Global information sharing: EUginius

GMO analysis and new genomic techniques

March 16th, Theo Prins (Wageningen food Safety Research)

GMO Database www.EUginius.eu

The **Eu**ropean **G**MO **ini**tiative for a **u**nified database **s**ystem

- A cooperation of

- In partnership with

- Shared standards with EURL GMFF (GMOMETHODS) and BCH (GMOGET)

Sharing information

- Reliable information for GMO detection and identification
  - GMOs authorised in the EU (Reg. 1829/2003, 258/97; Dir. 2001/18/EC; 90/220/EEC)
  - GMOs non-authorised in the EU that are market relevant (incl. authorised elsewhere)

- Stakeholders
  - (official) GMO analysis laboratories
  - (EU-MS) enforcement institutions

Features of the EUginius database

- 4 modules to explore data

- Manifold information crosslinked supporting GMO analysis
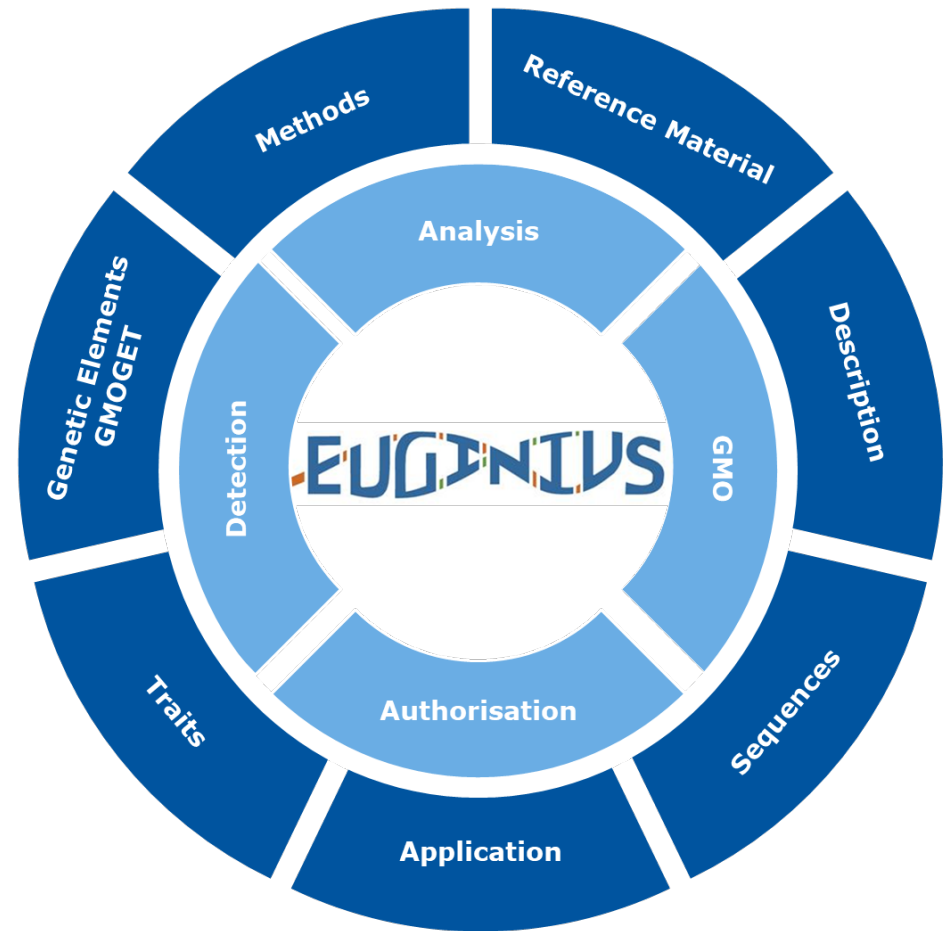
Genetic Element Thesaurus GMOGET

- Standardised naming developed together with BCH
- Elements linked to GMOs as well as to detection methods

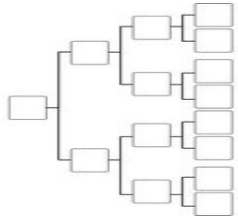| Genetic element	Donor	Trait	Definition	BCH record
Promoter				
➢ P-Cauliflower mosaic virus				
➢ P-e35S-CaMV	Cauliflower mosaic virus		35S promoter with duplicated enhancer region; inserted element length can vary between 307 – 615 bp. Kay R, et al. (1987)	100366
Coding sequence				
➢ CS-phosphinothricin N-acetyltransferase				
➢ CS-bar-STRHY	*Streptomyces hygroscopicus*	Herbicide tolerance > Glufosinate tolerance	Phosphinothricin acetyltransferase gene a.k.a. bar gene shares 85 % homology at the amino acid level with the pat gene. Thompson et al. (1987)	14972
Terminator				
➢ T-nopaline synthase				
➢ T-nos-RHIRD	*Agrobacterium tumefaciens*		3' untranslated region of the nopaline synthase gene . Bevan et al. (1983); Fraley et al. (1983)	100269

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Genetic Element Thesaurus GMOGET

BMC Bioinformatics

DATABASE   **Open Access**

GMO Genetic Elements Thesaurus (GMO-GET): a controlled vocabulary for the consensus designation of introduced or modified genetic elements in genetically modified organisms

Paulien Adamse[1*], Emilie Dagand[2], Karen Bohmert-Tatarev[2,3], Daniela Wahler[2], Manoela Miranda[4,5], Esther J. Kok[1] and Joachim Bendiek[2,6]
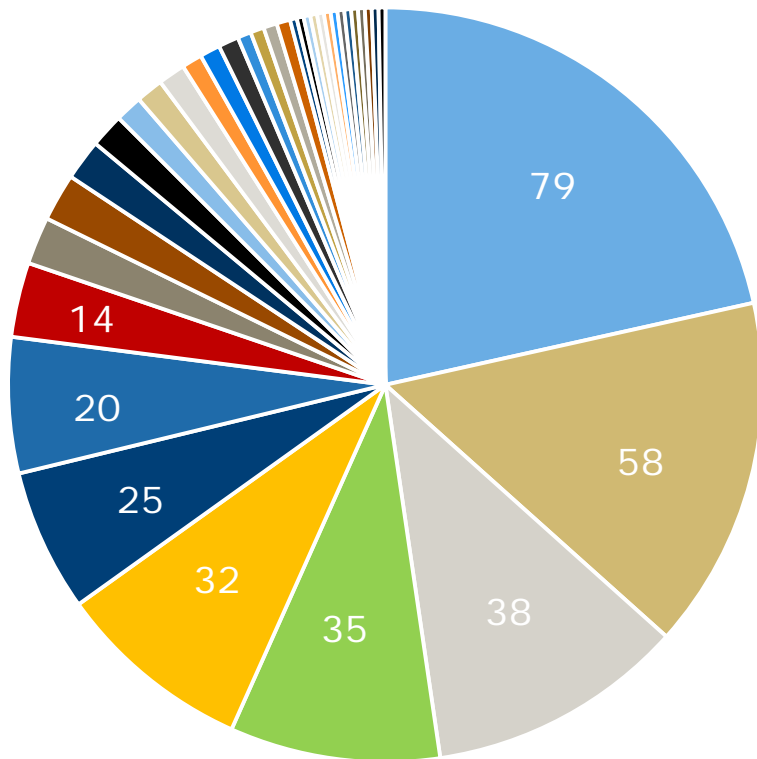
GMOs in EUginius

- 873 GMO entries (incl. stacks)

- 409 single events

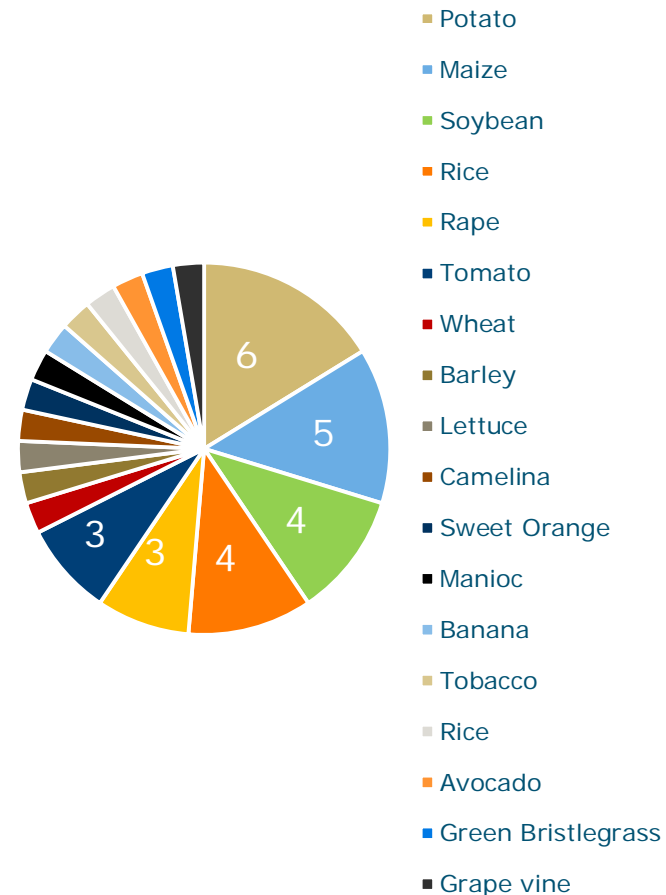### GMO entries
- single events -
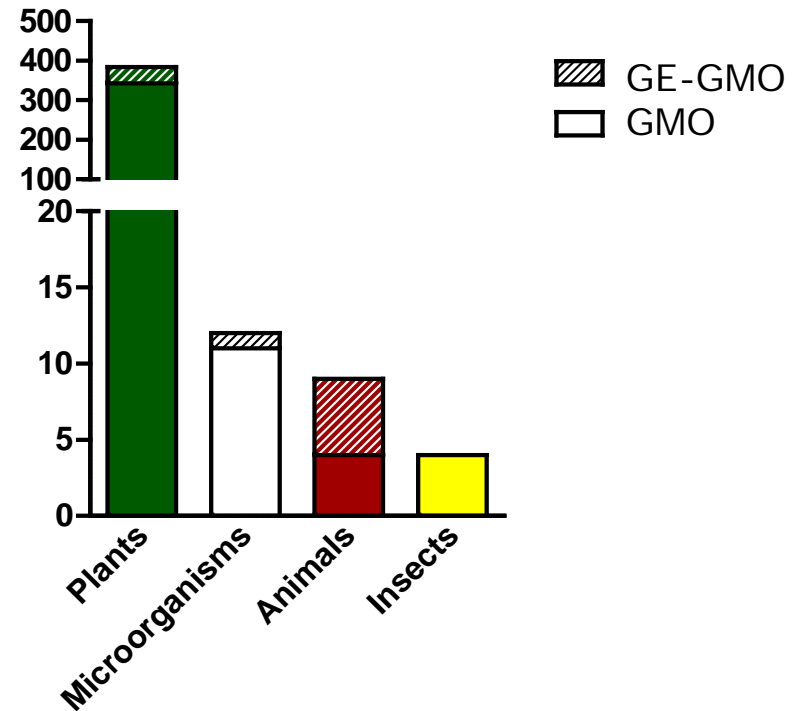
GMO plants in EUginius
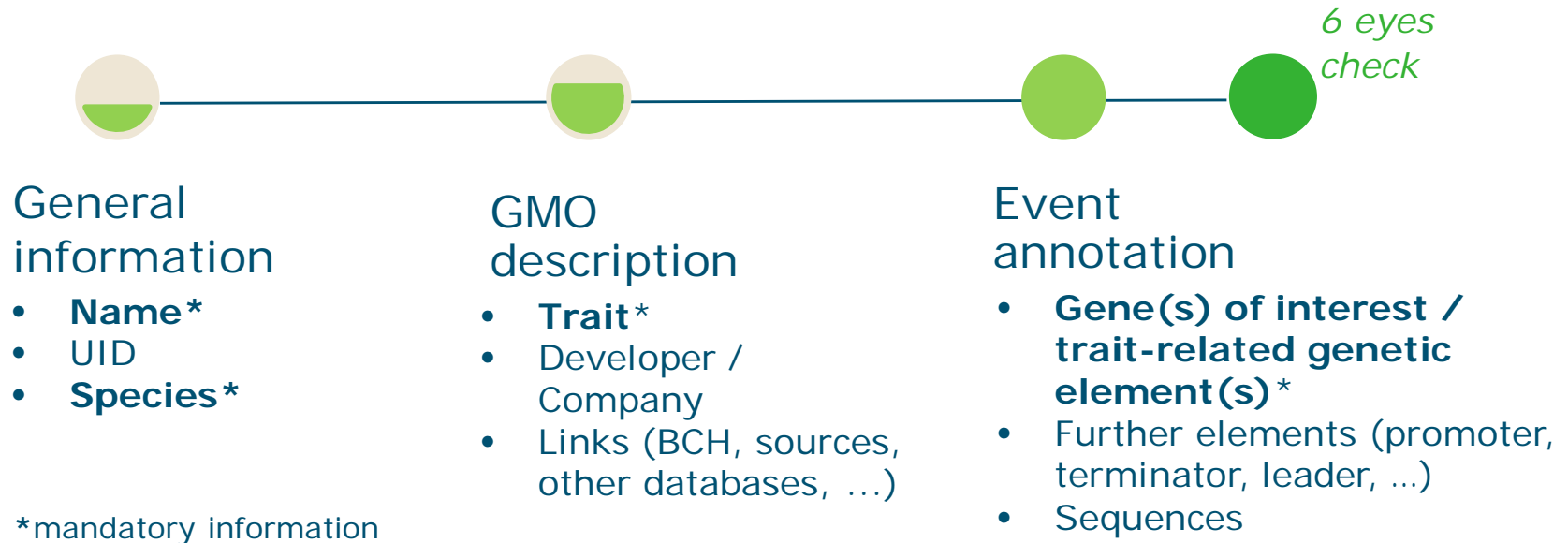
GMO plants
- 344 single events -

Legend:
- Maize
- Potato
- Cotton
- Soybean
- Rape
- Rice
- Pumpkin
- Tomato
- Wheat
- Sugar Cane
- Papaya
- Beetroot
- Safflower
- Apple
- Petunia
- Brassica rapa
- Chicory
- Alfalfa
- Melon
- Cucurbita pepo
- Common Bean
- Rose
- Barley
- Lettuce
- Creeping Bentgrass
- Pineapple
- Thale Cress
- Brassica oleracea
- American Chestnut
- Eucalyptus
- Common Gypsophila
- Flax
- Moth Orchid
- Plum
- Eggplant
- Cowpea

GE-GMO plants (NGT)
- 37 single events -

Legend:
- Potato
- Maize
- Soybean
- Rice
- Rape
- Tomato
- Wheat
- Barley
- Lettuce
- Camelina
- Sweet Orange
- Manioc
- Banana
- Tobacco
- Rice
- Avocado
- Green Bristlegrass
- Grape vine

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Information for GMO entries

6 eyes check

General information
- **Name***
- UID
- **Species***

*mandatory information

GMO description
- **Trait***
- Developer / Company
- Links (BCH, sources, other databases, …)

Event annotation
- **Gene(s) of interest / trait-related genetic element(s)***
- Further elements (promoter, terminator, leader, …)
- Sequences

+ **broad data set** (data from EU-applications, ww-applications, publications, patents…)

+ **fast reaction**

+ **reliable data**

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Examples of GE-GMOs in EUginius

■ **High-oleic soybean**

NGT: modifications of the *FAD2* gene; oil commercialised in the USA

Source: Genetic Literacy Project, 2019/03/01

■ **GABA tomato**

NGT: modifications of the *Slgad3* gene; Tomatoes commercialised in Japan

Source: sanatech-seed.com

■ **Heat tolerant cattle**

NGT: modification of the prolactin receptor gene (*prlr*) PRLR-SLICK cattle

Source: Realimage / Alamy Stock Photo

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Examples of GMOs in EUginius

- **MON95275 maize**

Insect resistant maize; *Agrobacterium*-mediated transformation

Source: BVL picture archive

- **PinkGlow pineapple EF2-114**

Altered color of the fruit through accumulation of lycopene; *Agrobacterium*-mediated transformation (RNAi)

Source: USDA/ARS, Peggy Greb

- **PBR cowpea 709A**

Insect resistant; *Agrobacterium*-mediated transformation

Source: NDLink.org

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GMOs detailed page

Information for GMO detection

- Ring-trial and in-house validated PCR based detection methods

- **ISO-, EU-RL GMFF verified, CEN-, national standards**

    - Element specific (62)

    - Construct specific (30)

    - Event specific (111)

    - Taxon specific (59)

- Methods with targets from element thesaurus are **automatically linked to appropriate GMOs** (verification level 1)

- **Additional verification data** (verification level 2 and 3) confirm or deny automatically assigned methods

GMO/method matrix

Custom-made

(here screening proposal for EU-authorized maize events*)

* Bonfini, L., "In Silico Proposal of Screening Strategies for Detecting EU Authorised GMOs." European Commission, Ispra, JRC131782, 2023.

GMO/method matrix

Matrix assists screening

Verification levels

- descriptive, based on element (+/-**1**)

- *in silico*, Sequence-based (+/-**2**)

- experimental (+/-**3**)

Tools to assist monitoring: Analysis tool

EUGENIUS

Home | GMO | Detection | Analysis | Authorisation | Search | Help

European GMO Initiative for a Unified Database System

GMO analysis tool

This tool is designed to support labs that analyse samples for the presence of GMOs. Based on data you found in your GMO analysis you can use the search module on this page to find additional information on possible GMO targets.

Step 1: select detected targets. The chosen method becomes relevant in case the output needs to be filtered for GMOs that have a verified detection method (+3).

Step 2: select target that has been analysed, but not positively detected (= select confirmed negative targets).

If detected targets can be explained by detected events, no further action will be necessary.

If targets are not explained by the detected events, the table **Suggested output** will list GMOs that could explain these targets.

Step 1:
Select species, GMOs and elements that have been detected in a laboratory analysis:

Species:	any (select to restrict options)
GMO:	any (select to restrict options)
Promoter:	any (select to restrict options)
Terminator:	any (select to restrict options)
Coding Sequence:	any (select to restrict options)
Other elements:	any (select to restrict options)
Construct:	any (select to restrict options)

Step 2:
Select species, GMOs and elements that have been searched for in a laboratory analysis, but were **NOT** detected:

Species:	any (select to restrict options)
GMO:	any (select to restrict options)
Promoter:	any (select to restrict options)
Terminator:	any (select to restrict options)
Coding Sequence:	any (select to restrict options)
Other elements:	any (select to restrict options)
Construct:	any (select to restrict options)

Analyse   Reset

Import result file (xlsx, see Help-file)

Select file   Choose File   No file chosen   Analyse result file

Enter results detected/not detected

Step 1:
Select species, GMOs and elements that have been detected in a laboratory analysis:

Species:
Brassica napus (QL-TAX-BN-003) (canola, oilseed rape, rapeseed) ×

GMO:
Ms11 (QT-EVE-BN-011) ×

Promoter:
any (select to restrict options)

Terminator:
T-nos-RHIRD (QL-ELE-00-006) ×

T-rbcS_E9-PEA (QL-ELE-00-024) ×

Coding Sequence:
CS-bar-STRHY (QL-ELE-00-014) ×

CS-barnase (QL-ELE-B'nase-F-n4/BNaseR-n3) ×

Other elements:
any (select to restrict options)

Construct:
any (select to restrict options)

Step 2:
Select species, GMOs and elements that have been searched for in a laboratory analysis, but were **NOT** detected:

Species:
Glycine max (QL-TAX-GM-001) (soybean) ×
Oryza sativa (QL-TAX-OS-002) (rice) ×
Zea mays (QL-TAX-ZM-003) (maize, corn) ×

GMO:
GTS 40-3-2 (QT-EVE-GM-005) ×
CV127 (QT-EVE-GM-011) ×
DP305423 (QT-EVE-GM-008) ×
VCO1981 (QT-EVE-ZM-001) ×

Promoter:
any (select to restrict options)

Terminator:
T-ocs-RHIRD (QL-ELE-T-OCS-F/T-OCS-R) ×

Coding Sequence:
any (select to restrict options)

Other elements:
any (select to restrict options)

Construct:
cpti/T-nos (QL-CON-cptiF2/tNOS-uni-R) ×

Analyse   Reset

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Everything explained? NO!

Confirmed output

Red 💡: detected target not present in the identified GMOs
Green ✅: detected target present in at least one identified GMO
Yellow ✖: target tested but not detected

Analysis report: The detected GMO is: Ms11. It can explain the presence of CS-bar-STRHY ✅, CS-barnase ✅, T-nos-RHIRD ✅ but not T-rbcS_E9-PEA 💡.

Refine Search

The identified GMOs and their elements

Species	GMO	Promoter	Terminator	Coding Sequence	Construct	EU authorisation
Brassica napus (canola, oilseed rape, rapeseed)	Ms11	P-nos-RHIRD P-rbcS-ARATH P-ta29-TOBAC	T-barnase-BACAM T-nos-RHIRD ✅ T-tr7-RHIRD	CS-bar-STRHY ✅ CS-barnase-BACAM ✅ CS-barstar-BACAM	P-SSU/bar	✖ 📎 ♿ ✿

But what about T-rbcs_E9-PEA. How can this be explained??

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>100 candidates

GMO prediction view

- Restrict list to GMOs from detected species ☐
- Restrict list to GMOs that are linked to methods with a +3 verification value (verified detection methods) ☐
- Exclude GMOs from the list that contain targets that were not detected ☐

Importance sorting (x/y): GMOs sorted first by (x) = number of detected target not present in the identified GMOs (unexplained), then by (y) = number of detected target present in the identified GMOs (explained)

GMOs that can explain one or more detected targets

Species	GMO	Importance sorting ☑	Promoter	Terminator	Coding Sequence	Construct	EU authorisation
Gossypium hirsutum (cotton)	MON1076	1 / 1	P-34S FMV ✖ P-35S-CaMV	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-cry1Ac-BACTU CS-nptII-ECOLX	P-35S/nptII	✖ ⬮ 🚜 ❄
Gossypium hirsutum (cotton)	MON1445	1 / 1	P-34S FMV ✖ P-35S-CaMV	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-aadA-ECOLX CS-CP4epsps-RHIRD CS-nptII-ECOLX	ctp2/cp4-epsps P-35S/nptII P-FMV/cp4-epsps	✖ ⬮ 🚜 ❄
Gossypium hirsutum (cotton)	MON1698	1 / 1	P-34S FMV ✖ P-35S-CaMV	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD CS-nptII-ECOLX	ctp2/cp4-epsps P-35S/nptII P-FMV/cp4-epsps	✖ ⬮ 🚜 ❄
Oryza sativa ✖ *(rice)*	LLRICE601	1 / 1	P-35S-CaMV	T-nos-RHIRD 💡	CS-bar-STRHY ✔	P-35S/bar	✖ ⬮ 🚜 ❄
Phaseolus vulgaris (bean)	INI-FMAPDF	1 / 1	P-35S-CaMV P-nos-RHIRD	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-nptII-ECOLX CS-pdf1.2-ARATH	P-nos/nptII	✖ ⬮ 🚜 ❄
Solanum tuberosum (potato)	1210 amk	1 / 1	P-34S FMV ✖ P-rbcS-ARATH	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD CS-cry3A-BACTT		✖ ⬮ 🚜 ❄
Solanum tuberosum (potato)	2904/1 kgs	1 / 1	P-34S FMV ✖ P-rbcS-ARATH	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD CS-cry3A-BACTT	ctp2/cp4-epsps	✖ ⬮ 🚜 ❄
Solanum tuberosum (potato)	ATBT04-06	1 / 1	P-35S-CaMV P-rbcS-ARATH	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-cry3A-BACTT CS-nptII-ECOLX	P-35S/nptII	✖ ⬮ 🚜 ❄
Solanum tuberosum (potato)	ATBT04-27	1 / 1	P-35S-CaMV P-rbcS-ARATH	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-aadA-ECOLX CS-cry3A-BACTT CS-nptII-ECOLX	P-35S/nptII	✖ ⬮ 🚜 ❄
Solanum tuberosum (potato)	ATBT04-30	1 / 1	P-35S-CaMV P-rbcS-ARATH	T-nos-RHIRD ✔ T-rbcS_E9-PEA 💡	CS-cry3A-BACTT CS-nptII-ECOLX	P-35S/nptII	✖ ⬮ 🚜 ❄

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Reduce possible outcome (assumptions)

GMO prediction view

- Restrict list to GMOs from detected species ☑
- Restrict list to GMOs that are linked to methods with a +3 verification value (verified detection methods) ☐
- Exclude GMOs from the list that contain targets that were not detected ☐

Importance sorting (x/y): GMOs sorted first by (x) = number of detected target not present in the identified GMOs (unexplained), then by (y) = number of detected target present in the identified GMOs (explained)

GMOs that can explain one or more detected targets

Species	GMO	Importance sorting ☑	Promoter	Terminator	Coding Sequence	Construct	EU authorisation
Brassica napus (canola, oilseed rape, rapeseed)	GT200	1 / 0	P-34S FMV ✖	T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD CS-gox-OCHAN	ctp2/cp4-epsps P-FMV/cp4-epsps	✖ 🌿 🚜 🌸
Brassica napus (canola, oilseed rape, rapeseed)	GT73	1 / 0	P-34S FMV ✖	T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD CS-gox-OCHAN	ctp2/cp4-epsps P-FMV/cp4-epsps	✖ 🌿 🚜 🌸
Brassica napus (canola, oilseed rape, rapeseed)	LBFLFK	1 / 0	P-arc5a-PHAVU P-cnl1-LINUS P-fae1-BRANA P-napA-BRANA P-pxr-LINUS P-sbp-VICFA P-setl-BRANA P-ubi4-PETCR P-usp-VICFA	T-35S-CaMV T-ahas-ARATH T-arc5a-PHAVU T-CDI-SOLTU T-fae1-ARATH T-ocs-RHIRD ✖ T-pxr-ARATH T-rbcS_E9-PEA 💡	CS-ahas-ARATH CS-delta12D-PHYSP CS-delta4D-DIALT CS-delta4D-THRSP CS-delta5D-THRSP CS-delta5E-OSTTA CS-delta6D-OSTTA CS-delta6E-THAPS CS-detla6E-PHYPA CS-omega3D-PHYIT CS-omega3D-PYTIR		✖ 🌿 🚜 🌸
Brassica napus (canola, oilseed rape, rapeseed)	MON88302	1 / 0	P-EF1alpha-ARATH	T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD	ctp2/cp4-epsps P-FMV/cp4-epsps	✖ 🌿 🚜 🌸
Brassica napus (canola, oilseed rape, rapeseed)	Ms1	0 / 3	P-nos-RHIRD P-rbcS-ARATH P-ta29-TOBAC	T-nos-RHIRD ✔ T-ocs-RHIRD ✖ T-tr7-RHIRD	CS-bar-STRHY ✔ CS-barnase-BACAM ✔ CS-nptII-ECOLX	P-nos/nptII P-SSU/bar	✖ 🌿 🚜 🌸

Remove GMOs w.o. not-detected elements

GMO prediction view

- Restrict list to GMOs from detected species ☑
- Restrict list to GMOs that are linked to methods with a +3 verification value (verified detection methods) ☐
- Exclude GMOs from the list that contain targets that were not detected ☑

Importance sorting (x/y): GMOs sorted first by (x) = number of detected target not present in the identified GMOs (unexplained), then by (y) = number of detected target present in the identified GMOs (explained)

GMOs that can explain one or more detected targets

Species	GMO	Importance sorting ☑	Promoter	Terminator	Coding Sequence	Construct	EU authorisation
Brassica napus (canola, oilseed rape, rapeseed)	MON88302	1 / 0	P-EF1alpha-ARATH	T-rbcS_E9-PEA 💡	CS-CP4epsps-RHIRD	ctp2/cp4-epsps P-FMV/cp4-epsps	❌ 📎 🚜 ❀
Brassica napus (canola, oilseed rape, rapeseed)	Ms11	0 / 3	P-nos-RHIRD P-rbcS-ARATH P-ta29-TOBAC	T-barnase-BACAM T-nos-RHIRD ✅ T-tr7-RHIRD	CS-bar-STRHY ✅ CS-barnase-BACAM ✅ CS-barstar-BACAM	P-SSU/bar	❌ 📎 🚜 ❀
Brassica napus (canola, oilseed rape, rapeseed)	Ms8	0 / 3	P-rbcS-ARATH P-ta29-TOBAC	T-barnase-BACAM T-nos-RHIRD ✅ T-tr7-RHIRD	CS-bar-STRHY ✅ CS-barnase-BACAM ✅	P-SSU/bar	❌ 📎 🚜 ❀
Brassica napus (canola, oilseed rape, rapeseed)	Rf3	0 / 2	P-rbcS-ARATH P-ta29-TOBAC	T-barstar-BACAM T-nos-RHIRD ✅ T-tr7-RHIRD	CS-bar-STRHY ✅ CS-barstar-BACAM	P-SSU/bar	❌ 📎 🚜 ❀
Brassica napus (canola, oilseed rape, rapeseed)	DHA	0 / 1	P-35S-CaMV P-cnl1-LINUS P-cnl2-LINUS P-fae1-ARATH P-FP1-BRANA	T-cnl1-LINUS T-cnl2-LINUS T-le1-SOYBN T-nos-RHIRD ✅	CS-delta12D-LACKL CS-delta4D-REBSA CS-delta5D-REBSA CS-delta5E-PYRCR CS-delta6D-MICPC CS-delta6E-PYRCR CS-omega3D-KOMPG CS-pat-STRVR	P-35S/pat	❌ 📎 🚜 ❀

Check for MON88302 canola presence

GMO prediction view

- Restrict list to GMOs from detected species ☑
- Restrict list to GMOs that are linked to methods with a +3 verification value (verified detection methods) ☑
- Exclude GMOs from the list that contain targets that were not detected ☑

Importance sorting (x/y): GMOs sorted first by (x) = number of detected target not present in the identified GMOs (unexplained), then by (y) = number of detected target present in the identified GMOs (explained)

GMOs that can explain one or more detected targets

| Species	GMO	Importance sorting ☑	Promoter	Terminator	Coding Sequence	Construct	EU authorisation
Brassica napus (canola, oilseed rape, rapeseed)	MON88302	1 / 0	P-EF1alpha-ARATH	T-rbcS_E9-PEA	CS-CP4epsps-RHIRD	ctp2/cp4-epsps P-FMV/cp4-epsps	

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Search options

EUginius support in GMO analysis

- Reliable data
- Broad method and GMO datasets
- Clear quality level
- Combined information
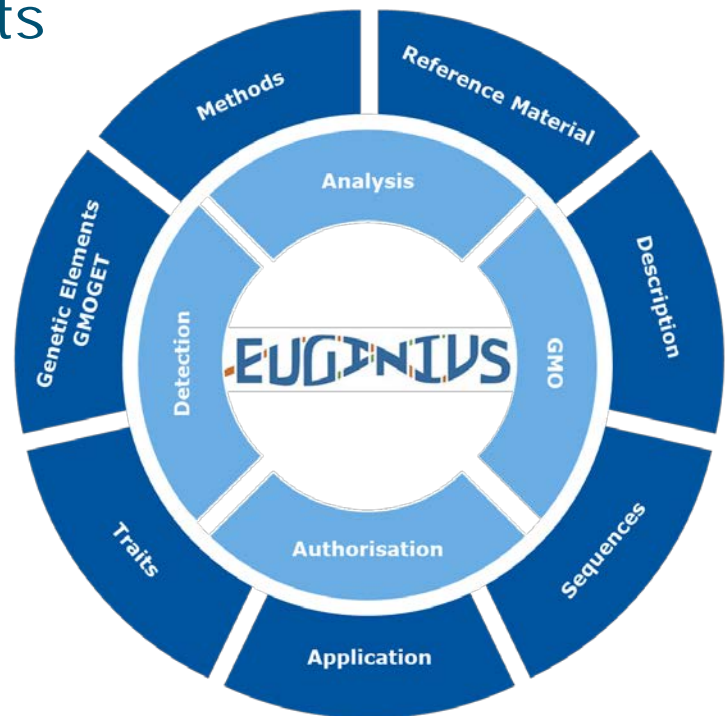
Thank you for your attention

WFSR:

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