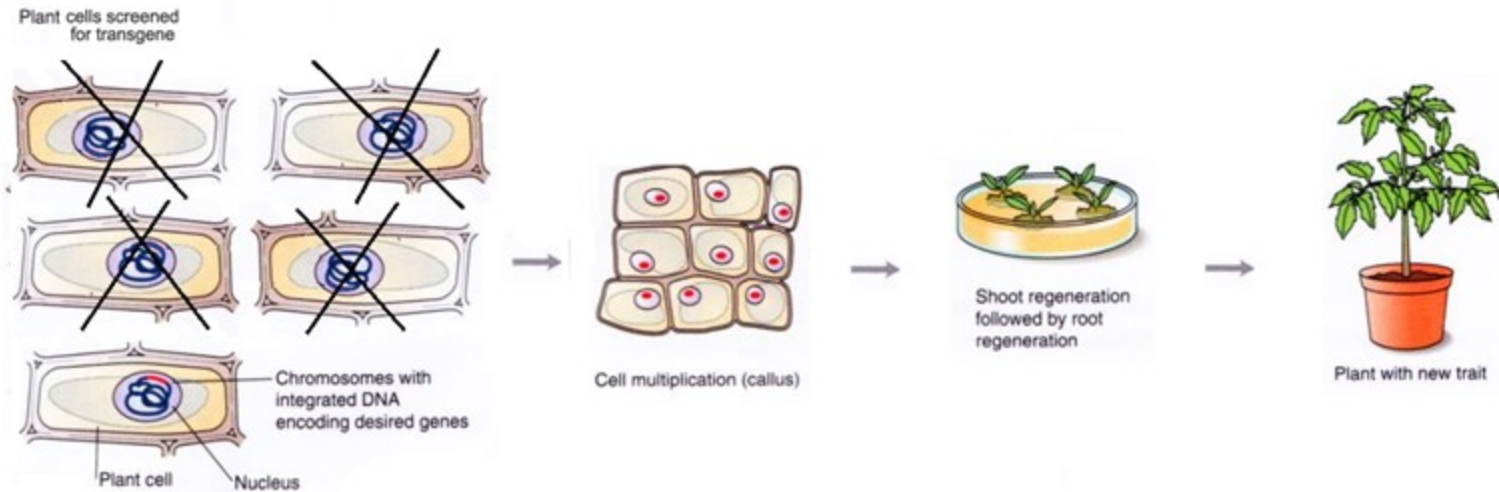
- After the DNA-coated particles have been delivered to the cells, the DNA is used as a template for transcription (transient expression), and sometimes it integrates into a plant chromosome ('stable' transformation).



# GENETIC ENGINEERING – MODERN BIOTECHNOLOGY

## LMO PLANT GENERATION


- Transformed cells are then selected, e.g., with the help of a marker gene
- Then are treated with a series of plant hormones, such as auxins and gibberellins, to divide and differentiate into an entire plant.
- The new plant that originated from a successfully transformed cell has new traits that are heritable (LMO).



### Use of Terms

For the purposes of this Protocol:

- (g) "Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;
  
- (i) "Modern biotechnology" means the application of:
  - a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or
  - b. Fusion of cells beyond the taxonomic family,that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;



| Record ID                             | Unique identification        | Identity & transformation event  | Organism  | Description  |
|---------------------------------------|------------------------------|--|---|--|
| <a href="#">BCH-LMO-SCBD-114444-1</a> | AAT-709AA-4                  | <b>Pod Borer-resistant cowpea</b><br>AAT709A                             | Vigna unguiculata<br>Cowpea, Black eyed<br>pea                                    | Resistance to diseases and pests - Insects - Lepidoptera (butterflies and moths), Resistance to antibiotics - Kanamycin                        |
| <a href="#">BCH-LMO-SCBD-14752-6</a>  | ACS-BN011-5                  | <b>Navigator™ canola</b><br>Oxy-235                                      | Brassica napus<br>Turnip, Rapeseed,<br>Canola Plant, Oilseed<br>Rape, Rape, BRANA | Resistance to herbicides - Bromoxynil  |
| <a href="#">BCH-LMO-SCBD-15101-6</a>  | ACS-BN010-4                  | <b>Falcon™ rapeseed</b><br>GS40/90pHoe6/Ac                               | Brassica napus<br>Turnip, Rapeseed,<br>Canola Plant, Oilseed<br>Rape, Rape, BRANA | Resistance to herbicides - Glufosinate   |
| <a href="#">BCH-LMO-SCBD-14753-6</a>  | ACS-BN001-4                  | <b>InVigor™ canola</b><br>RF1 (B93-101)                                  | Brassica napus<br>Turnip, Rapeseed,<br>Canola Plant, Oilseed<br>Rape, Rape, BRANA | Resistance to herbicides - Glufosinate, Resistance to antibiotics - Kanamycin, Changes in physiology and/or production - Fertility restoration |
| <a href="#">BCH-LMO-SCBD-14754-5</a>  | ACS-BN002-5                  | <b>InVigor™ canola</b><br>RF2 (B94-2)                                    | Brassica napus<br>Turnip, Rapeseed,<br>Canola Plant, Oilseed<br>Rape, Rape, BRANA | Resistance to herbicides - Glufosinate, Resistance to antibiotics - Kanamycin, Changes in physiology and/or production - Fertility restoration |
| <a href="#">BCH-LMO-SCBD-14755-7</a>  | ACS-BN003-6                  | <b>InVigor™ canola</b><br>RF3  | Brassica napus<br>Turnip, Rapeseed,<br>Canola Plant, Oilseed<br>Rape, Rape, BRANA | Resistance to herbicides - Glufosinate, Changes in physiology and/or production - Fertility restoration  |
| <a href="#">BCH-LMO-SCBD-116285-1</a> | ACS-BN003-6 ×<br>MON-00073-7 | <b>Herbicide tolerant, male fertility restoring canola</b><br>RF3 × RT73 | Brassica napus<br>Turnip, Rapeseed,<br>Canola Plant, Oilseed                      | Resistance to herbicides - Glufosinate, Glyphosate, Changes in physiology and/or production - Reproduction, Fertility restoration              |



# UNIQUE IDENTIFIERS

## WHAT IS A UNIQUE IDENTIFIER?

- It is a digital alphanumeric code for each living-modified plant approved for commercial use, including food or feed.
- Unique Identifiers are generated by the developers of a new transgenic plant and included in the dossiers that they forward to national authorities during the safety assessment process.
- Once approved, national authorities can forward the unique identifier to the OECD Secretariat for inclusion in the OECD's product database, from which the information is automatically shared with the Biosafety Clearing House.

# UNIQUE IDENTIFIERS

## UNDERSTANDING THE CODE

2 or 3 alphanumeric digits to designate the applicant

5 or 6 alphanumeric digits to designate the transformation event

One numerical digit for verification (to reduce errors by ensuring the integrity of the alphanumeric code)

MON = Monsanto  
SYN = Syngenta  
DAS = Dow Agro-Science  
BCS = Bayer Crop-Science

MON-15985-7

SYN-EV176-9

DAS-Ø15Ø7-1

# GENETIC ENGINEERING – MODERN BIOTECHNOLOGY

## WHAT IS A STACKED LMO?

- It is an LMO possessing new traits resulting from more than one transformation cassette. It can be produced by several approaches, including conventional cross-breeding involving two LMOs that are either single transformation events or already stacked events, the transformation of an LMO, or simultaneous transformation with different transformation cassettes or vectors.
- Accordingly, the cassettes containing the transgenes and other genetic elements inserted in the original transformation events may be physically unlinked (i.e., located separately in the genome) and can segregate independently.
- Stacked LMOs may occur in the field in cross-pollinating plants like maize (corn) if more than one LMO are planted near each other.

# UNIQUE IDENTIFIERS

## UNDERSTANDING THE CODE

- For stacked LMOs, the unique identifiers show the multiple combined GM events.

**BCS-BNØ12-7** X **ACS-BNØØ3-6** X **MON-883Ø2-9**

**BCS-GHØØ2-5** X **BCS-GHØØ4-7**

LMO with 3 stacked events

LMO with 2 stacked events



## EXERCISES



# SEARCHING FOR INFORMATION

## CASE STUDY (CSF108):

You have recently been given a food product that indicates that it contains a genetically modified organism identified as 'SYN-EV176-9'. Use the BCH to answer the following questions.

Q1. What type of organism is 'SYN-EV176-9'?

Q2. How has 'SYN-EV176-9' been modified from its parent organism (i.e., what new characteristics does it display)?

Q3. Is 'SYN-EV176-9' known by any other names?

Q4. What gene has been inserted into 'SYN-EV176-9'? Where did the gene come from?

Q5. Have any countries approved 'SYN-EV176-9' for human food, animal feed, or processing? Which ones?

# SEARCHING FOR INFORMATION

## CASE STUDY (CSFI08):

Q6. Have any countries decided that 'SYN-EV176-9' cannot be used for any reason? If so, why?

Q7. Where could you go for further information about this organism?

Q8. What product does the inserted gene produce?

Q9. What other organisms in the BCH have the same inserted traits as 'SYN-EV176-9'?

Q10. What genes have been inserted into the other organisms to give these same traits?



# SEARCHING FOR INFORMATION

## CASE STUDY (CSF115):

You are a phytosanitary officer in the Czech Republic. You are inspecting a field planted with MON-ØØ81Ø-6 - YieldGard™ maize.

Q. What stacked events can be present with this event?

Thank you !

For more information, please email

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