

JRC GMO-Matrix: an *in silico* decision support tool for Genetically Modified Organisms detection



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Content

1. Presentation of the EU-RL GMFF
2. The bioinformatics infrastructure of the EU-RL GMFF
3. The JRC GMO-Matrix application

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The Joint Research Centre (JRC) is a Directorate-General of the European Commission under the responsibility of the European Commissioner for Science and Research.



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EC-JRC: 7 Institutes in 5 EU Member States

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IES - *Ispra, Italy*

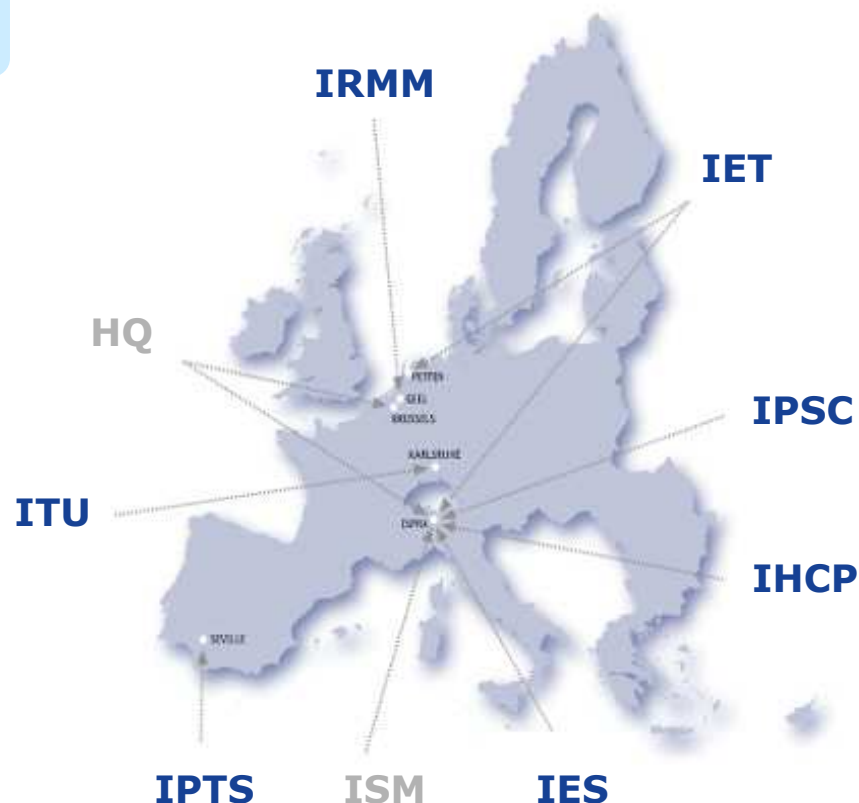
Institute for Environment and Sustainability

IHCP - *Ispra, Italy*

Institute for Health and Consumer Protection

IPTS - *Seville, Spain*

Institute for Prospective Technological Studies





European Union Reference Laboratory for Genetically Modified Food and Feed (EU-RL GMFF)

- Labelling of authorised Genetically Modified Organisms (GMOs)
- Prevent un-authorised GMOs on the European market

Two legal mandates:

1. Regulation (EC) No 1829/2003 → validation of methods for detection and quantification of GM events
2. Regulation (EC) No 882/2004 → official controls performed to ensure the verification of compliance with feed and food law

1st mandate of the EU-RL GMFF under Reg. (EC) No 1829/2003 (and implementing Regulations)

Core activity: Validation of detection methods for GM Food and Feed as part of the EU authorisation procedure
(No method – no authorisation)

Provision of **control samples**

Provision of **guidance documents**

Role in **dispute settlements**

Impact: Standardised methods delivering robust analytical results
Better implementation of legal limits

<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>

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2nd mandate of the EU-RL GMFF under Reg. (EC) No 882/2004

Coordination of EU National Reference Laboratories (**NRLs**)

Providing NRLs with **reference analytical methods**

Coordinating the accurate application of analytical methods, *e.g.* by organising **comparative testing** and by ensuring an appropriate follow-up

Coordinating the application of **new analytical methods**

Conducting **training** courses for the benefit of staff from NRLs in the EU and of laboratories responsible for feed and food analysis **in third countries.**

Role in **emergency situations** (unauthorised GMOs on EU market)

Impact:
Europe

Analytical controls are harmonised throughout

20 May 2014 Rome

GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis

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JOINT RESEARCH CENTRE
European Union Reference Laboratory for GM Food and Feed

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GMOMETHODS:
EU Database of Reference Methods for GMO Analysis

Home

Search for Select by GMO Unique Identifier:

Quantitative GMO detection PCR methods

- GMO specific
 - Event specific
 - Maize
 - Soybean
 - Cotton
 - Oilseed rape
 - Potato
 - Rice
 - Sugar beet
 - Construct specific
 - Element specific
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)

Qualitative GMO detection PCR methods

- GMO specific
 - Event-specific
 - Construct-specific
 - Element-specific
 - Cauliflower Mosaic Virus 35S promoter (CaMV P-35S)
 - Figwort Mosaic Virus 35S promoter (P-FMV)
 - Neomycin phosphotransferase II gene (nptII)
 - Nopaline synthase terminator (T-nos)
 - Phosphinothricin N-acetyltransferase gene (bar)
- Taxon specific
 - Validated independently
 - Validated in combination with other method(s)
 - Plant-specific

Released the GMOMethods app for iPad on 20-12-2011.



Last update

Date	ID	Description
28/06/2013	QT-ELE-00-004	Quantitative PCR method for detection of Cauliflower Mosaic Virus 35S promoter.
25/01/2013	BCS-GH004-7	Quantitative PCR method for

Available at: <http://gmo-crl.jrc.ec.europa.eu/gmomethods/>

Criteria for Methods Selection

The concept of “reference method” per se is not strictly defined in EU legislation on GMOs.

Selection criteria for inclusion of methods*:

- Validated in a collaborative trial, according to the principles and requirements of ISO 5725 and/or IUPAC protocol
- Verified by the EU-RL GMFF in the context of compliance with a EU legislative act.

For qualitative analysis, only the general ISO 5725 and/or the IUPAC principles and definitions are evaluated (i.e. availability of data on the method specificity and compliance with provisions on number of participating laboratories)

*criteria under revision

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The Central Core Sequence Information System (CCSIS)

COMMISSION REGULATION (EC) No 641/2004

*"the applicant shall submit **the full sequence of the insert(s), together with the base pairs of the host flanking sequences** needed to establish an event-specific detection method."*

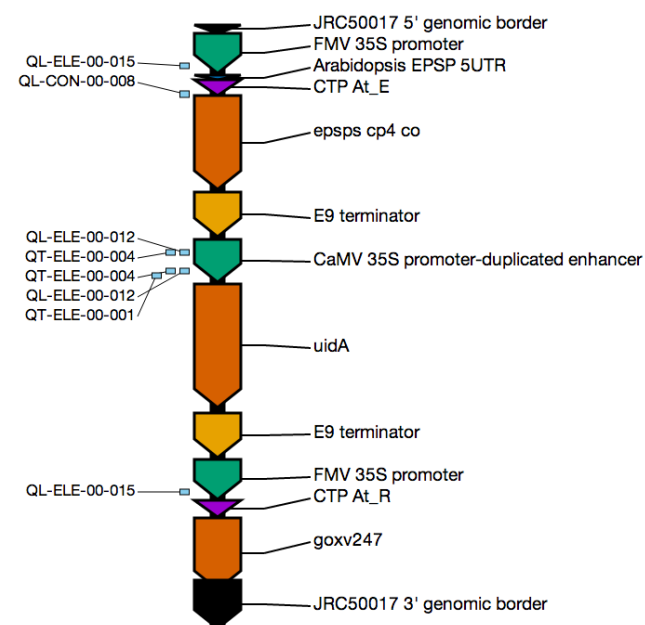
*"The **CRL** shall enter these data in a **molecular database**."*

*"By running **homology searches**, the **CRL** will thus be in a position to **assess the specificity** of the proposed method."*

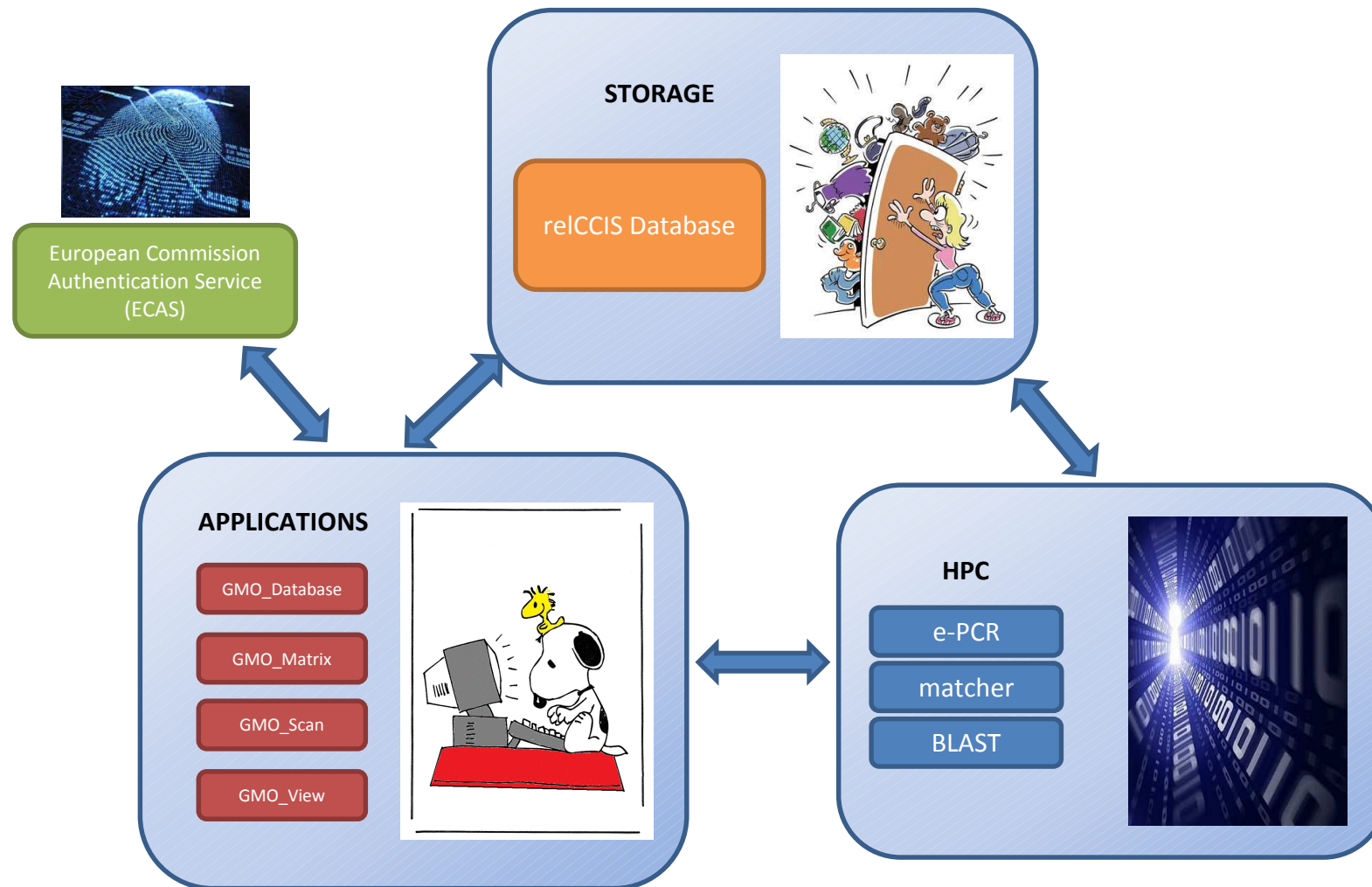
- The CCSIS is the **implementation** of this database, and is integrated with **Bioinformatics Tools** (developed in-house and/or installed locally)

CCSIS records are annotated

...ACAACGTTTCGTCAAGTTCAATGCATCAGTTTCATTGCGCACACACC
AGAATCCTACTGAGTTCGAGTATTATGGCATTGGGAAAACTGTTTT
CTTGTACCATTTGTTGTGCTTGTAAATTTACTGTGTTTTTATTTCGGT
TTTCGCTATCGAACTGTGAAATGGAAATGGATGGAGAAGAGTTAATG
AATGATATGGTCCTTTTGTTCATTCTCAAATTAATATTATTTGTTTT
TTCTCTTATTTGTTGTGTGTTGAATTTGAAATATAAGAGATATGCA
AACATTTTGTTTTGTGAGTAAAAATGTGTCAAATCGTGGCCTCTAATGA
CCGAAGTTAATATGAGGAGTAAAACTTGTAGTTGTACCATTATGC
TTATTCACCTAGGCAACAAATATATTTTCAGACCTAGAAAAGCTGCAA
ATGTTACTGAATACAAGTATGTCCTCTTGTGTTTTAGACATTTATGA
ACTTTCCTTTATGTAATTTCCAGAATCCTTGTGAGATTCTAATCAT
TGCTTTATAATTATAGTTATACCTCATGGATTTGTAGTTGAGTATGAA
AATATTTTAAATGCATTTTATGACTTGCCAATTGATTGACAACATG
CATCAATCGACCTGCAGCCACTCGAAGCGGCCGCTTCAAGCTTCTG
CAGGTCCGATGTGAGACTTTTCAACAAAGGGTAATATCCGGAACCT
CCTCGGATTCCATTGCCAGCTATCTGTCACTTTATTGTGAAGATAG
TGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTCGATAAAGGA
AAGGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGG
ACCCCCACCCACGAGGAGCATCGTGAAAAAGAAGACGTTTCCAACCA
CGTCTTCAAAGCAAGTGGATTGATGTGATGGTCCGATGTGAGACTTT
TCAACAAAGGGTAATATCCGGAACCTCCTCGGATTCCATTGCCAG
CTATCTGTCACTTTATTGTGAAGATAGTGGAAGGAAGGTGGCTCC
TACAAATGCCATCATTCGATAAAGGAAGGCCATCGTTGAAGATGC
CTCTGCCGACAGTGGTCCCAAAGATGGACCCACCCACGAGGAGCA
TCGTGGAAGAAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGAT
TGATGTGATAT...



GMO Event GTSB77 sugarbeet (SY-GTSB77-8)



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The JRC GMO-Matrix application

Predicts (in silico) **which GMO detection method(s)** will detect
which GMO event(s)

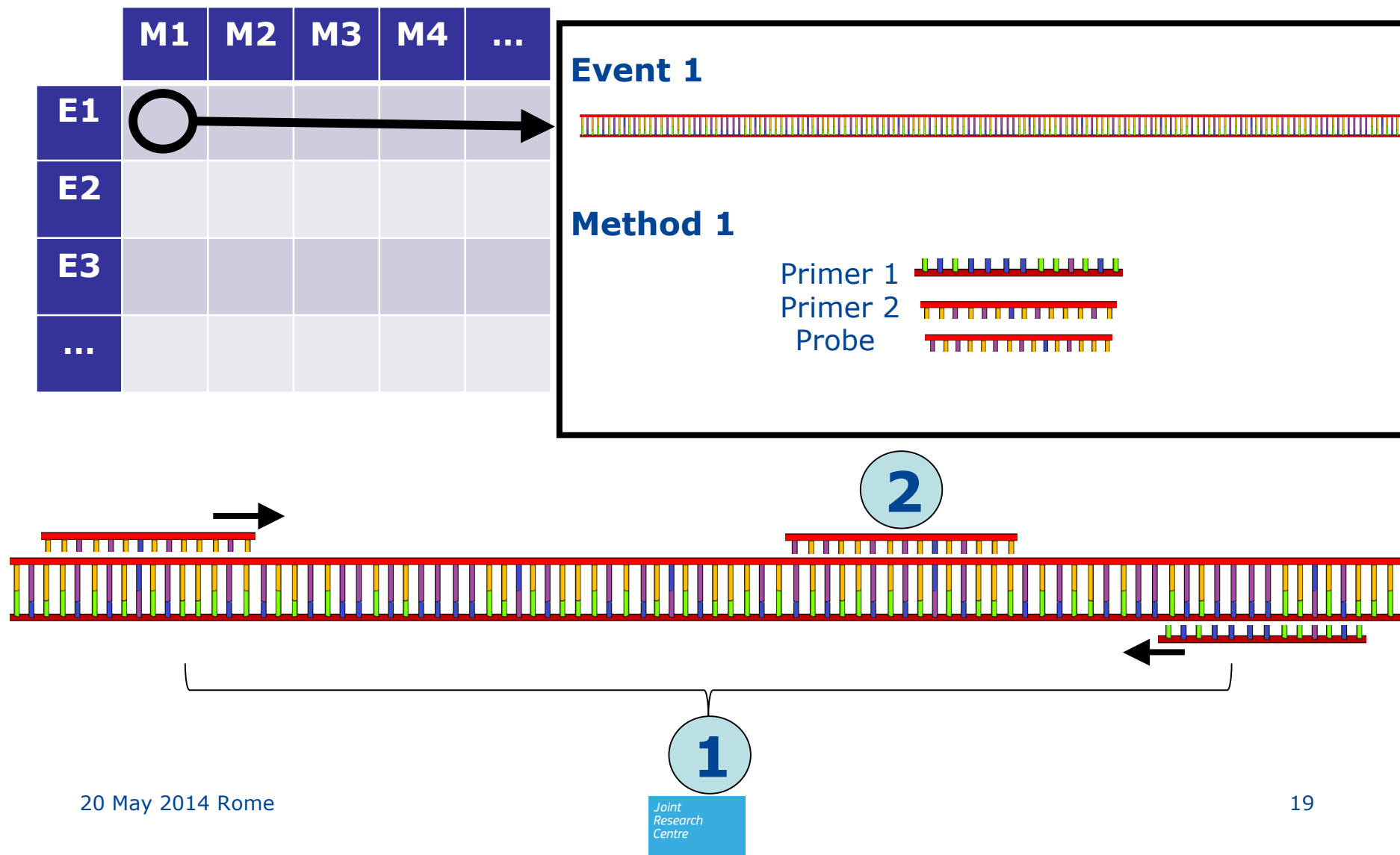
- Links the information between the JRC GMOMETHODS database and the CCSIS

GMO Matrix concept

- 1) User selects GMO(s) to analyse
- 2) User selects detection method(s) to analyse
- 3) The application builds and presents a two-dimensional table (matrix)

Columns:
Rows:
Each cell:

Methods
GMOs
Prediction



Question

A rapeseed sample needs to be tested, and three methods (CTP2-CP4 EPSPS, P-FMV and T-nos) are available. Are there some known GMOs that won't be detectable?

Select taxon(s):

✖ Rapeseed (Brassica napus)

Select specific GMO(s):

Select methods:

CCSIS

Event-specific

Construct-specific

✖ QL-CON-00-008 (CTP2-CP4 EPSPS)

Element-specific

✖ QL-ELE-00-015 (P-FMV)

✖ QL-ELE-00-007 (T-nos)

Custom

Show

Export as CSV

GMO Event GT73 Rapeseed (MON-00073-7)

GMO Event MS8 Rapeseed (ACS-BN005-8)

GMO Event RF3 Rapeseed (ACS-BN003-6)

GMO Event T45 Rapeseed (ACS-BN008-2)

GMO Event Topas 19/2 Rapeseed (ACS-BN007-1)

GMO Event Rf1 Rapeseed (ACS-BN001-4)

GMO Event Ms1 Rapeseed (ACS-BN004-7)

GMO Event Rf2 Rapeseed (ACS-BN002-5)

GMO Event MON 88302 Rapeseed (MON-88302-9)

GMO Event 73496 rapeseed (DP-073496-4)

	QL-CON-00-008 (CTP2-CP4 EPSPS)	QL-ELE-00-015 (P-FMV)	QL-ELE-00-007 (T-nos)
GMO Event GT73 Rapeseed (MON-00073-7)	0/0	0/0 0/0	-
GMO Event MS8 Rapeseed (ACS-BN005-8)	-	-	0/0
GMO Event RF3 Rapeseed (ACS-BN003-6)	-	-	0/0 0/0
GMO Event T45 Rapeseed (ACS-BN008-2)	-	-	-
GMO Event Topas 19/2 Rapeseed (ACS-BN007-1)	-	-	-
GMO Event Rf1 Rapeseed (ACS-BN001-4)	-	-	0/0
GMO Event Ms1 Rapeseed (ACS-BN004-7)	-	-	0/0
GMO Event Rf2 Rapeseed (ACS-BN002-5)	-	-	0/0
GMO Event MON 88302 Rapeseed (MON-88302-9)	0/0	0/0	-
GMO Event 73496 rapeseed (DP-073496-4)	-	-	-

Question

A sample was tested with 4 methods, two were positive (CaMV35S-CTP and T-nos), and two were negative (bar and nptII). What known GMO event(s) could be present in the sample?

Select positive method(s):

CCSIS

Event-specific

Construct-specific

☒ QT-CON-00-009 (CaMV P-35S-CTP)

Element-specific

☒ QL-ELE-00-007 (T-nos)

Custom

Maximum number of events:

Show

Select negative method(s):

CCSIS

Event-specific

Construct-specific

Element-specific

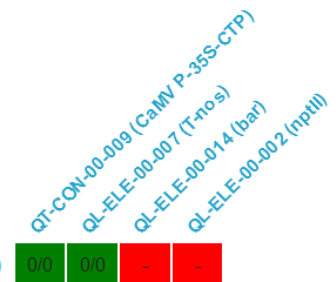
☒ QL-ELE-00-014 (bar) ☒ QL-ELE-00-002 (nptII)

Custom

The following events or combinations of events match the selected pattern:

GMO Event 40-3-2 Roundup Ready Soybean (MON-04032-8)

GMO Event 40-3-2 Roundup Ready Soybean (MON-04032-6)



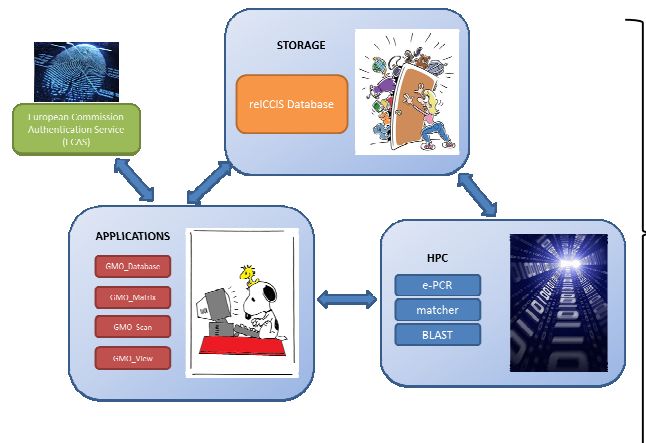
A public version of the JRC GMO-Matrix

The functionality of the JRC GMO-Matrix can support GMO control laboratories in planning and evaluating their GMO screening strategies.

Main concerns:

- Restrict access to the other applications developed for in-house bioinformatics support
- Protect the information contained in the CCSIS

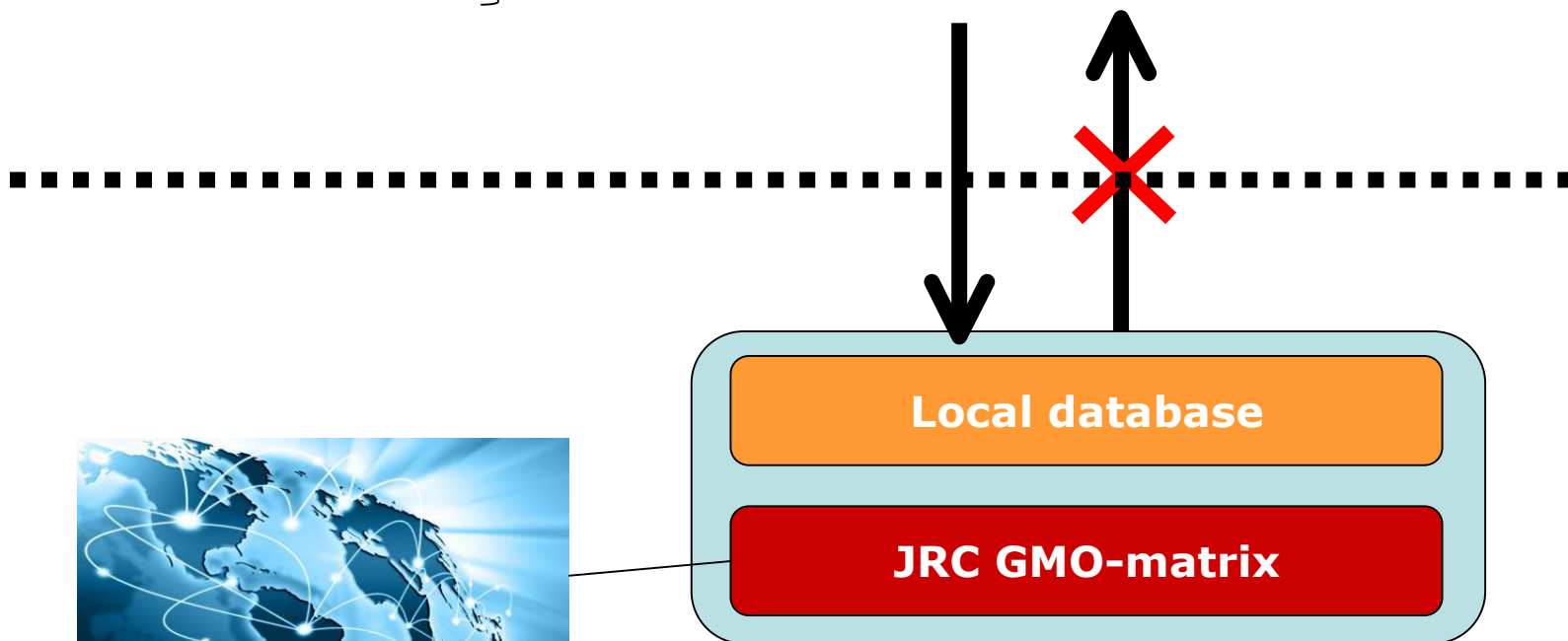
Solution: An **independent** application, with its **own database**, containing only **pre-computed values**.



Local script that performs the calculations for **every combinations of detection methods and GMO events**

Final conclusion:

- 0 - No detection predicted
- 1 - Detection predicted, but imperfect annealing
- 2 - Detection predicted, perfect annealing



<http://gmo-crl.jrc.ec.europa.eu/jrcgmomatrix/>

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EU-RL GMFF Home

Legal basis

Tasks and duties

Guidance documents

Status of dossiers

Methods database

JRC GMO-Matrix

JRC GMO-Matrix

Event finder

[Prespotted plates]

Capacity building

ENGL

Emergencies/
Unauthorised GMOs

Contacts

JRC GMO-Matrix

1) Select GMO(s):

By taxon(s)

and/or

Specific GMO(s)

2) Select method(s):

Event-specific

and/or

Construct-specific

and/or

Element-specific

Please select at least one taxon

Please select at least one method

Try an example matrix: All cotton events vs 'pat' and 'bar' element-specific methods

All rapeseed events vs all rapeseed event-specific methods

All soybean events vs all CaMV 35S and T-nos specific methods

Strengths and weaknesses

Strength: Sequence-based

The same elements have different sequences in different events

Detects "unexpected" amplifications

Can easily accommodate new detection methods and new events

Weakness: Sequence-based

Requires the full (and correct) sequence of the GMO event



European
Commission

A large, close-up image of the European Union flag, which is blue with twelve yellow stars arranged in a circle. The flag is waving in the wind against a clear blue sky.

Thank you for your attention!

Joint
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