5-Day Theoretical and Practical Training Workshop on Laboratory Identification of Species, Screening of Living Modified Organisms and Detection of Plant Pathogens

Screening and identification of living modified organisms – theoretical training

Mojca Milavec

Minsk, 13.2.2024

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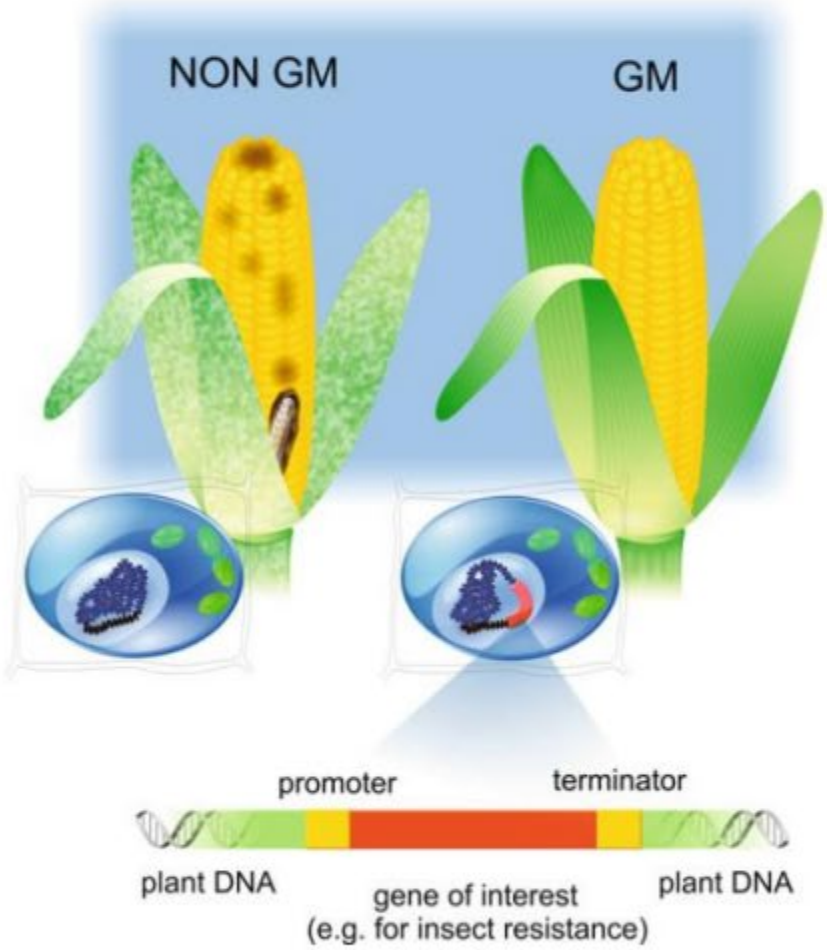
Outline

- General information
- Pre-analytical steps
- LMO screening
- LMO identification

General information



Living Modified Organisms (LMOs)/Genetically Modified Organisms (GMOs)

…by the Cartagena Protocol on Biosafety (article 3):

(g) "Living modified organism" means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology;

(h) "Living organism" means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids

(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.

… by the EU legal definition[1]:

"genetically modified organism (GMO)" means an organisms, with the exception of humans, in which the genetic material has been altered through the use of biotechnological methods, in a way that does not occur naturally by mating and/or natural recombination.

[1] Directive 2001/18/EC of the European Parliament and the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EC

Standards, guidelines, recommendations….

ISO 24276:2006 Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions

ISO 21571:2005 Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction

ISO 21569:2005 Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods

Series of ISO/TS 21569

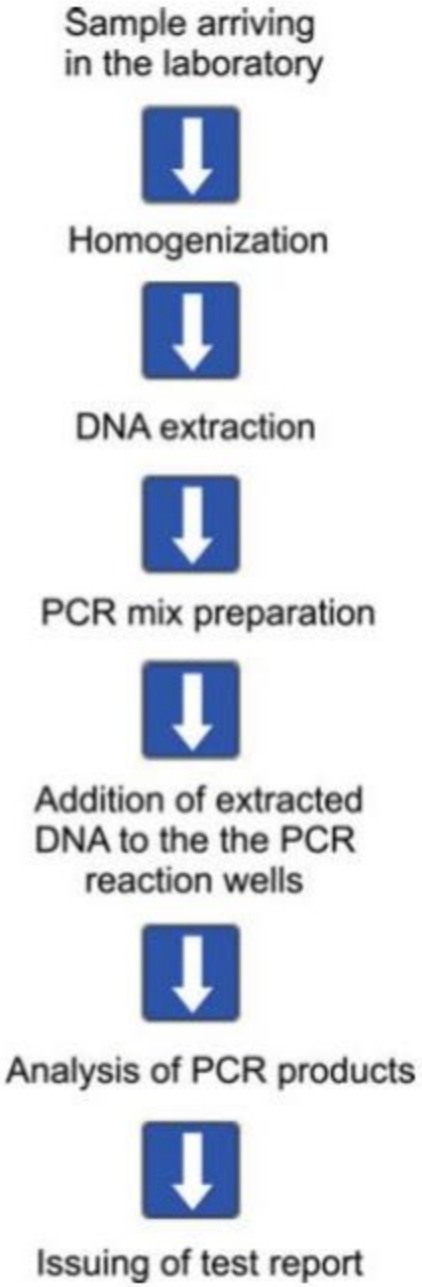
ISO 21570:2005 Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods

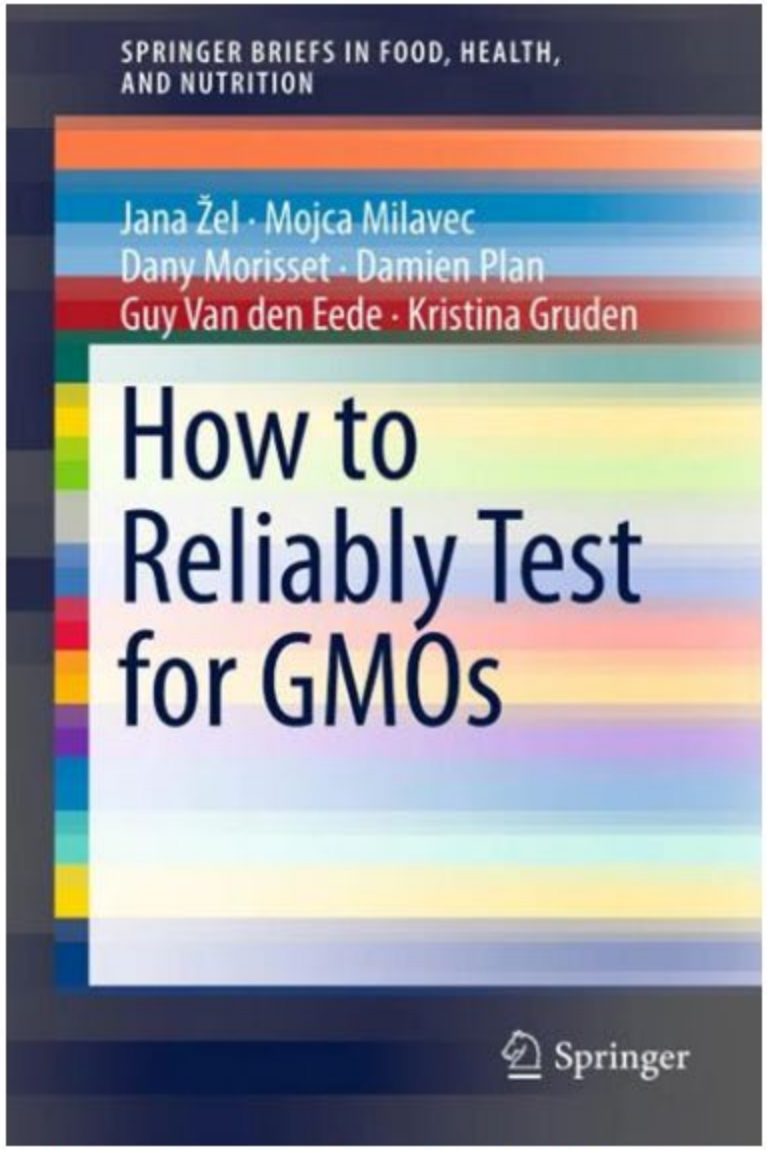
Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing (European Network of GMO Laboratories - ENGL)

Definition of minimum performance requirements for analytical methods of GMO testing. Part 2. (ENGL)

Nucleic acid!

Fig. 3 Scheme showing unidirectional route of sample in GMO testing from the first contact with the customer, through analyses in the laboratory, and issue of the final test report to the customer. Wherever possible, separate rooms (or chambers) should be assured for performing each stage of the procedure





https://link.springer.com/book/10.1007/978-1-4614-1390-5

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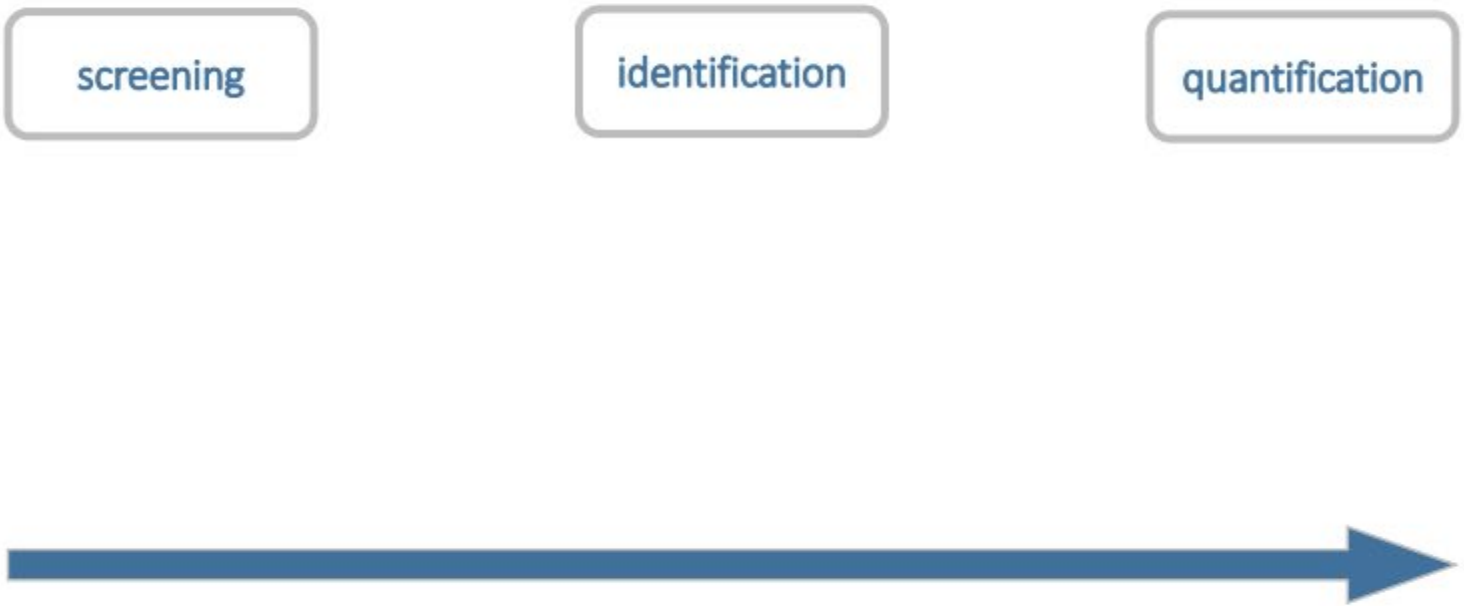
Genetically Modified Organisms/Living Modified Organisms

Screening and identification are possible, if the inserted sequence is known.

Three partite PCR-based testing scheme



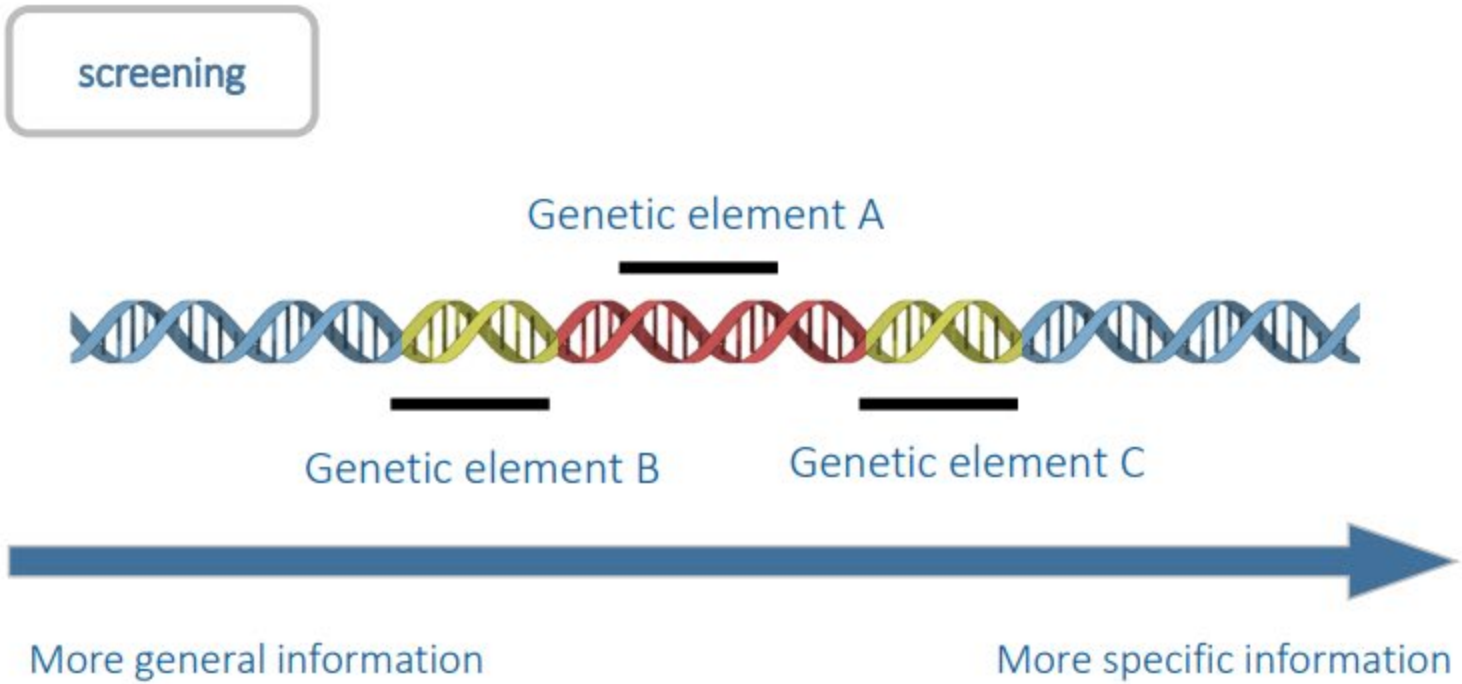
Three partite PCR-based testing scheme

screening

More general information

More specific information

Three partite PCR-based testing scheme



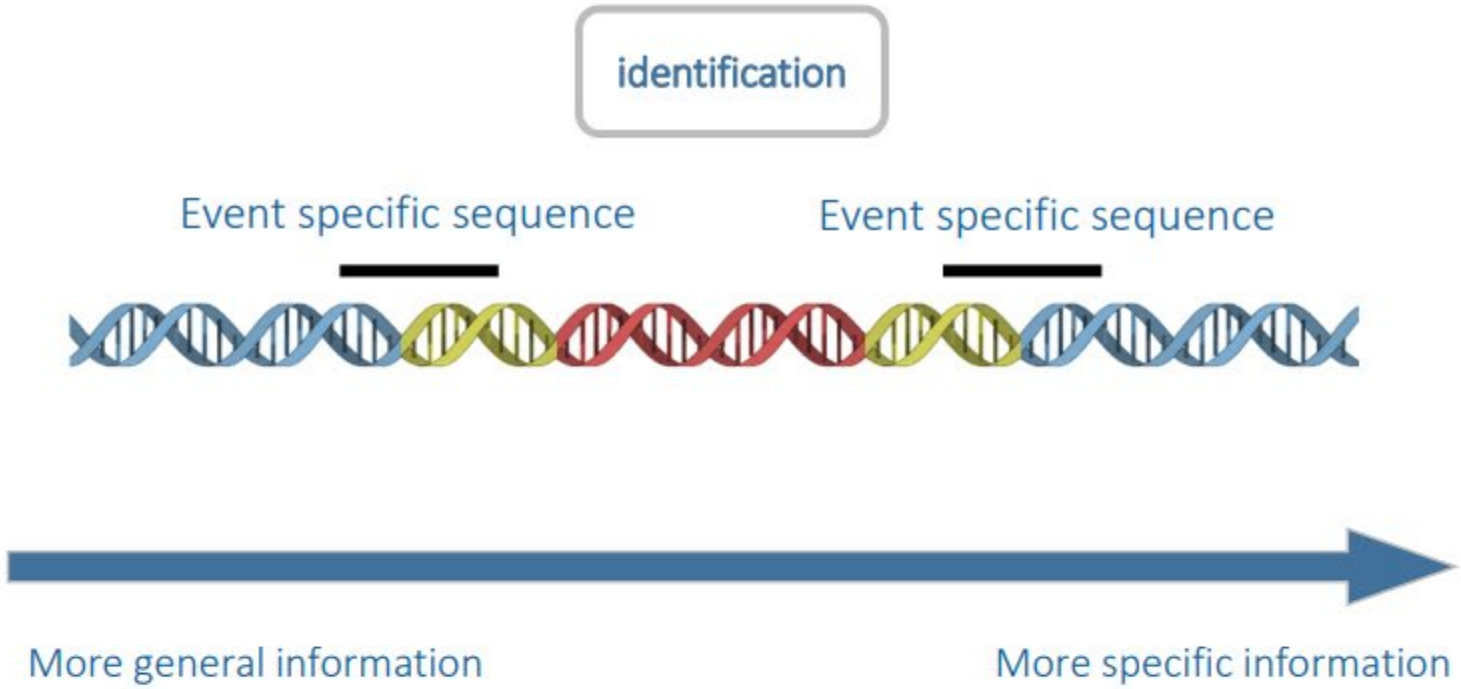
Three partite PCR-based testing scheme

identification

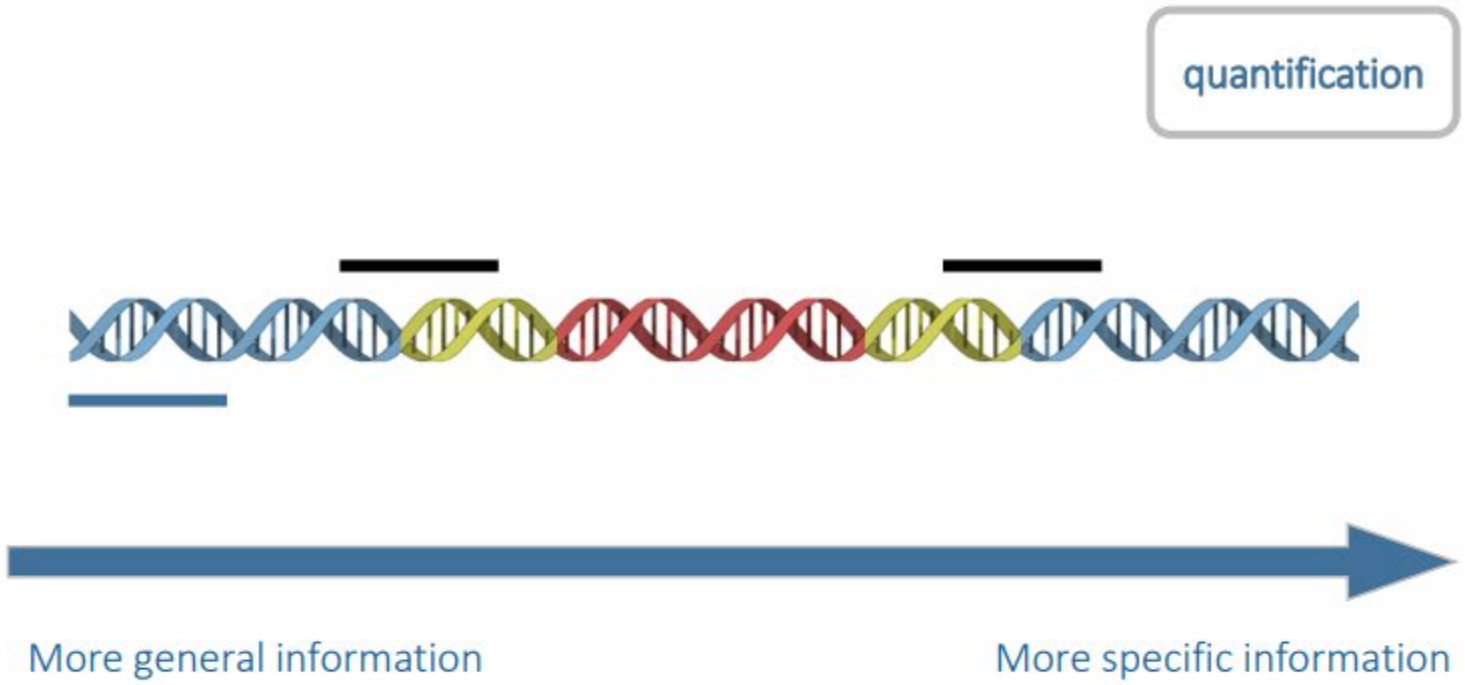
More general information

More specific information

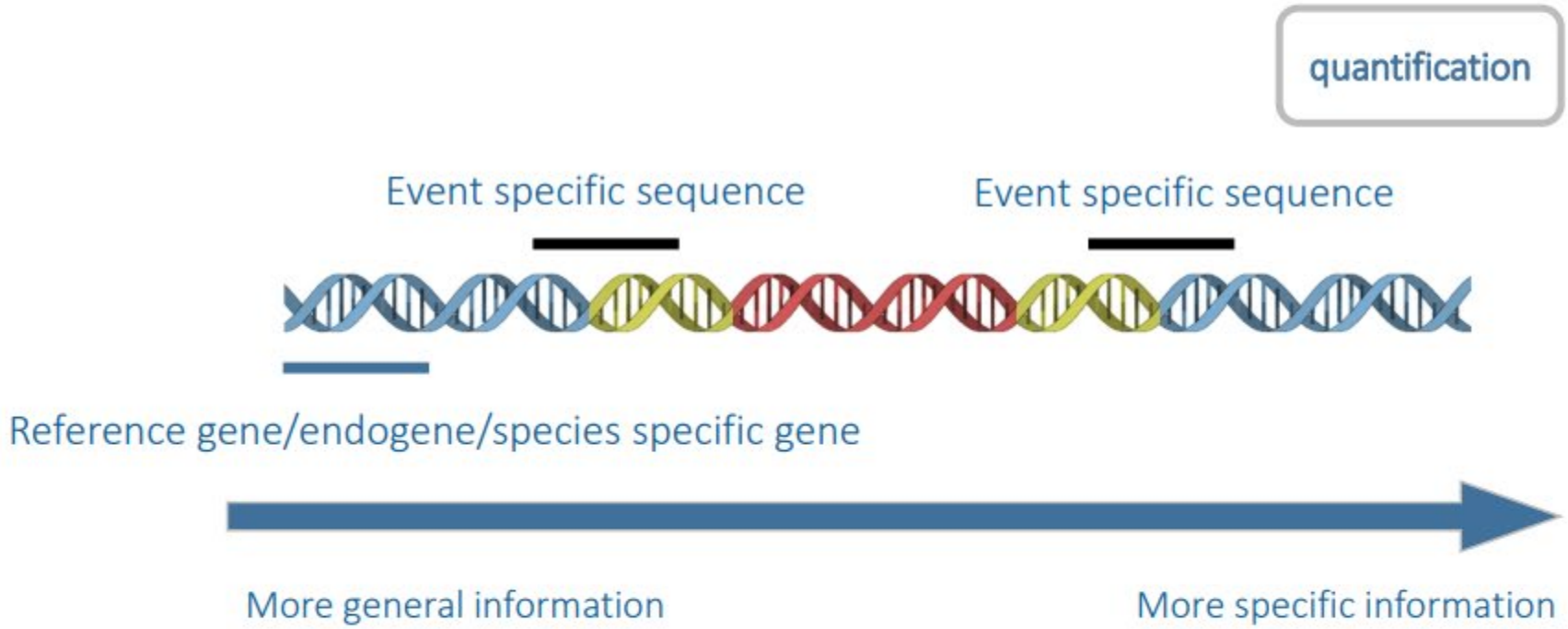
Three partite PCR-based testing scheme



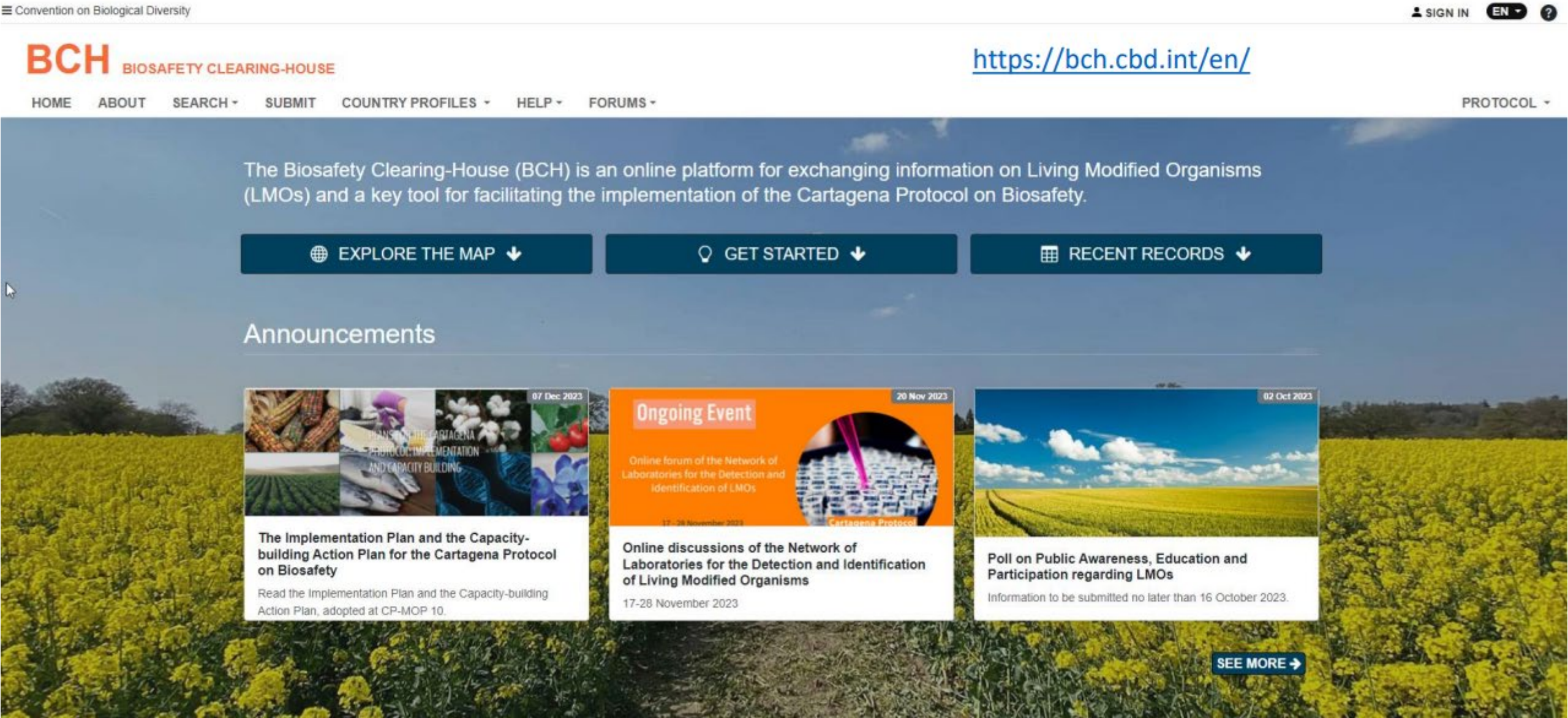
Three partite PCR-based testing scheme



Three partite PCR-based testing scheme



Sources of information on LMOs



Living Modified Organism (LMO) Registry

Registries

LMO Registry

Organism Registry

Genetic Element Registry

The LMO Registry provides summary information on all living modified organisms registered in the BCH, including transformation events, genetic modifications and the unique identification code (if available) for each record. Links to all decisions and risk assessment reports that refer to these organisms are accessible through the records in the registry.

Click here to perform an in-depth search of all LMO records available in this Registry.

Total records: 960

Export

Record ID	Unique identification	Identity & transformation event	Organism	Description
BCH-LMO-SCBD-114444-1	AAT-7Ø9AA-4	**Pod Borer-resistant cowpea** AAT709A	Vigna unguiculata Cowpea, Black eyed pea	Resistance to diseases and pests - Insects - Lepidoptera (butterflies and moths), Resistance to antibiotics - Kanamycin
BCH-LMO-SCBD-14752-6	ACS-BNØ11-5	**Navigator™ canola** Oxy-235	Brassica napus Turnip, Rapeseed, Canola Plant, Oilseed Rape, Rape, BRANA	Resistance to herbicides - Bromoxynil
BCH-LMO-SCBD-15101-6	ACS-BNØ1Ø-4	**Falcon™ rapeseed** GS40/90pHoe6/Ac	Brassica napus Turnip, Rapeseed, Canola Plant, Oilseed Rape, Rape, BRANA	Resistance to herbicides - Glufosinate
BCH-LMO-SCBD-14753-6	ACS-BNØØ1-4	**InVigor™ canola** RF1 (B93-101)	Brassica napus Turnip, Rapeseed, Canola Plant, Oilseed Rape, Rape, BRANA	Resistance to herbicides - Glufosinate, Resistance to antibiotics - Kanamycin, Changes in physiology and/or production - Fertility restoration
BCH-LMO-SCBD-14754-5	ACS-BNØØ2-5	**InVigor™ canola** RF2 (B94-2)	Brassica napus Turnip, Rapeseed, Canola Plant, Oilseed Rape, Rape, BRANA	Resistance to herbicides - Glufosinate, Resistance to antibiotics - Kanamycin, Changes in physiology and/or production - Fertility restoration
BCH-LMO-SCBD-14755-9	ACS-BNØØ3-6	**InVigor™ canola** RF3	Brassica napus Turnip, Rapeseed, Canola Plant, Oilseed Rape, Rape, BRANA	Resistance to herbicides - Glufosinate, Changes in physiology and/or production - Fertility restoration

LIVING MODIFIED ORGANISM (LMO) | BCH-LMO-SCBD-14768-7 | PDF | Print | Share | Compare

Decisions on the LMO | Risk Assessments

PUBLISHED: 05 JUN 2006 LAST UPDATED: 15 JAN 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.

Name

CDC Triffid flax modified for herbicide resistance EN

Transformation event

FP967

Does this LMO have a unique identifier?

Yes

Unique identifier

CDC-FLØØ1-2

Developer(s)

- ORGANIZATION: UNIVERSITY OF SASKATCHEWAN | BCH-CON-SCBD-4941-2

ORGANIZATION:

University of Saskatchewan
Website: http://www.usask.ca/

Description

Linseed tolerant to the herbicide sufonylurea through insertion of the acetolactate synthase (als) gene. Neomycin phosphotransferase II (neo) confers resistance to the antibiotic kanamycin and the nos gene codes for nopaline synthase; these were used as selectable markers. EN

Recipient Organism or Parental Organisms

The term "Recipient organism" refers to an organism (either already modified or non-modified) that was subjected to genetic modification, whereas "Parental organisms" refers to those that were involved in cross breeding or cell fusion.

BCH-ORGA-SCBD-12087-4 | Organism | Linum usitatissimum (Flax, Flax, Linseed, LINUS)

Crops

Characteristics of the modification process

Vector

pGH6 derived from pGV3850 | EN

Techniques used for the modification

Agrobacterium-mediated DNA transfer

Genetic elements construct

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-15001-5 | Neomycin Phosphotransferase II | Escherichia coli (ECOLX)

Protein coding sequence | Resistance to antibiotics (Kanamycin)

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BCH-GENE-SCBD-15171-5 | Nopaline Synthase Gene | Agrobacterium tumefaciens (Agrobacterium)

Protein coding sequence | Selectable marker genes and reporter genes

BCH-GENE-SCBD-103932-4 | Acetohydroxy acid synthase gene promoter | Arabidopsis thaliana (Thale cress, Mouse-ear cress, Arabidopsis, ARATH)

Promoter

BCH-GENE-SCBD-103933-4 | Acetohydroxy acid synthase gene terminator | Arabidopsis thaliana (Thale cress, Mouse-ear cress, Arabidopsis, ARATH)

Terminator

BCH-GENE-SCBD-100270-6 | Nopaline Synthase Gene Promoter | Agrobacterium tumefaciens (Agrobacterium)

Promoter

BCH-GENE-SCBD-100269-8 | Nopaline Synthase Gene Terminator | Agrobacterium tumefaciens (Agrobacterium)

Terminator

BCH-GENE-SCBD-48073-8 | Acetohydroxy acid synthase gene | Arabidopsis thaliana (Thale cress, Mouse-ear cress, Arabidopsis, ARATH)

Protein coding sequence | Resistance to herbicides (Imidazolinone, Sulfonylurea)

LMO characteristics

Modified traits

Resistance to herbicides
- Sulfonylurea

Resistance to antibiotics
- Kanamycin

Common use(s) of the LMO

Food
Feed

Detection method(s)

External link(s)

European Union Reference Laboratory - Detection of flax CDC Triffid (FP967) *[English]*

CDC-FLØØ1-2 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) *(JRC) [English]*

[No Title]

Additional Information

Additional Information

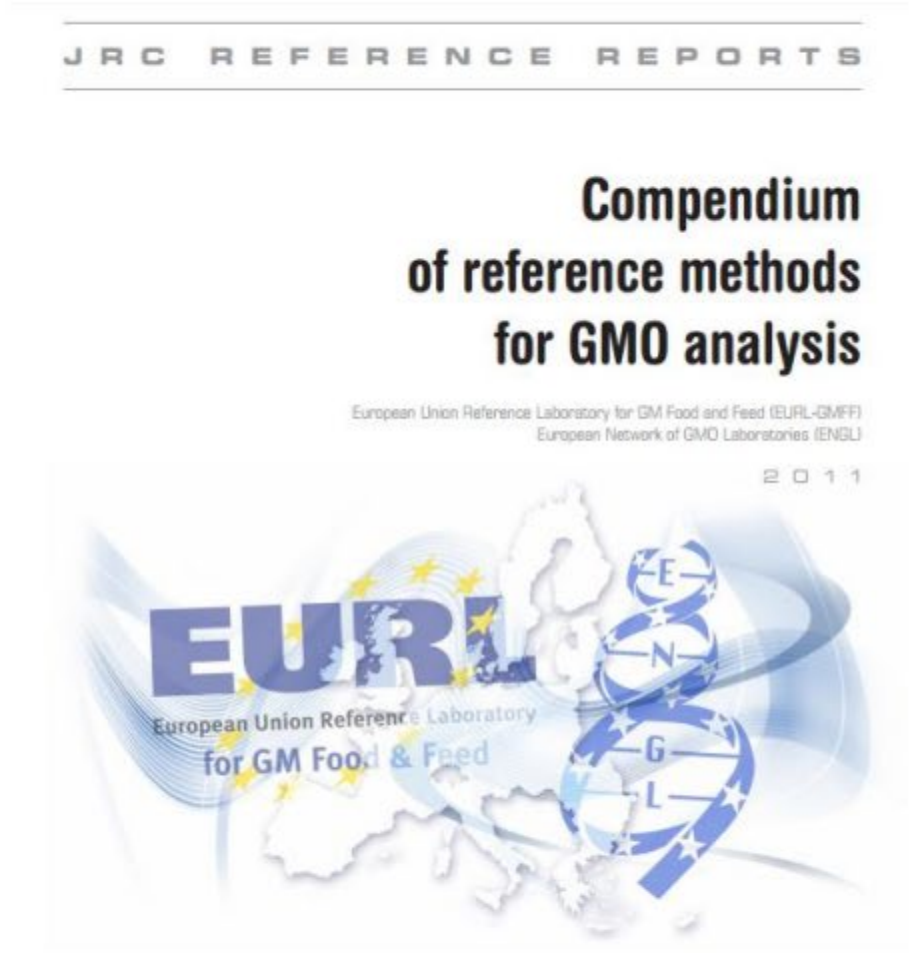
Sulfonylurea herbicides, such as triasulfuron and metsulfuron-methyl, target and bind to the enzyme acetolactate synthase (ALS) thereby inhibiting the biosynthesis of the branched chain amino acids valine, leucine and isoleucine and resulting in the accumulation of toxic levels of alpha-ketoglutarate. In addition to its native ALS gene, CDC Triffid contains an als gene from a chlorsulfuron tolerant line of Arabidopsis thaliana. This variant als gene differs from the wild type A. thaliana gene by one nucleotide and the resulting ALS enzyme differs by one amino acid from the wild type ALS enzyme. The inserted als gene is linked to its native promoter and terminator. Enzyme extracts from CDC Triffid exhibited a slightly higher ALS activity compared to its non-modified counterpart cv. Norlin. Whereas the statistical significance of this higher activity could not be verified, it may be expected due to the presence of at least two additional copies of the als gene in CDC Triffid.	EN

Other relevant website addresses and/or attached documents

Biotechnology Consultation Note to the File BNF No. 000050 - FDA *[English]*

Sources of information on methods for detection of LMOs

- Compendium of reference methods for GMO analysis
- EURL-GMFF web page
- Scientific literature
- Commercial providers



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Compendium of reference methods for GMO analysis

(https://publications.jrc.ec.europa.eu/repository/bitstream/JRC64876/gmo-jrc_reference%20report_2011_publ.pdf)

Chapter 1: Quantitative GMO detection PCR methods

Chapter 2: Qualitative GMO detection PCR methods

EURL-GMFF web page (https://gmo-crl.jrc.ec.europa.eu/gmomethods/)

European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF)

Home | What we do | Tools | Our publications | ENGL | National Reference Laboratories | Useful links | Contacts

Home > GMOMETHODS

GMOMETHODS

Last update: 22/11/2023

GMOMETHODS provides information on EU reference methods for GMO Analysis.

The tool assists control laboratories in selecting the appropriate methods, supplies core data on the experimental protocol and information on methods performance, ring-trial design, plasmid standards, reference materials and links to published articles or validation reports.

The assays are DNA-based detection methods that have been validated according to the principles and requirements of international standards and can assure therefore consistent and reproducible results in the analysis. Data is retrieved from peer-reviewed journals and final reports of collaborative studies. Few assays have been verified by the EURL GMFF for EU legal purposes.

Perform your search by keyword, select a GMO unique identifier or click a link in the section below.

keyword Search or by GMO unique identifier:

Quantitative methods

- GMO specific
  - Event specific

Qualitative methods

- GMO specific
  - Event specific

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Perform your search by keyword, select a GMO unique identifier or click a link in the section below.

epsps Search or by GMO unique identifier:

Records 1-9 of 9 1

Results for query [epsps]

Nr	Relevance	ID	Title
1		QL-ELE-00-029	Qualitative LAMP method for detection of CP4 epsps gene (Li et al., 2018).
2		QL-CON-00-008	Qualitative PCR method for detection of the junction between the chloroplast transit peptide 2 and the CP4 epsps gene (Grohmann et al., 2009).
3		QT-CON-00-001	Quantitative PCR method for detection of the junction between the chloroplast transit peptide and the CP4 epsps gene.
4		QT-CON-00-002	Quantitative PCR method for detection of the junction between the CTP sequence and the CP4 epsps gene (Hird et al., 2003).
5		QL-ELE-00-019	Qualitative PCR method for detection of CP4 epsps gene (Barbau-Piednoir et al., 2014).
6		QT-CON-00-008	Quantitative PCR method for detection of the junction between an optimized transit peptide sequence and the point mutated epsps gene from maize.
7		QT-CON-00-003	Quantitative PCR method for detection of the junction between the CaMV35S promoter and the CTP sequence (ISO/FDIS 21570:2005).
8		QL-CON-00-006	Qualitative PCR method for detection of the junction between the CaMV35S promoter and the chloroplast transit peptide sequence (EU-Project SMT4-CT96-2072:1998).
9		QL-CON-00-001	Qualitative PCR method for detection of the junction between the CaMV35S promoter and the chloroplast transit peptide sequence (ISO/FDIS 21569:2005).

GMOMETHODS

Last update: 22/11/2023

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The tool assists control laboratories in selecting the appropriate methods, supplies core data on the experimental protocol and information on methods performance, ring-trial design, plasmid standards, reference materials and links to published articles or validation reports.

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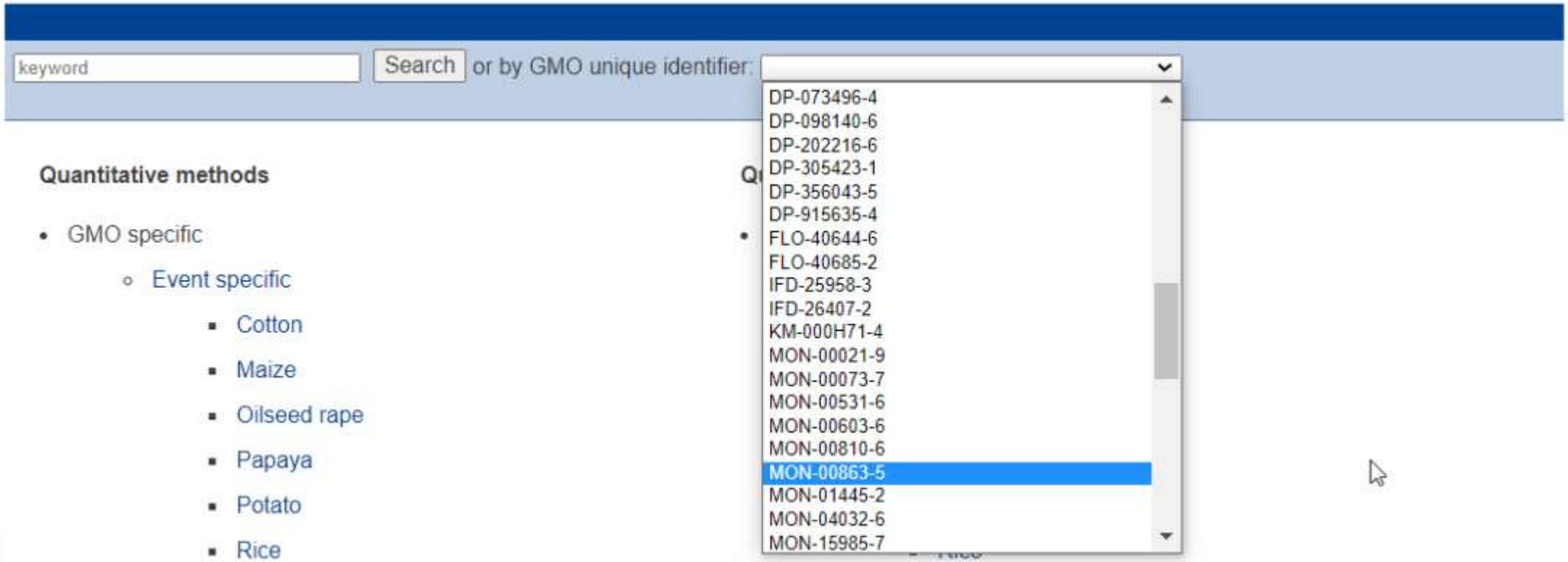
Perform your search by keyword, select a GMO unique identifier or click a link in the section below.



Quantitative methods

- GMO specific
  - Event specific
    - Cotton
    - Maize
    - Oilseed rape
    - Papaya
    - Potato
    - Rice

Perform your search by keyword, select a GMO unique identifier or click a link in the section below.

ac:MON-00863-5 Search or by GMO unique identifier:

View Entry Download View in GMO matrix View in GMO amplicons

Entry information

Entry name	**QT-EVE-ZM-009**; SV 0; linear; genomic DNA; STS; SYN; 84 BP.
Primary accession	**MON-00863-5**

Description

Description	Quantitative PCR method for detection of maize event MON863 (Mazzara et al., 2005).
Keywords	event_specific
From	Zea mays (maize) - event MON863 (MON-00863-5)

References

1 Mazzara M., Foti N., Price S., Paoletti C., Van Den Eede G.;**"Event-Specific Method for the Quantitation of Maize Line MON 863 Using Real-Time PCR - Validation Report and Protocol";** Online Publication (2005).

BSHOP	LBNA21830
Reference Position	1-84

2 **"PCR reactions set up and amplification conditions";** Online Publication (2010).

PCR	QT-EVE-ZM-009.pdf
Reference Position	1-84

Cross-references

GMOMETHODS	QT-TAX-ZM-011;

Features

Key	From	To	Length	Qualifier	Value
sts	1	84	84	standard_name	PCR 84 bp amplicon
				note	event-specific RT-PCR
				target	5' integration border region (IBR) between the insert of maize event MON 863 and the maize host genome
primer_bind	1	23	23	standard_name	Primer forward: MON863 primer F
				note	TGTTACGGCCTAAATGCTGAACT
				target	5'-host genome

Sequence information

Length: **84** BP, A count: **15**, C count: **19**, G count: **16**, T count: **23**, Other count: **11**

tgttacggcc taaatgctga actnntgacc ctacttgttc ggatgggtgt tcannnnnnn
nngtaccaag ctttccgatc ctac

https://food.r-biopharm.com/analytes/genetically-modified-organisms/

https://www.invitek.com/en/gmo-detection

https://www.techno-path.com/product/gmo-detection-kits/

+ foodproof®GMO Screening Kit, 2 Target

– foodproof® GMO Screening Kit, 4 Target

The foodproof GMO Screening Kit detects and differentiates the 35S promoter (cauliflower mosaic virus), the NOS terminator (Agrobacterium tumefaciens), the bar gene (Streptomyces hygroscopicus) and the FMV promoter (figwort mosaic virus). This multiplex real-time PCR kit can be used to detect genetically modified plants in food and animal feed. Additionally as a control, the kit allows the detection of plant DNA in the sample.]

Product Sheet

+ foodproof® GMO Screening 1 LyoKit, 3 Targets

+ foodproof® GMO Screening 2 LyoKit, 5 Targets

+ foodproof® Plant Taxon Screening LyoKit

Pre-analytical steps

Pre-analytical steps - Sampling

A **representative sample** must be obtained from the material to be examined (e.g. container of maize kernels should be examined, while typical laboratory sample is 10 000 kernels).

If possible, taking samples for the subsequent official analysis should be carried out by so-called official sampler or inspection agency.

There are different sampling guidelines, depending on the goods to be sampled.

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International Rules for Seed Testing

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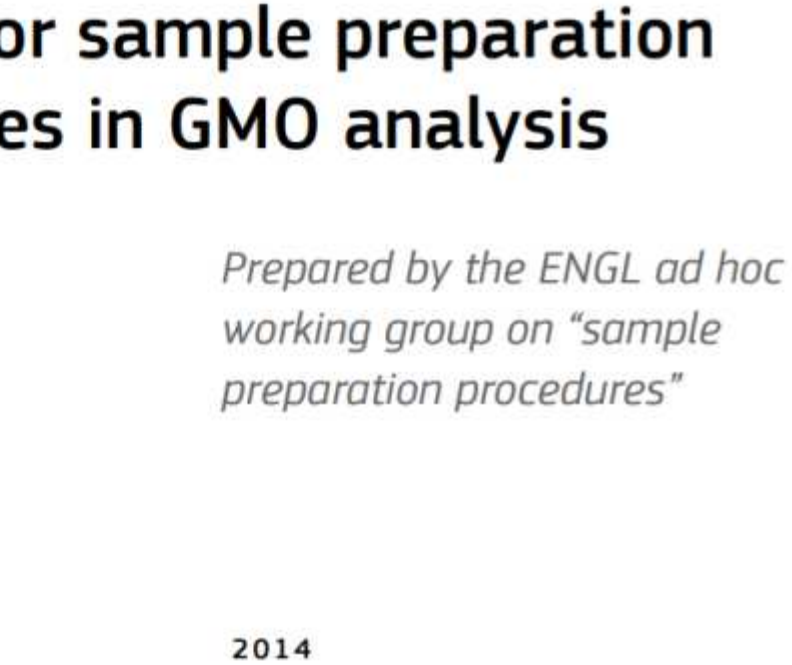
Pre-analytical steps – Subsampling of the laboratory sample

In general the whole laboratory sample is homogenised to obtain a test sample for the analysis. Sometimes mass reduction (sub-sampling) has to be done. The procedure followed has to be documented.

These guidelines are based on that of an existing standard (ISO 6498:2012, Animal feeding stuffs Guidelines for sample preparation) and adapted for GMO detection and different matrices (food, feed and seeds).

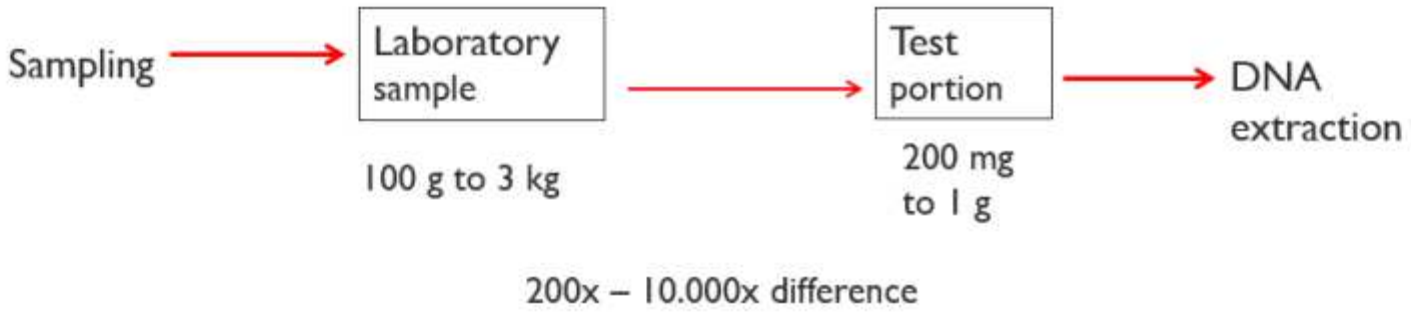


Pre-analytical steps – Particle size reduction and homogenisation

Sample preparation procedure is necessary:

- to achieve greater effectiveness of DNA extraction;

- to ensure homogeneity and the equal representation of GMOs in the sample.



It is necessary to avoid cross-contamination. Particle size reduction step is the step with the highest risk.

After grinding and/or homogenisation of the sample at least two test portions (at least 200 mg each, see CEN/ISO 21571:2005) is gained by dividing the analytical sample in a representative way.

Pre-analytical steps - extraction

Several methods are available for DNA extraction that are suitable for different matrices. A procedure found to be suitable for DNA extraction of one kind of matrix may not be suitable for a different kind of matrix.

Each sample is extracted in at least two parallels.

Regardless the method used, quality controls are always included in extraction procedure. For each extraction series it is recommended to perform:

- environment control: possible environment contamination is checked. A tube with a volume of water equal to the elution volume of samples is opened during DNA extraction procedure;
- extraction blank control: negative control of extraction. All steps of DNA extraction are the same as for samples only that water is used instead of a sample.

Pre-analytical steps - extraction

Fos some samples (e.g. cotton) additional DNA purification is needed for removal of components, that could influence (inhibit) PCR reaction, thus causing false negative result.

Extracted DNA should be of appropriate quantity and quality!

Quality and quantity can be assessed using various methods such as absorbance (optical density), agarose gel electrophoresis or the use of fluorescent DNA binding dyes. Methods have different requirements in terms of equipment needed, ease of use and calculations to be considered. Results between methods are not comparable.

Quality and quantity can also be assessed by analysing species specific gene using qPCR.

Screening for LMOs

Broad screening for elements present in many LMOs including from different species.

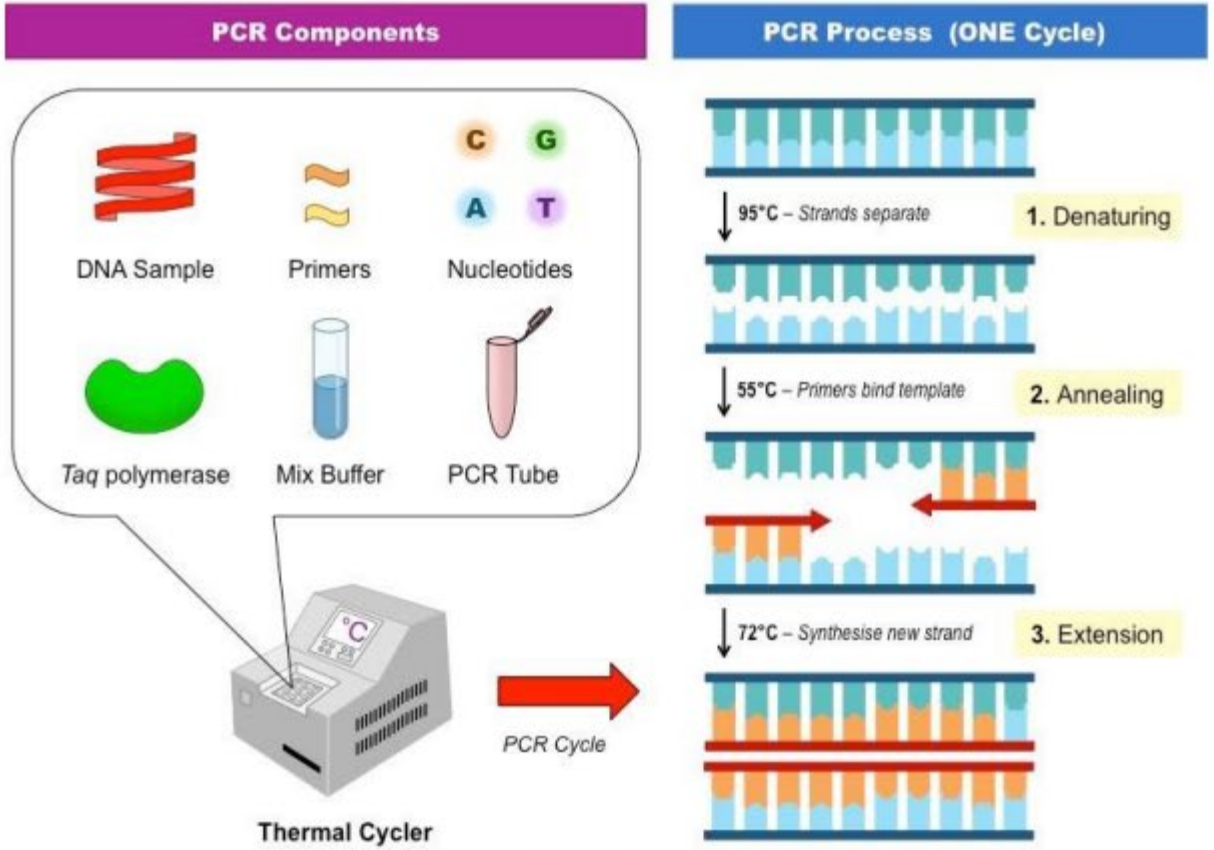
screening

More general information

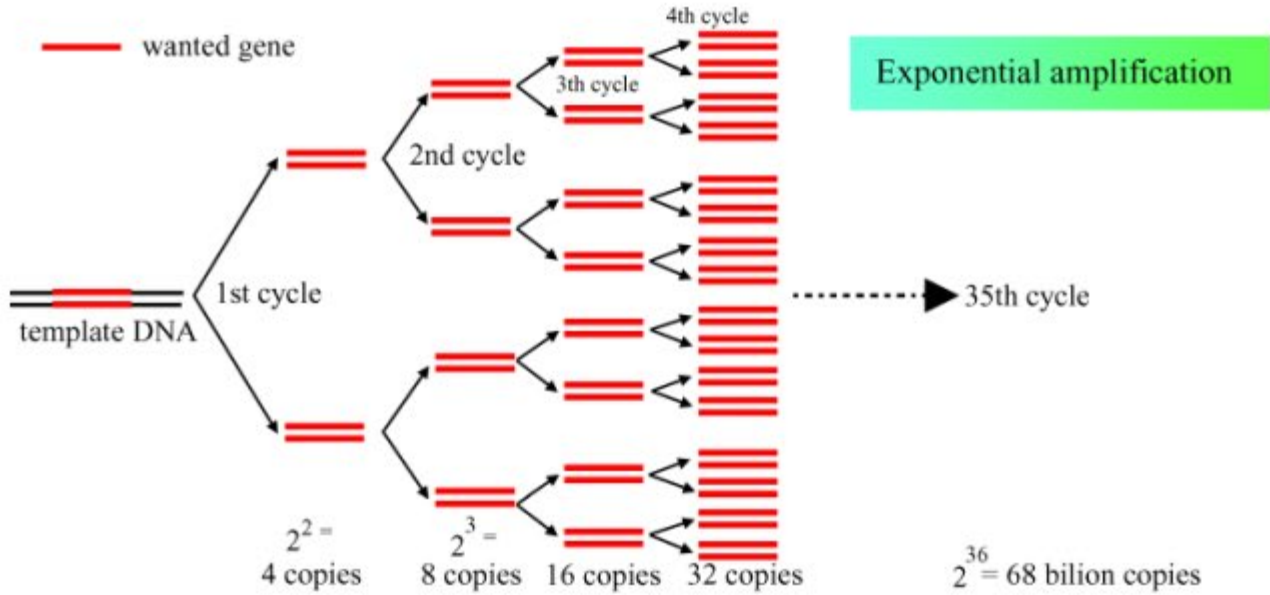
More specific information

Polymerase Chain Reaction (PCR)

Enables detection of specific nucleic acid sequences (targets).

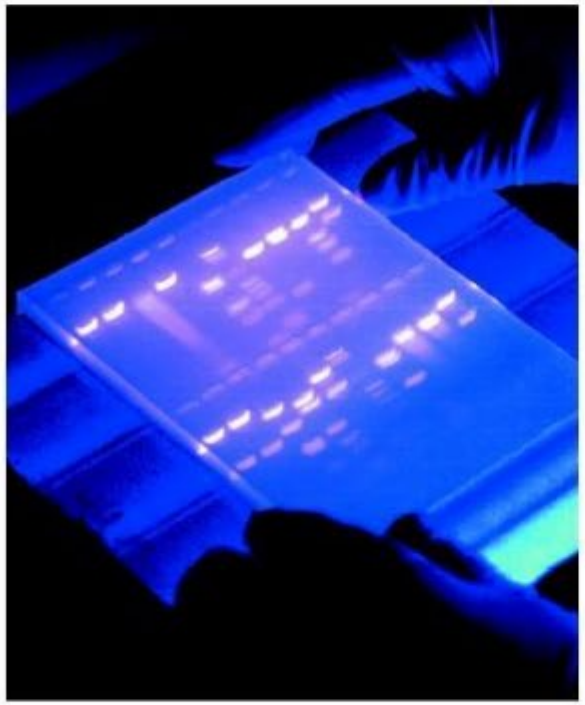


http://ib.bioninja.com.au/standard-level/topic-3-genetics/35-genetic-modification-and/pcr.html



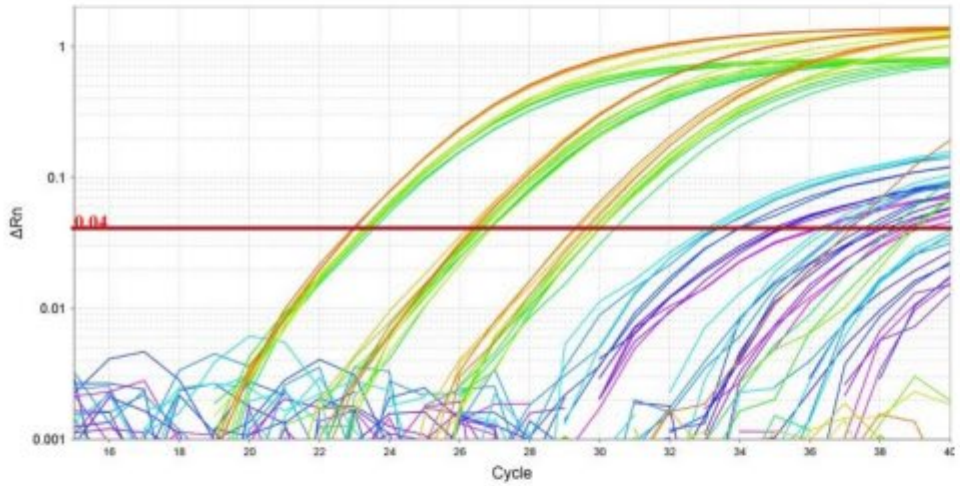
Evolution of PCR

1983
PCR



- endpoint results
- **qualitative** (target present or not present)

1996
Real-time PCR (qPCR)



- results in real time (although point results - Cq values - are mainly used)
- **relative quantification** (e.g. compared to calibrant, compared to reference sample)

Quality control

For PCR (targeted element):

- Positive control (DNA extracted from CRM if possible or plasmid)
- Negative control (water instead of DNA - no-template control, NTC)

For extraction (targeted element):

- Negative extraction control
- Control of environment
- For quality control of extracted DNA (species specific gene)

For potential inhibition (dilutions of extracted DNA)

Common screening elements

Confirmation of presence of genetic element(s) is indication of presence of LMOs in tested sample.

The same genetic element can be present in different LMOs.

Examples of elements/constructs present in many LMOs:

- CaMV P-35s - Cauliflower mosaic virus promoter
- tNOS - nopaline synthase terminator from Agrobacterium tumefaciens
- ctp2-cp4-epsps - junction region between the chloroplast transit peptide 2 (CTP2) sequence from the Arabidopsis thaliana epsps gene and the CP4 epsps gene from Agrobacterium tumefasciens (CP4 EPSPS)
- bar - phosphinothricin N-acetyltransferase from bacterium Streptomyces hygroscopicus
- pat - phosphinothricin N-acetyltransferase from bacterium Streptomyces viridochromogenes
- Cry IAb – gene for insecticidal proteins produced by Bacillus thuringiensis during sporulation
- nptII – neomycin phosphotransferase that inactivates aminoglycoside antibiotics
- P-FMV - Figwort mosaic virus promoter

An example for screening - plan

Experiment ID	.ixo
Analyst:	

Pipets used	
DNA 1 A	
manual 0.1uL-2.5uL	□
manual 0.5uL-10uL	□
multistep 0.2uL-10uL	□

DNA 1 B	
manual (0.5uL-10uL)	□
manual (10uL-100uL)	□
multistep (0.2uL-10uL)	□
multistep (2.0uL-20uL)	□
multistep (5.0uL-120uL)	□

Plazmids C	
manual (0.5uL-10uL)	□
manual (2.0uL-20uL)	□
manual (10uL-100uL)	□
manual (20uL-200uL)	□
manual (100uL-1000uL)	□

TARGET:		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	A																								
5-plex	B	NTC1		ID491			G222/23 -1 1x		G222/23 -2 1x		G223/23 -1 1x		G223/23 -2 1x		G224/23 -1 1x		G224/23 -2 1x		G225/23 -1 1x		G225/23 -2 1x				
5-plex	C						G226/23 -1 1x		G226/23 -2 1x		G227/23 -1 1x		G227/23 -2 1x		G236/23 -1 1x		G236/23 -2 1x								
5-plex	D						G239/23 -1 1x		G239/23 -2 1x										NKI1 IZ076/23		NKI2 IZ076/23		OE IZ076/23		NTC2
	E																								
	F																								
	G																								
	H																								

384 well plate, 10 µL reactions

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An example for screening - results

CaMV P35s

We	Sample Name	Cq	Comment
B1	NTC1		NTC OK.
D24	NTC2		NTC OK.
B3	ID491	32,42	Pos. control OK.
B4	ID491	32,56	Pos. control OK.
D18	NKI1 IZ076/23		NKI1 OK.
D19	NKI1 IZ076/23		NKI1 OK.
D20	NKI2 IZ076/23		NKI2 OK.
D21	NKI2 IZ076/23		NKI2 OK.
D22	OE IZ076/23		OE OK.
D23	OE IZ076/23		OE OK.

Well	Sample Name	Cq	Comment
B6	G222/23 -1 1x	31,03	pozitivno
B7	G222/23 -1 1x	31,05	
B8	G222/23 -2 1x	31,53	
B9	G222/23 -2 1x	31,37	
B10	G223/23 -1 1x		ni zaznano
B11	G223/23 -1 1x		
B12	G223/23 -2 1x		
B13	G223/23 -2 1x	43,13	
B14	G224/23 -1 1x	36,7	vprašljivo
B15	G224/23 -1 1x	38,22	
B16	G224/23 -2 1x		info run, ker ponavljamo izolacijo.
B17	G224/23 -2 1x		
B18	G225/23 -1 1x		ni zaznano
B19	G225/23 -1 1x	35,96	
B20	G225/23 -2 1x	42,46	
B21	G225/23 -2 1x	39,85	
C6	G226/23 -1 1x		ni zaznano
C7	G226/23 -1 1x		
C8	G226/23 -2 1x		
C9	G226/23 -2 1x		
C10	G227/23 -1 1x		ni zaznano
C11	G227/23 -1 1x		
C12	G227/23 -2 1x		
C13	G227/23 -2 1x		

ctp2-cp4-epsps

We	Sample Name	Cq	Comment
B1	NTC1		NTC OK.
D24	NTC2		NTC OK.
B3	ID491	31	Pos. control OK.
B4	ID491	30,73	Pos. control OK.
D18	NKI1 IZ076/23		NKI1 OK.
D19	NKI1 IZ076/23	46,47	NKI1 OK.
D20	NKI2 IZ076/23		NKI2 OK.
D21	NKI2 IZ076/23		NKI2 OK.
D22	OE IZ076/23		OE OK.
D23	OE IZ076/23		OE OK.

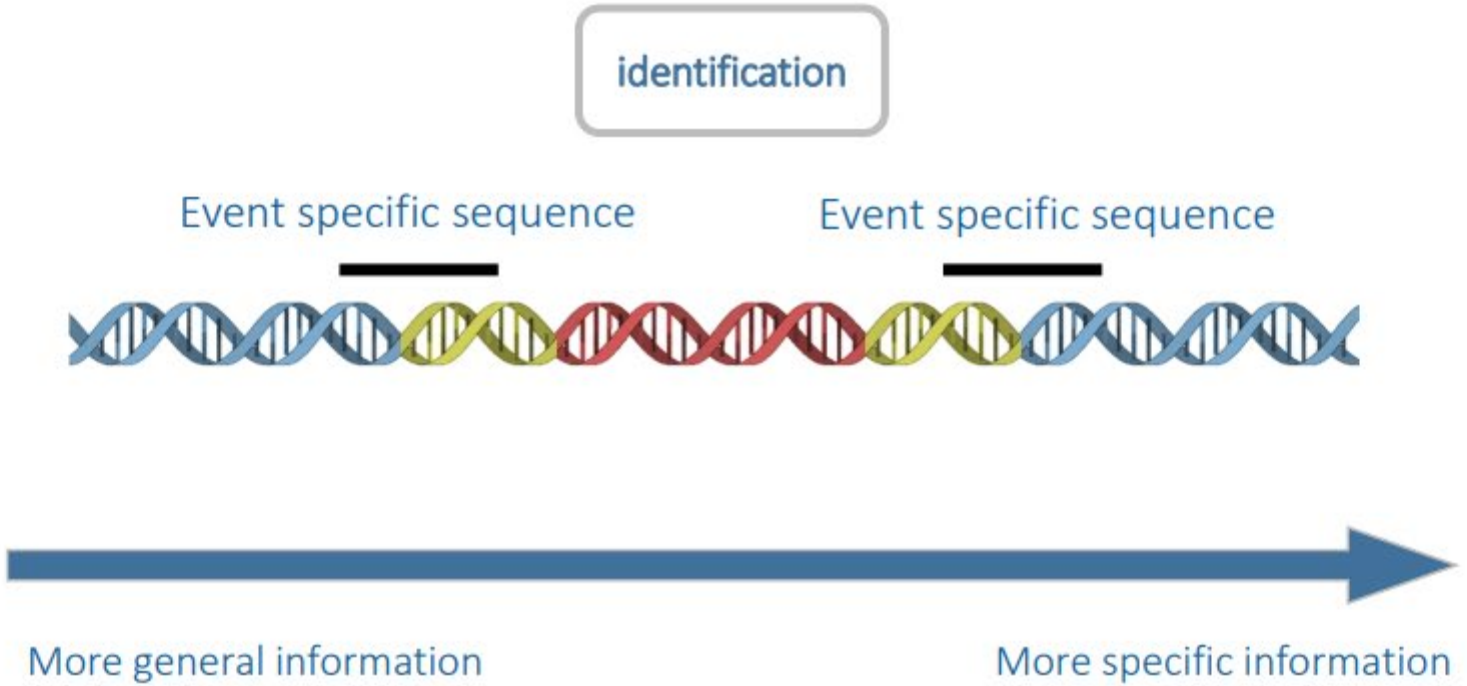
Last Cq is 39,5.

Well	Sample Name	Cq	Comment
B6	G222/23 -1 1x		ni zaznano
B7	G222/23 -1 1x		
B8	G222/23 -2 1x		
B9	G222/23 -2 1x		
B10	G223/23 -1 1x		ni zaznano
B11	G223/23 -1 1x		
B12	G223/23 -2 1x		
B13	G223/23 -2 1x		
B14	G224/23 -1 1x		ni zaznano
B15	G224/23 -1 1x		
B16	G224/23 -2 1x		
B17	G224/23 -2 1x		
B18	G225/23 -1 1x		ni zaznano
B19	G225/23 -1 1x		
B20	G225/23 -2 1x		
B21	G225/23 -2 1x		
C6	G226/23 -1 1x		ni zaznano
C7	G226/23 -1 1x		
C8	G226/23 -2 1x		
C9	G226/23 -2 1x		
C10	G227/23 -1 1x		ni zaznano
C11	G227/23 -1 1x		
C12	G227/23 -2 1x		
C13	G227/23 -2 1x		

An example for screening - results

Amplicon		p35S	tNOS	EPSPS	bar	pat
Well	Sample Name	Cq	Cq	Cq	Cq	Cq
B1	NTC1	/	/	/	/	/
D24	NTC2	/	/	/	/	/
B3	ID491	32,42	32,01	31	32,42	32,46
B4	ID491	32,56	31,82	30,73	32,67	32,47
D18	NKI1 IZ076/23	/	/	/	/	/
D19	NKI1 IZ076/23	/	/	46,47	/	/
D20	NKI2 IZ076/23	/	/	/	/	/
D21	NKI2 IZ076/23	/	/	/	/	/
D22	OE IZ076/23	/	/	/	/	/
D23	OE IZ076/23	/	/	/	/	45,04
Amplicon		p35S	tNOS	EPSPS	bar	pat
Well	Sample Name	Cq	Cq	Cq	Cq	Cq
B6	G222/23 -1 1x	31,03	/	/	/	/
B7	G222/23 -1 1x	31,05	/	/	/	/
B8	G222/23 -2 1x	31,53	/	/	/	/
B9	G222/23 -2 1x	31,37	/	/	/	/
B10	G223/23 -1 1x	/	/	/	/	/
B11	G223/23 -1 1x	/	/	/	/	/
B12	G223/23 -2 1x	/	/	/	/	/
B13	G223/23 -2 1x	43,13	/	/	/	/
B14	G224/23 -1 1x	36,7	/	/	/	/
B15	G224/23 -1 1x	38,22	/	/	/	/
B16	G224/23 -2 1x	/	/	/	/	/
B17	G224/23 -2 1x	/	/	/	/	/
B18	G225/23 -1 1x	/	34,67	/	/	/
B19	G225/23 -1 1x	35,96	35,24	/	/	/
B20	G225/23 -2 1x	42,46	35,32	/	/	/
B21	G225/23 -2 1x	39,85	35,64	/	/	/

Identification of LMOs



Based on results of screening some LMOs can be excluded. This can decrease time and costs of testing.

Sample should be tested for all LMOs that can be potentially present (based on results of screening). Was enough DNA extracted?

Quality control:

Only for PCR (targeted element):

- Positive control (CRM if possible)
- Negative control (no-template control, NTC)
- Dilutions of DNA – if needed

GMO-Matrix (https://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/matrices/full)

GMO-Matrix

1) Select GMO(s) *

By taxon(s)

Specific GMO(s)

2) Select legal status

Regulation (EC) No 1829/2003

- [x] Authorised events
- [x] Events in authorised stacks
- [x] Pending events
- [x] Expired events
- [x] Withdrawn events
- [x] Unauthorised events

Directive 2001/18/EC

- [ ] Authorised events
- [ ] Pending events
- [ ] Withdrawn events

3) Select method(s) *

Event-specific

Construct-specific

Element-specific

** required field*

Show | Cancel | Export as CSV

GMO-Matrix

1) Select GMO(s) *

By taxon(s): × Rapeseed (Brassica napus)

Specific GMO(s)

2) Select legal status

Regulation (EC) No 1829/2003

- [x] Authorised events
- [x] Events in authorised stacks
- [x] Pending events
- [x] Expired events
- [x] Withdrawn events
- [x] Unauthorised events

Directive 2001/18/EC

- [ ] Authorised events
- [ ] Pending events
- [ ] Withdrawn events

3) Select method(s) *

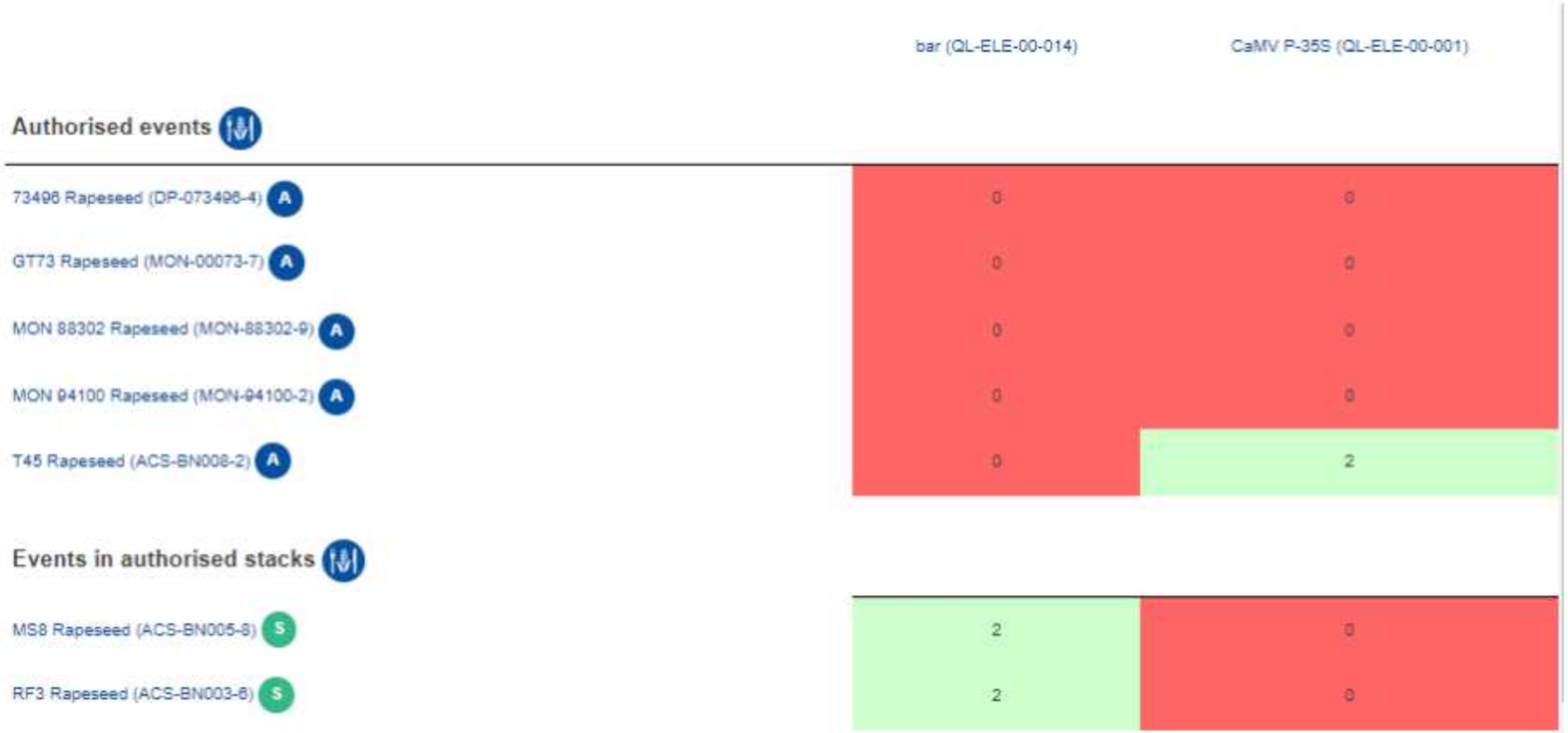
Event-specific

Construct-specific

Element-specific: × bar (QL-ELE-00-014), × CaMV P-35S (QL-ELE-00-001)

** required field*

Show | Cancel | Export as CSV



	bar (QL-ELE-00-014)	CaMV P-35S (QL-ELE-00-001)
Authorised events		
73496 Rapeseed (DP-073496-4) A	0	0
GT73 Rapeseed (MON-00073-7) A	0	0
MON 88302 Rapeseed (MON-88302-9) A	0	0
MON 94100 Rapeseed (MON-94100-2) A	0	0
T45 Rapeseed (ACS-BN008-2) A	0	2
Events in authorised stacks		
MS8 Rapeseed (ACS-BN005-8) S	2	0
RF3 Rapeseed (ACS-BN003-6) S	2	0

If both, CAMV P35s and bar are present four lines can be excluded and three lines of LMO rapeseed could be present. This should be confirmed through analysis.

In–house screening matrix

Laboratores can build their in-house screening matrix based on tested species and available methods

		02G-Pos47	02G-Pos15	02G-Pos35	02G-Pos58	02G-Pos59	02G-Pos65				
Event (Unique identifier)	**Species**	**P35S**	**tNOS**	**P35S:: bar**	**P35S-HSP70**	**P35S-PAT**	**CTP2-CP4-EPSPS**	**tNOS**	**P35S**	**bar**	**pat**
GT73 (MON-ØØØ73-7)	oilseed rape	0	0	0	0	0	1	0	0	0	0
T45 (ACS-BNØØ8-2)	oilseed rape	1	1[b]	1[b]	0	1	0	0	1	0	1
Topas 19/2 (ACS-BNØØ7-1)	oilseed rape	1	0	0	0	1	0	0	1	0	1
DP073496 (DP-Ø73496-4)	oilseed rape	0	0	0	0	0	0	0	0	0	0
Ms11 (BCS BNØ12-7)	oilseed rape	0	1	NR	NR	NR	0	1	0	1	0
MON94100 (MON-941ØØ-2)	oilseed rape	0	0	0	0	0	0	0	0	0	0
OXY-235 (ACS-BNØ11-5)	oilseed rape	1	1	NR	NR	NR	0	1	1	0	0
MON88032 (MON-88302-9)	oilseed rape	0	0	NR	NR	0	1	0	0	0	0
MS1 (ACS-BNØØ4-7)	oilseed rape	0	1	NR	NR	0	0	1	0	1	0
Rf1 (ACS-BNØØ1-4)	oilseed rape	0	1	NR	NR	0	0	1	0	1	0
Rf2 (ACS-BNØØ2-5)	oilseed rape	0	1	NR	NR	0	0	1	0	1	0
MS8 (ACS-BNØØ5-8)	oilseed rape	0	1	1	0	0	0	1	0	1	0
RF3 (ACS-BNØØ3-6)	oilseed rape	0	1	1	0	0	0	1	0	1	0
LBFLFK Lokus 1 (BPS-BFLFK-2)	oilseed rape	0	0	NR	NR	NR	0	0	0	0	0
LBFLFK Lokus 2 (BPS-BFLFK-2)	oilseed rape	0	0	NR	NR	NR	0	0	0	0	0

An example for identification - plan

Experiment ID	.ixo
Analyst:	

Pipets used	
DNA 1 A	
manual 0.1uL-2.5uL	□
manual 0.5uL-10uL	□
multistep 0.2uL-10uL	□

DNA 1 B	
manual (0.5uL-10uL)	□
manual (10uL-100uL)	□
multistep (0.2uL-10uL)	□
multistep (2.0uL-20uL)	□
multistep (5.0uL-120uL)	□

Plazmids C	
manual (0.5uL-10uL)	□
manual (2.0uL-20uL)	□
manual (10uL-100uL)	□
manual (20uL-200uL)	□
manual (100uL-1000uL)	□

TARGET:		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	A																								
DAS40278	B	NTC1		ID651			G221/23 -1 1x		G221/23 -2 1x		G225/23 -1 1x		G225/23 -2 1x												
DAS40278	C						G228/23 -1 1x		G228/23 -2 1x		G229/23 -1 1x		G229/23 -2 1x												
DAS40278	D						G230/23 -1 1x		G230/23 -2 1x																NTC2
	E																								
73496-4	F	NTC1		PK			G222/23 -1 1x		G222/23 -2 1x		G223/23 -1 1x		G223/23 -2 1x												
73496-4	G						G226/23 -1 1x		G226/23 -2 1x		G227/23 -1 1x		G227/23 -2 1x		G239/23 -1 1x		G239/23 -2 1x								NTC2

Maize DAS40278 (DAS-40278-9) and oilseed rape 73496-4 (DP-073496-4) do not contain any screening elements used in our laboratory. They should always be tested.

384 well plate, 10 µL reactions

An example for identification - result

We	Sample Name	Cq	Comment
B1	NTC1	Undetermined	NTC OK.
D24	NTC2	Undetermined	NTC OK.
B3	ID651	31,65803939	Pos.control OK.
B4	ID651	31,46042856	Pos.control OK.
B6	G221/23 -1 1x	Undetermined	ni zaznano
B7	G221/23 -1 1x	Undetermined	
B8	G221/23 -2 1x	Undetermined	
B9	G221/23 -2 1x	Undetermined	
B10	G225/23 -1 1x	Undetermined	ni zaznano
B11	G225/23 -1 1x	Undetermined	
B12	G225/23 -2 1x	Undetermined	
B13	G225/23 -2 1x	Undetermined	
C6	G228/23 -1 1x	Undetermined	ni zaznano
C7	G228/23 -1 1x	Undetermined	
C8	G228/23 -2 1x	Undetermined	
C9	G228/23 -2 1x	Undetermined	
C10	G229/23 -1 1x	Undetermined	ni zaznano
C11	G229/23 -1 1x	Undetermined	
C12	G229/23 -2 1x	Undetermined	
C13	G229/23 -2 1x	Undetermined	
D6	G230/23 -1 1x	Undetermined	ni zaznano
D7	G230/23 -1 1x	Undetermined	
D8	G230/23 -2 1x	Undetermined	
D9	G230/23 -2 1x	Undetermined	

DAS40278 (DAS-40278-9) in maize samples.

We	Sample Name	Cq	Comment
F1	NTC1	Undetermined	NTC OK.
G24	NTC2	Undetermined	NTC OK.
F3	PK	26,40724731	Pos.control OK.
F4	PK	26,32239206	Pos.control OK.
F6	G222/23 -1 1x	Undetermined	ni zaznano
F7	G222/23 -1 1x	Undetermined	
F8	G222/23 -2 1x	Undetermined	
F9	G222/23 -2 1x	Undetermined	
F10	G223/23 -1 1x	Undetermined	ni zaznano
F11	G223/23 -1 1x	Undetermined	
F12	G223/23 -2 1x	Undetermined	
F13	G223/23 -2 1x	Undetermined	
F14	G224/23 -1 1x	Undetermined	ni zaznano
F15	G224/23 -1 1x	Undetermined	
F16	G224/23 -2 1x	Undetermined	
F17	G224/23 -2 1x	Undetermined	
G6	G226/23 -1 1x	Undetermined	ni zaznano
G7	G226/23 -1 1x	Undetermined	
G8	G226/23 -2 1x	Undetermined	
G9	G226/23 -2 1x	Undetermined	
G10	G227/23 -1 1x	Undetermined	ni zaznano
G11	G227/23 -1 1x	Undetermined	
G12	G227/23 -2 1x	Undetermined	
G13	G227/23 -2 1x	Undetermined	
G14	G239/23 -1 1x	Undetermined	ni zaznano
G15	G239/23 -1 1x	Undetermined	
G16	G239/23 -2 1x	Undetermined	
G17	G239/23 -2 1x	Undetermined	

73496-4 (DP-073496-4) in oilseed rape samples.

Pre-prepared plates for identification

As the number of LMOs is increasing number of tests following screening is also increasing.

In addition number of LMOs without common screening elements is increasing.

To maintain analysis time and cost efficient, pre-prepared plates were introduced…..

		MON87701	DP-305423-1	CV127	MON87769	MON87708	MON87751	MON40-3-2	DP356043	MON89788	GMB151	MON87705	A2704-12	A5547-127	FG72	DAS68416	DAS44406	DAS81419	SYHT0H2							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Določanje
NTC1	A	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1							
poz. control	B	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control							
Sample1	C	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1							
	D	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1							
Sample2	E	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2							
	F	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2							
	G																									
	H																									
	I																									
	J																									
	K																									
	L																									
	M																									
	N																									
poz. control	O	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control							
NTC2	P	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2							

		DAS40278	MON810	MON87403	GA21	MIR604	MIR162	5307	MON89034	MON87460	MON87411	NK603	MON88017	MON87427	DAS1507	DAS59122	DP4114	T25	Bt11	MZHG0JG	MZIR098	3272	MON863	Bt176	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
NTC1	A	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	NTC1	
poz. control	B	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	
Sample1	C	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	
	D	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	Sample 1	
Sample 2	E	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	
	F	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	Sample 2	
	G																								
	H																								
	I																								
	J																								
	K																								
	L																								
	M																								
	N																								
poz. control	O	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	poz. control	
NTC2	P	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	NTC2	

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Article

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Development and Validation of Duplex, Triplex, and Pentaplex Real-Time PCR Screening Assays for the Detection of Genetically Modified Organisms in Food and Feed

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Methodology article

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Critical points of DNA quantification by real-time PCR – effects of DNA extraction method and sample matrix on quantification of genetically modified organisms

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