UN environment programme

THE BCH III PROJECT

gef

Detection and identification of GMOs

Prof Ossama AbdelKawy

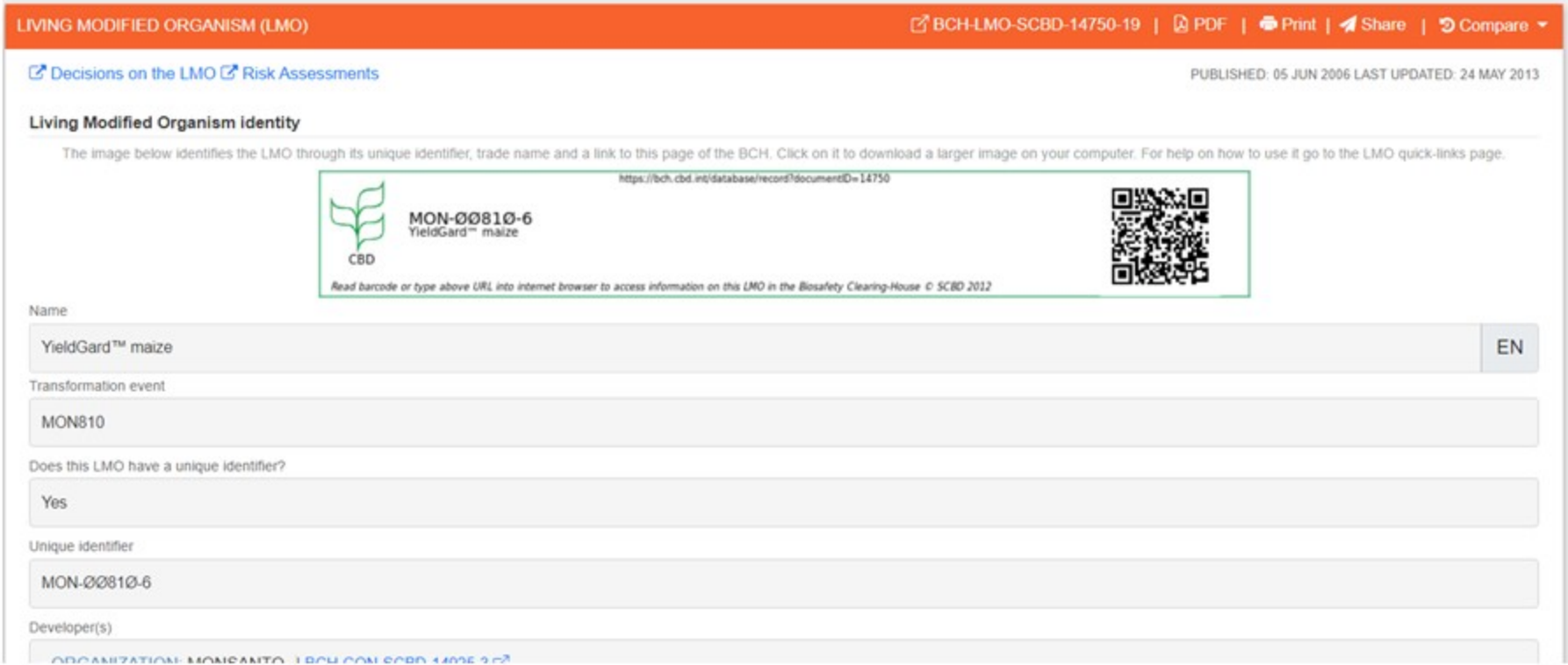


CEE REGIONAL BCH TRAINING WORKSHOP

2024

LMO RECORD

https://bch.cbd.int/en/database/record?documentID=14750



LIVING MODIFIED ORGANISM (LMO)

BCH-LMO-SCBD-14750-19 | PDF | Print | Share | Compare

Decisions on the LMO | Risk Assessments

PUBLISHED: 05 JUN 2006 LAST UPDATED: 24 MAY 2013

Living Modified Organism identity

The image below identifies the LMO through its unique identifier, trade name and a link to this page of the BCH. Click on it to download a larger image on your computer. For help on how to use it go to the LMO quick-links page.

https://bch.cbd.int/database/record?documentID=14750

CBD

MON-ØØ81Ø-6
YieldGard™ maize

Read barcode or type above URL into internet browser to access information on this LMO in the Biosafety Clearing-House © SCBD 2012

Name

YieldGard™ maize EN

Transformation event

MON810

Does this LMO have a unique identifier?

Yes

Unique identifier

MON-ØØ81Ø-6

Developer(s)

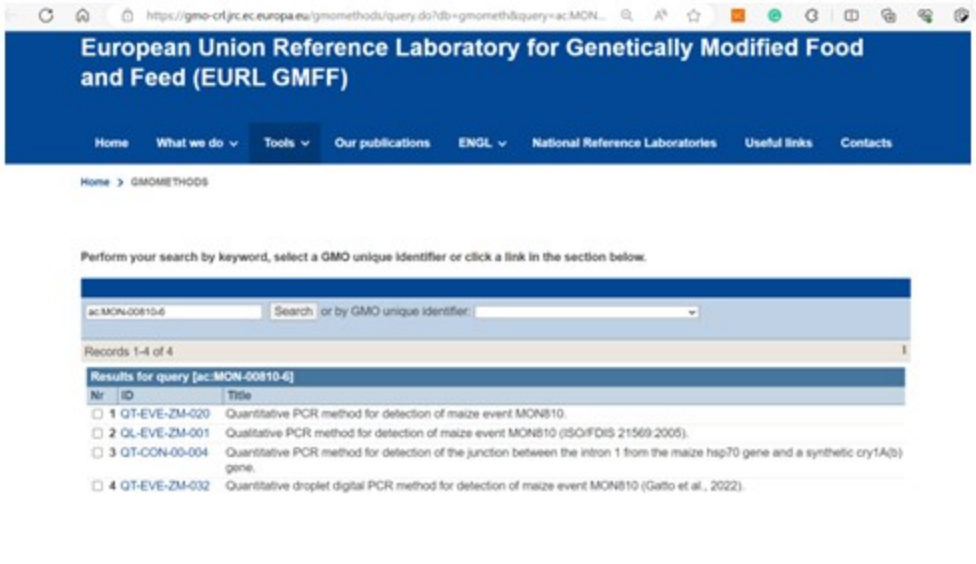
ORGANIZATION: MONSANTO, LBCH CON SCBD 14026 2

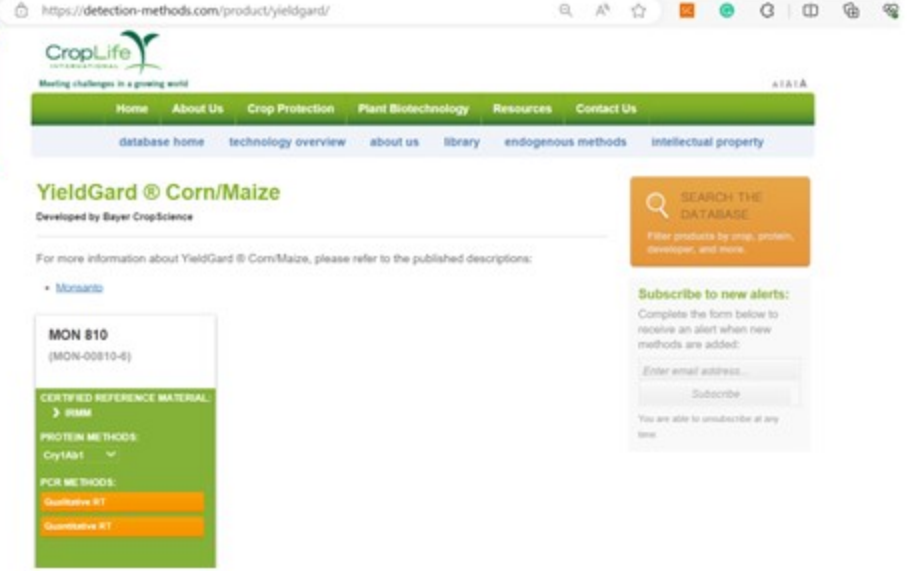
DETECTION AND IDENTIFICATION OF LMOS

Detection method(s)

External link(s)

- MON-ØØ81Ø-6 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) *[English]*
- MON-ØØ81Ø-6 - CropLife International Detection Methods Database *[English]*
- MON-ØØ81Ø-6 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) *(JRC) [English]*
- MON-ØØ81Ø-6 - CropLife International Detection Methods Database *(CropLife) [English]*





GMOs DETECTION

BRIEF OVERVIEW OF MOST COMMONLY USED METHODS

- LMOs are often developed by inserting one or more "genes of interest" which are mostly DNA molecules encoding proteins that confer particular traits of interest, such as insect resistance or herbicide tolerance.

- Either the DNA or the protein can be targeted for detecting or identifying LMOs.

- Both approaches, i.e. protein- or DNA-based methods, have advantages and disadvantages, and the adoption of one over the other, or both, will depend largely on the available expertise, infrastructure to handle samples, laboratory equipment and regulatory requirements.

GMOs DETECTION

GENERAL CONSIDERATIONS FOR DETECTION

- **Sampling**: determines how representative the result is of the lot from which it was taken. Sampling strategies must take into account the heterogeneity of the sample, the lot size, the sample size and particle size of the test portion being analyzed and the impact of this on the limits of detection (LOD) or quantification (LOQ).

- **Extraction method**: for proteins or DNA from the sample must take into account the matrix of the sample (the constituent complexity of the sample) to ensure that "matrix effects" due to an "interferent" do not affect the outcome of the results.

- **Reference material**: (a food matrix containing a specific amount of a specific GMO) during GMO testing that can be used as an external standard, to validate a method or determine method sensitivity and specificity.

GMOs DETECTION

GENERAL CONSIDERATIONS FOR DETECTION

- **Method validation:** Ensure that different testing methods give comparable results that are reliable and repeatable.

It is also important to establish minimum performance criteria in terms of specificity and sensitivity, accuracy and reproducibility

GMOs DETECTION

PROTEIN-BASED METHODS FOR LMO DETECTION

- Detect proteins that are manufactured in the cell according to the information coded by the transgenic (GMO) DNA by antibody recognition of an epitope specific to the transgenic protein.

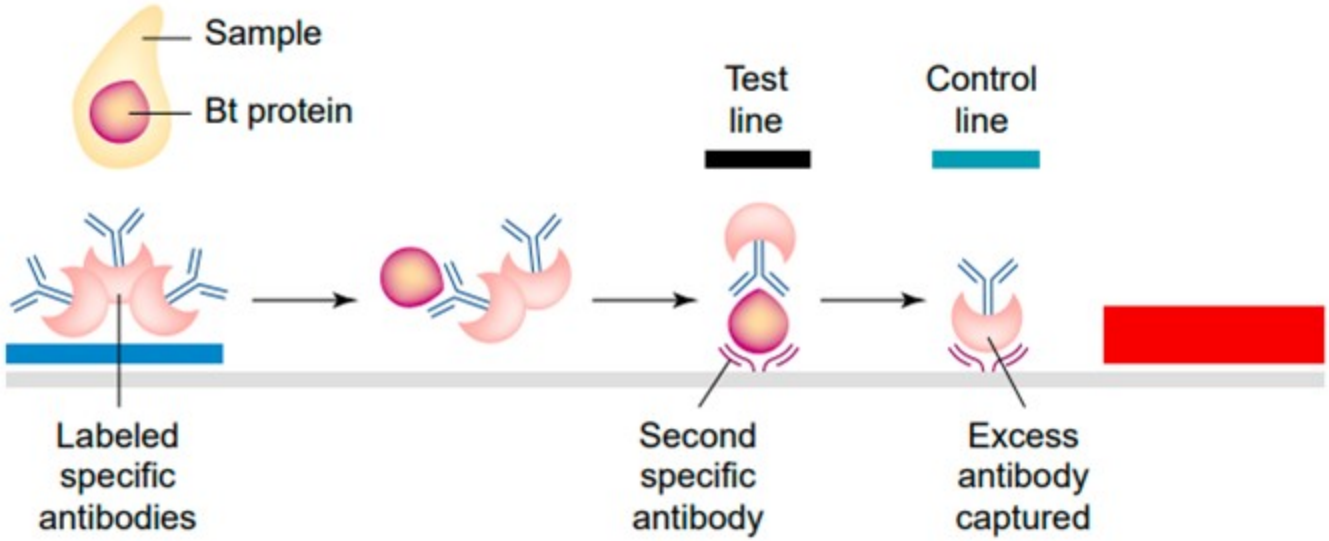
- Eg. Roundup Ready GM soy has been genetically engineered to resist the glyphosate herbicide Roundup by inserting a gene that codes for a glyphosate-tolerant version of a plant enzyme, CP4 epsps.

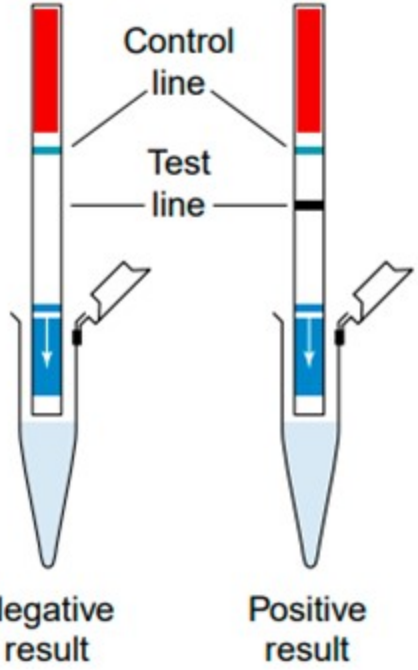
- Since antibody production is extremely complex and costly, detection using these methods typically relies on the availability of commercial antibodies.

- The total crude proteins are extracted from a sample by adding water or buffer followed by sample homogenization. Then the testing is either in the form of a lateral flow strip test, a micro-well format as an enzyme-linked immunosorbent analysis (ELISA) or a gel electrophoresis protein immunoblot (also known as western blot)

GMOs DETECTION

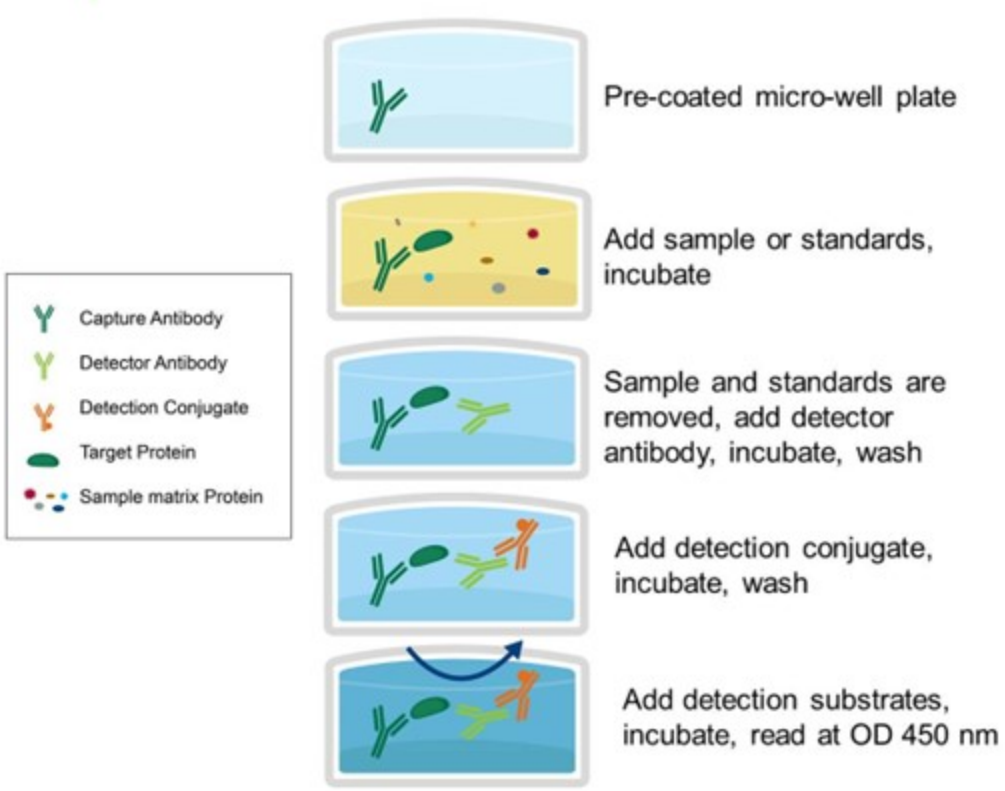
LATERAL FLOW STRIP TESTING

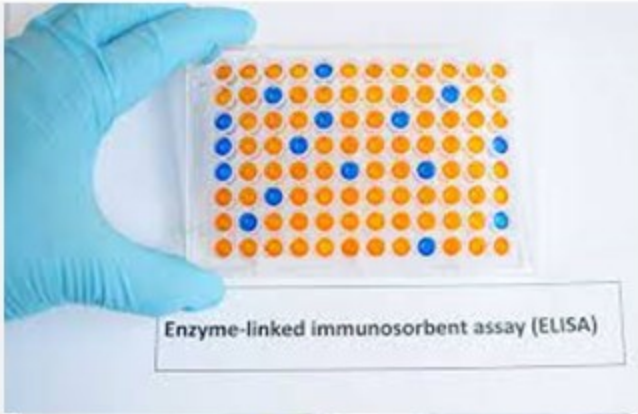




GMOs DETECTION

ELISA BASED APPROACH

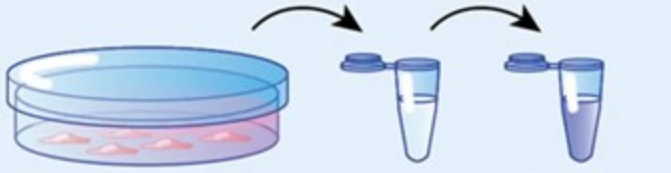




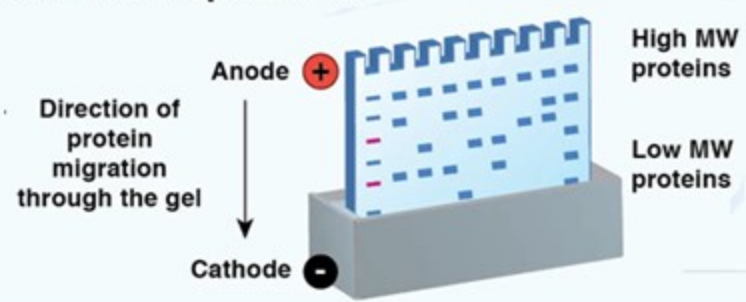
GMOs DETECTION

WESTERN BLOTTING

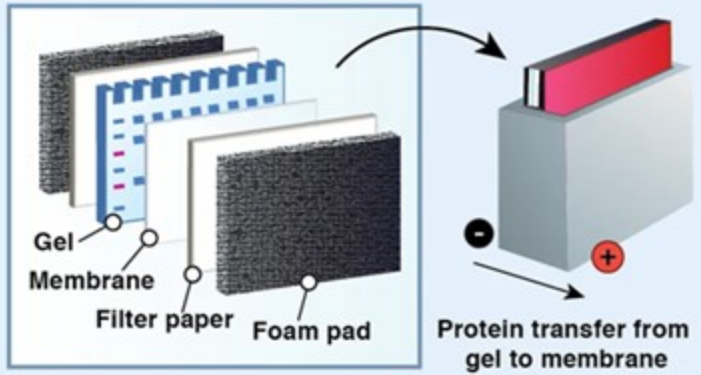
1 Sample preparation



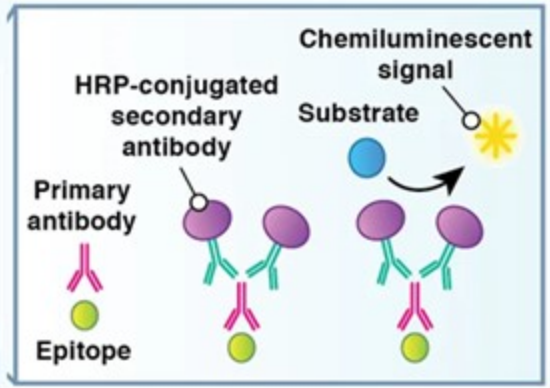
2 Gel electrophoresis



3 Membrane transfer

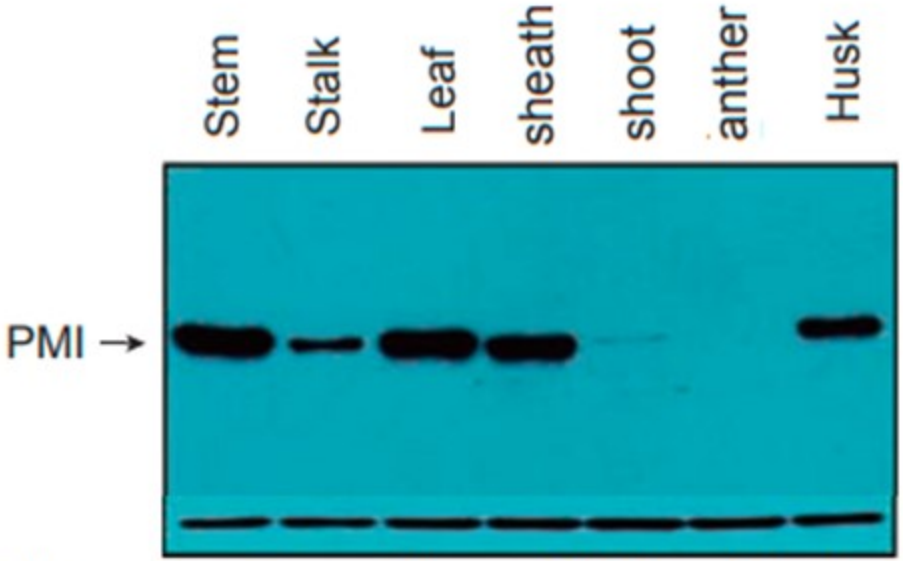


4 Immunodetection



GMOs DETECTION

WESTERN BLOTTING



The expression pattern of PMI protein in transgenic rice.

GMOs DETECTION

PROTEIN-BASED METHODS FOR LMO DETECTION

- Can not detect inserted genetic elements that do not produce a protein, such as regulatory sequences and new gene silencing technologies using double-stranded RNA (dsRNA)

- Rely on the specific recognition of an antigen in the transgenic protein by an antibody; therefore, any changes in the tertiary structure of the protein render the method ineffective. Such conformational changes are sometimes induced during sample processing, where the samples are subjected to heat and/or chemical treatment.

- The detection capability is also affected by the expression level of the transgenic protein, can vary between different parts (tissues) of the LMO or different stages of its life cycle and can be influenced by external factors such as climate and soil conditions.

GMOs DETECTION

DNA-BASED METHODS FOR LMO DETECTION

- Are based mainly on the polymerase chain reaction (PCR). PCR is a method that employs synthetic DNA oligonucleotides, so-called “primers”, to replicate or “amplify” targeted regions of an inserted DNA sequence in the LMO. The amplified product can then be detected to determine whether or not DNA originating from an LMO is present in a sample.

- Following the extraction of DNA from a sample, target sequences only found in the LMO are amplified using primers that have been designed to specifically bind the target sequence during the PCR reaction.

- PCR-based methods can be used on raw and processed products as long as DNA can be extracted from the sample.

GMOs DETECTION

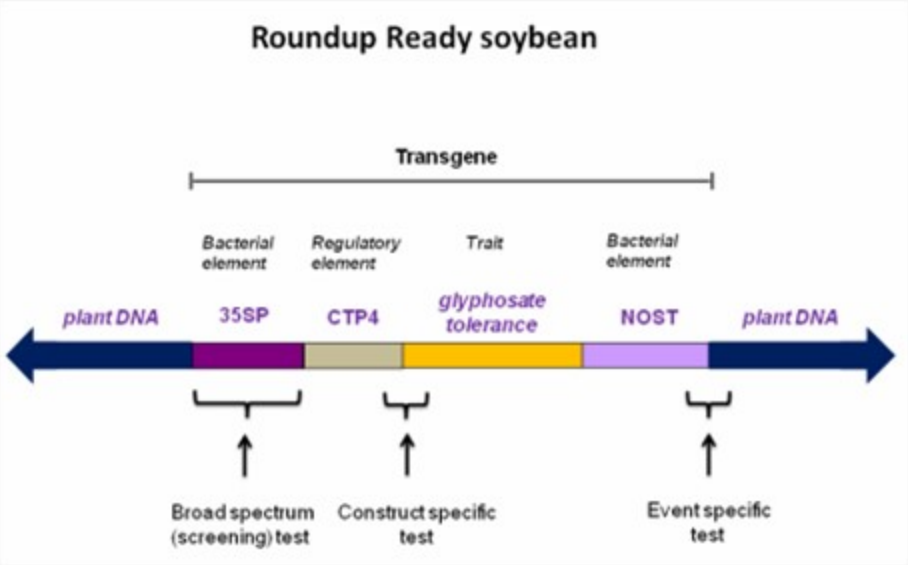
DNA-BASED METHODS FOR LMO DETECTION

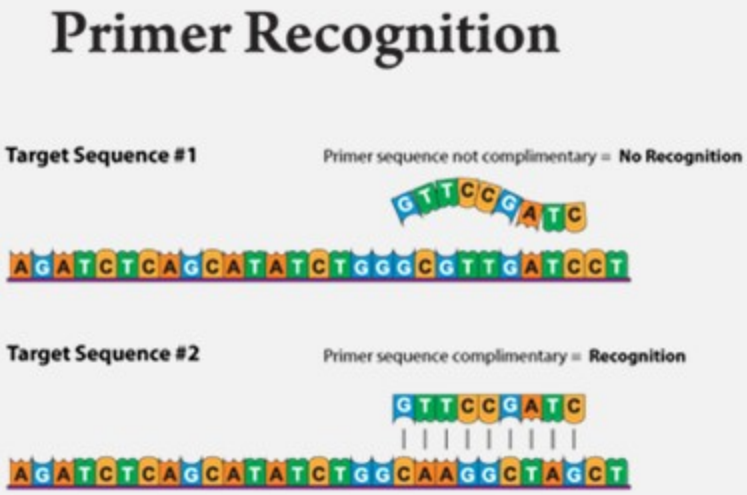
- Depending on the combination of primers, the PCR detection can be:

  - **GMO screening** is used to determine whether a sample contains GMOs by detecting regulatory elements (promoter and terminator sequences) commonly associated with GMOs. For example, the 35S promoter and NOS terminator are found in over 90 per cent of all commercial maize and soybean GMOs.
  - **Transgene-specific** identification identifies a specific gene, for example Cry1Ab, Cry9c (insect resistance) or EPSPS (herbicide tolerance).
  - **Construct-specific** methods target the region between two DNA elements found within a particular transgene construct, such as the promoter and gene.
  - **Event-specific** detection where the PCR target sequence is a junction between the host DNA and the inserted gene construct

GMOs DETECTION

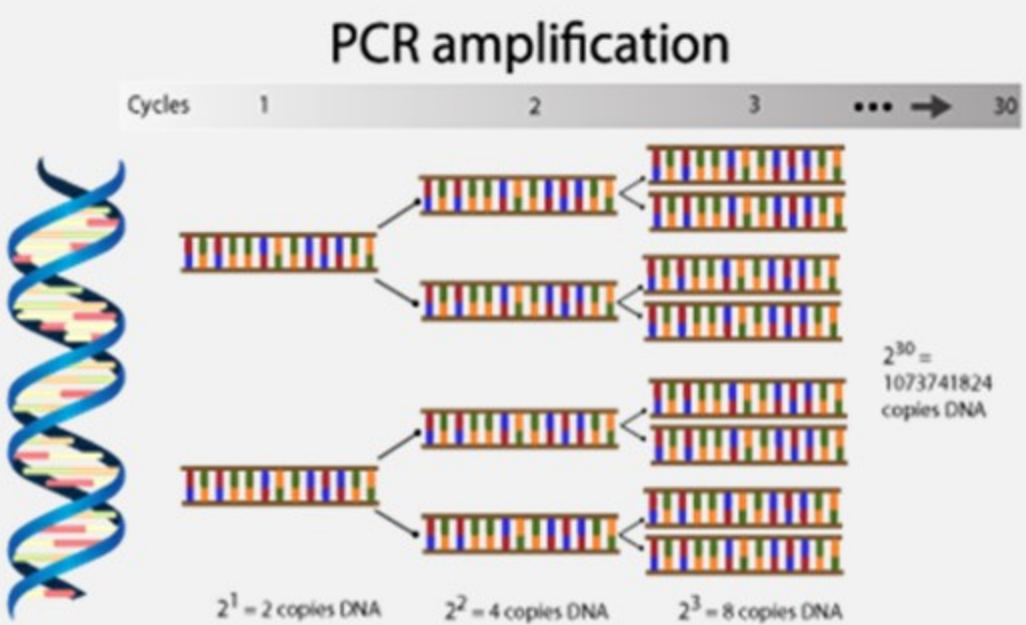
DNA-BASED METHODS FOR GMO DETECTION





GMOs DETECTION

DNA-BASED METHODS FOR GMO DETECTION



GMOs DETECTION

DNA-BASED METHODS FOR GMO DETECTION

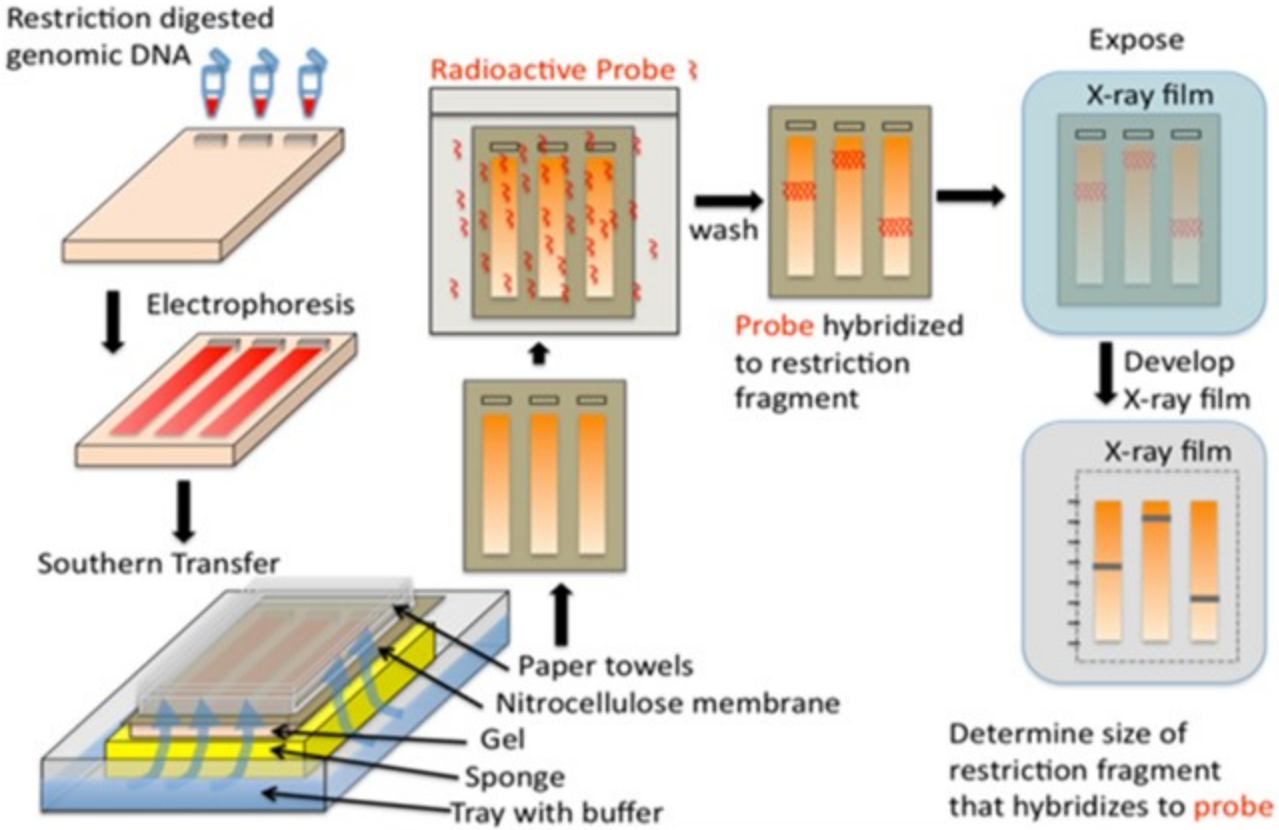
- Different methods can be used to confirm the PCR result including:

After PCR is completed

- Restriction enzyme cleavage of the PCR product, then visualization in the gel matrix using dyes such as ethidium bromide or SYBR Green that binds selectively to the dsDNA
- Southern blotting through hybridization with a DNA probe (specific for the target sequence),
- Direct sequencing of the PCR product
- Nested PCR through a two-step amplification approach that amplifies the target sequence followed by a second amplification of a smaller internal region of the product of the first amplification.

DNA-BASED METHODS FOR GMO DETECTION

SOUTHERN BLOTTING



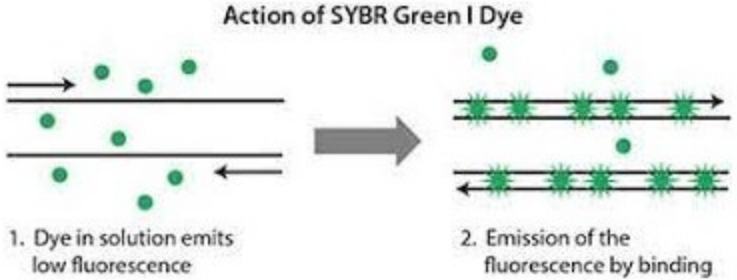
GMOs DETECTION

DNA-BASED METHODS FOR GMO DETECTION

- Different methods can be used to confirm the PCR result, including:

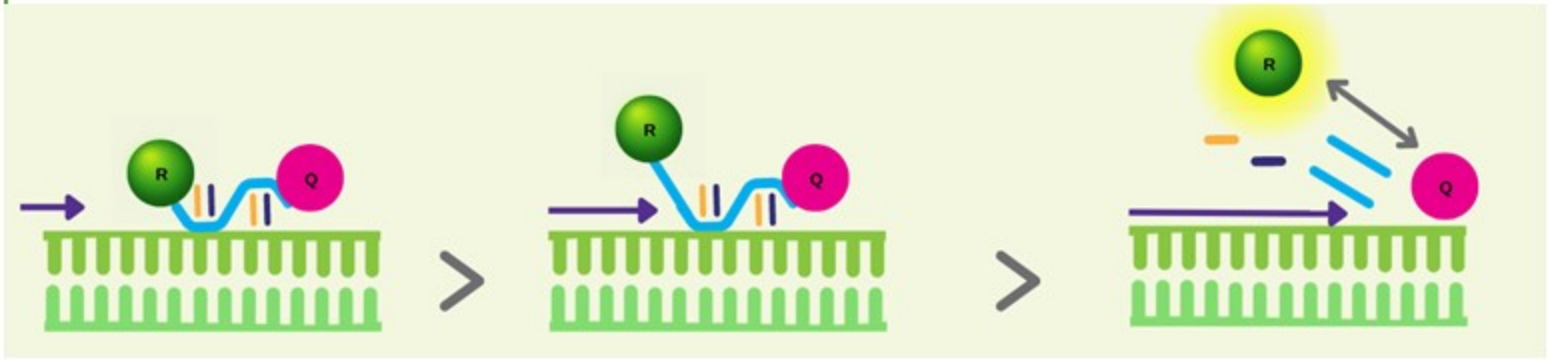
In real time using fluorescent dye or fluorescent probes:

- Double-stranded DNA-binding dye (SYBR Green I) is used as a non-specific detection system for all amplified DNA fragments, or
- Fluorescent probes that recognize an internal segment of the PCR target sequence (hybridization (FRET) probes or hydrolysis (Taqman) probes)



GMOs DETECTION

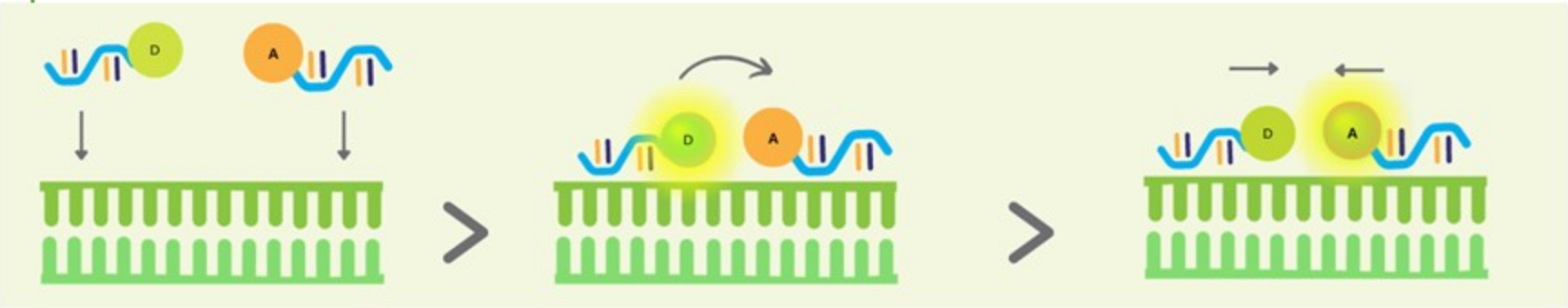
DNA-BASED METHODS FOR GMO DETECTION



- The hydrolysis probe binds to the complementary sequence. The polymerase (purple arrow) separates the fluorescent reporter from the probe, which also separates it from the quencher. Separation from the quencher leads to reporter fluorescence.

GMOs DETECTION

DNA-BASED METHODS FOR GMO DETECTION



- Dual hybridization probes require a donor and acceptor fluorophore. The two probes must come in close contact with each other. This proximity allows electronic transfer from the donor to the fluorophore, and then the acceptor fluoresces.

GMOs DETECTION

DNA-BASED METHODS FOR GMO DETECTION

- DNA has greater chemical stability than proteins, which allows it to withstand chemical and heat treatments.

- DNA is also present in all cells, and therefore, any part of the organism can be used for testing.

- PCR methods are more versatile than protein methods, and PCR can be easily used to screen a sample for the presence of several potential LMOs simultaneously.

- It is impossible to distinguish a sample containing material from different single transformation events from another sample containing one or more stacked LM events.

GMOs DETECTION

BRIEF OVERVIEW OF MOST COMMONLY USED METHODS

Parameter	Protein-based			DNA-based			
	Lateral flow strip	ELISA	Western blot	Southern blot	Qualitative PCR[d]	QC-PCR and limiting dilution	Real-time PCR
Ease of use	Simple	Moderate	Difficult	Difficult	Difficult	Difficult	Difficult
Needs special equipment	No	Yes	Yes	Yes	Yes	Yes	Yes
Sensitivity	High	High	High	Moderate	Very high	High	High
Duration[e]	10 min	30–90 min	2 d	6 h[f]	1.5 d	2 d	1 d
Cost/sample (US$)	2	5	150	150	250	350	450
Gives quantitative results	No	Yes[g]	No	No	No	Yes	Yes[h]
Suitable for field test	Yes	Yes[g]	No	No	No	No	No
Employed mainly in	Field testing	Test facility	Academic labs	Academic labs	Test facility	Test facility	Test facility

[a]Abbreviations: ELISA, enzyme-linked immunosorbant assay; GM, genetically modified; QC-PCR, quantitative-competitive PCR; rDNA, recombinant deoxyribonucleic acid. [b]Modified from [52]. [c]Near infra-red detects structural changes (not DNA or protein), is fast (<1 min) and inexpensive (~US$1); [d]Including nested PCR and GMO Chip; [e]Excluding time allotted for sample preparation; [f]When nonradioactive probes are used; otherwise 30 h with ^{32}P-labeled probes; [g]As in the antibody-coated tube format; [h]With high precision.

EXERCISES


SUBJECT
RESEARCH
METHOD
KNOWLEDGE
CASE STUDY
CONCLUSION
CRITERIA
EVIDENCE
DATA


Case Study
research
subject
case
studies
knowledge
cases
may
theoretical
single
study
local
thus
focus
whether
retrospective
Outlier
evidence
criteria
information
object
based
quantitative
in-depth
circumstances
researchers
multiple


case studies

SEARCHING FOR INFORMATION

CASE STUDY (CSFI16):

You are a phytosanitary officer in Kenya. You received documentation for a cottonseed shipment to be imported from the United States (USA) for food processing. Use the BCH to answer the following questions:

Q1. What GM cotton might be in your shipment?

Q2. Are all of them approved to be imported or domestically used in Kenya?

Q3. How will you proceed if the shipment is labeled as 'GMO-free?'

Q4. How will you proceed if the shipment is labeled as it might contain GMO?

SEARCHING FOR INFORMATION

CASE STUDY (CSFI18)

A company based in Malawi is willing to import corn for cultivation from Malawi trade partners in the SADC Region. Use the BCH to answer the following questions:

Q1. Which corn GM varieties can be in the shipments?

Q2. Are all of them approved in Malawi?

Q3. Are any of those varieties banned in any European country, and why?

Q4. If you are a phytosanitary officer in Malawi, how will you proceed if the corn shipment is labeled GMO-free?

Thank you !

For more information, please email

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