2. Introduction

Regulation (EU) No 2017/625 ([3]) states that the European Union Reference Laboratories (EURLs) for feed and food are responsible, amongst others, for "providing national reference laboratories with details and guidance on the methods of laboratory analysis, testing or diagnosis, including reference methods". To that end, the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF) has developed a database of EU reference methods for GMO analysis, called "GMOMETHODS" ([4]) available at https://gmo-crl.jrc.ec.europa.eu/gmomethods/. All the assays are DNA-based detection methods that have been validated in a collaborative trial according to the principles and requirements of ISO 5725 ([5]) and/or the International Union of Pure and Applied Chemistry (IUPAC) ([6]) protocol or that have been verified by the EURL GMFF for EU legal purposes.

Up to 25 February 2021 the GMOMETHODS database (see Figure 1) provides information on 197 different polymerase chain reaction (PCR) methods allowing identification of 92 single GM events. These methods cover all the GMOs that have been authorised in the EU, or whose authorisation is pending or has expired. The authorisation status of the GM events is retrieved from the EU Commission GMO Register website for authorisation under Regulation (EC) 1829/2003 ([7]) and from the JRC Deliberate Release and Placing on the EU Market of GMOs - GMO Register website ([8]) for notifications authorised under Directive 2001/18/EC ([9]).

Moreover, the database presents analytical tests for 25 taxon-specific genes and 35 'element-specific' screening assays. Additional 22 'construct-specific' methods included in the database may in some instances, also be used for screening purposes. The data outlined in Table *1* provide an overview on the methods included in the GMOMETHODS database up to 25 February 2021.

Table 1. GMO and taxon-specific methods included in the GMOMETHODS database according to specificity and purpose of the analysis

Target	Specificity	Purpose		Total
		Qualitative	Quantitative	
GMO	Event-specific	12	77	89
	Construct-specific	14	8	22
	Element-specific	31	4	35
Taxon	Species-specific	16	34	50
	Plant-specific	1	0	1
	Total methods	**74**	**123**	**197**

The screening assays allow detecting 19 single or combined genetic elements (see Table 2) used in the development of a large number of GMOs approved worldwide [(10)].

Table 2. Screening targets detected by the EU reference methods

N	Screening Target	Abbreviation
1	Cauliflower Mosaic Virus 35S promoter	CaMV P-35S
2	*Cauliflower Mosaic Virus 35S* terminator	*CaMV T-35S*
3	*Cauliflower Mosaic Virus 35S* terminator from pCAMBIA vectors	*T-35SpCAMBIA*
4	*CP4 epsps* gene from *Agrobacterium tumefaciens type I and II*	*CP4-epsps*
5	*Cry1Ab/Ac* modified genes derived from *Bacillus thuringiensis*	*cry1Ab/Ac*
6	*Cry1A(b)* synthetic gene derived from *Bacillus thuringiensis*	*cry1A(b)*
7	*Cry1Ac* synthetic gene derived from *Bacillus thuringiensis*	*cry1Ac*
8	*E9* terminator from *Pisum sativum*	*T-E9*
9	*Figwort Mosaic Virus 35S* promoter	*P-FMV*

concomitant presence of an authorised GMO presenting similar screening elements. The presence of unauthorised events in the food or feed samples analysed, therefore, cannot be formally excluded.

Another aspect to be considered in the analysis is that some sequences targeted by the screening methods are derived from organisms that may be naturally present in food and feed samples and may therefore produce false positive results. It is therefore important to test the food and feed samples in parallel with PCR methods specific for those donor organisms (i.e. for the occurrence of the *Cauliflower Mosaic Virus (CaMV)* in samples containing ingredients from *Brassicaceae* or for contamination by *Agrobacterium* species in samples resulting positive to nos terminator assays. Since the E9 terminator (*tE9*) element present in many GM events is derived from the pea genome, a method targeting a pea endogenous gene (i.e. lectin) may also be used in parallel with the E9 assay to exclude the presence of pea derived products in the sample.

Ultimately, the proposals included in this document may contribute to the design of more efficient screening strategies for EU legislative enforcement and inspection controls and be an important reference for international commercial trade. Availability of reference methods that have been selected according to internationally accepted standard protocols is indeed necessary for ensuring global applicability of safety and trade issues.

4. Rationale for the screening proposal

The screening approaches proposed were determined following the criteria of maximal coverage, best performance and minimum laboratory workload. Most of the methods selected are already implemented on the Pre-Spotted Plates (PSPs) developed for GMO screening. They are based on the results of *in silico* analyses performed on the JRC-GMO-Matrix application on 25 February 2021. A rationale is provided below for the selection of the genetic targets and corresponding EU reference methods.

To reduce laboratory workload, two duplex assays for detecting respectively the P-35S/T-nos and pat/bar elements were selected in place of the single methods used on the PSPs. The primers, probes and amplification conditions for targeting the P-35S and T-nos elements in the duplex assay are identical to those of the single methods included on the PSPs. The primers and probes targeting the bar element in the second duplex assay are also identical to the method included on the PSPs while those for the pat element are different. Both duplex methods could be considered in line with the minimum performance requirements defined in the ENGL document and in particular, with the practicability condition setting the maximum volume of the PCR reactions to 25 µL.

The method **QL-ELE-00-016** targeting the Cry1Ab/Ac sequences is included in the proposal to allow detection of soybean GM events MON87701 and MON87751. This method is also included on the PSPs. The only other EU reference method (QL-ELE-00-020) detecting these soybean events was not selected because it is using a SYBR Green approach for amplicon detection.

The **QT-CON-00-008** method targeting the junction between an optimised transit peptide sequence and the point-mutated epsps gene from maize (CTP2-CP4EPSPS) is included in the proposal for detecting the cotton GM events GHB614 and GHB811. These events are not detected *in silico* by any other screening method.

The **QL-ELE-00-024** method targeting the tE9 element is included in the proposal for detecting GM sugar beet H7-1, oilseed rape GT73 and MON88302 and soybean MON89788, MON87769, MON87705 and MON87708. All these events are only partially covered by the other remaining methods QL-CON-00-008 (included on the PSPs), QL-ELE-00-010 and QL-ELE-00-015, which do not detect soybean events MON89788 and MON87708.

The event-specific method **QT-EVE-ZM-004** targeting maize DAS-40278-9 is included in the proposal because the event is not detected *in silico* by any screening EU reference method.

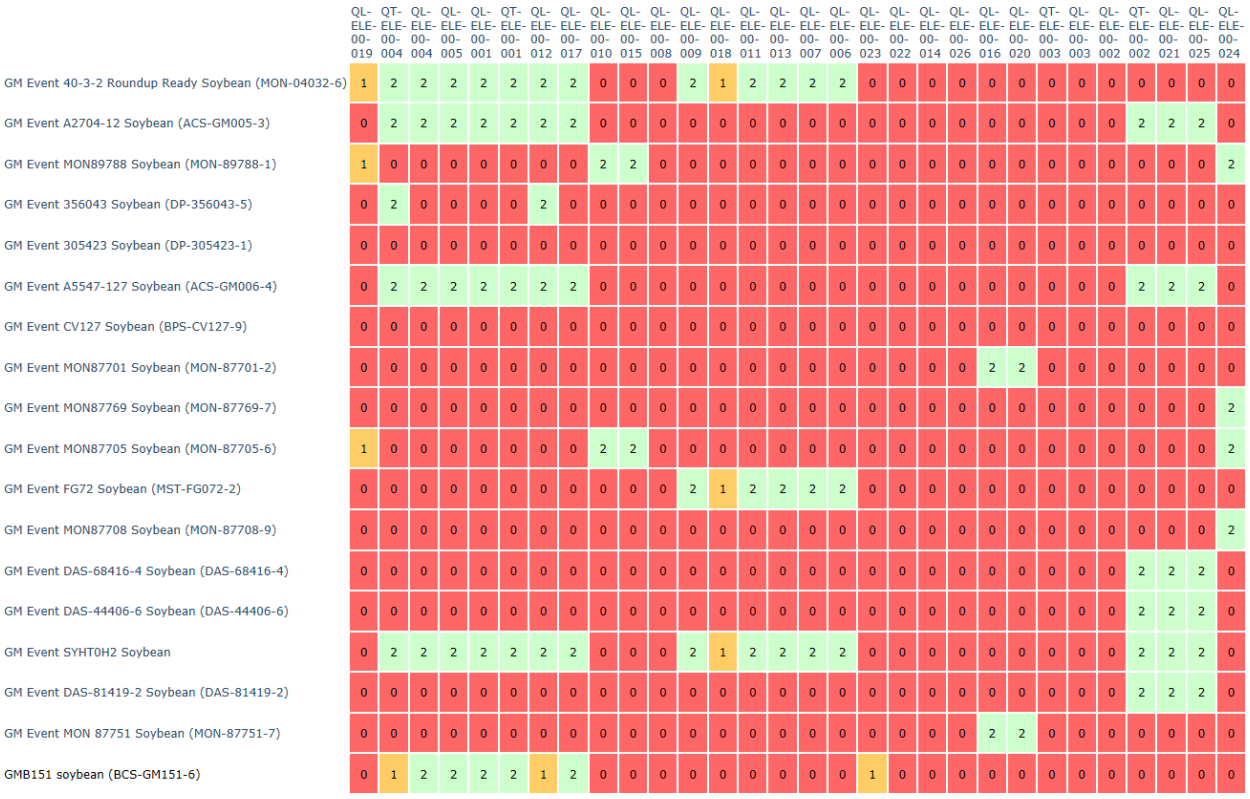
The event-specific method **QT-EVE-BN-009** targeting oilseed rape 73496 is included in the proposal because the event is not detected *in silico* by any screening EU reference method.

The event-specific methods **QT-EVE-GM-008** and **QT-EVE-GM-011** targeting respectively soybean 305423 and BPS-CV127-9 are included in the proposal because these events are not detected *in silico* by any screening EU reference method.

Figures

Figure 1. GMOMETHODS database home page

Figure 2. JRC GMO-Matrix output example



	QL-ELE-00-019	QT-ELE-00-004	QL-ELE-00-004	QL-ELE-00-005	QL-ELE-00-001	QT-ELE-00-001	QL-ELE-00-012	QL-ELE-00-017	QL-ELE-00-010	QL-ELE-00-015	QL-ELE-00-008	QL-ELE-00-009	QL-ELE-00-018	QL-ELE-00-011	QL-ELE-00-013	QL-ELE-00-007	QL-ELE-00-006	QL-ELE-00-023	QL-ELE-00-022	QL-ELE-00-014	QL-ELE-00-026	QL-ELE-00-016	QL-ELE-00-020	QT-ELE-00-003	QL-ELE-00-003	QL-ELE-00-002	QT-ELE-00-002	QL-ELE-00-021	QL-ELE-00-025	QL-ELE-00-024
GM Event 40-3-2 Roundup Ready Soybean (MON-04032-6)	1	2	2	2	2	2	2	2	0	0	0	2	1	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
GM Event A2704-12 Soybean (ACS-GM005-3)	0	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0
GM Event MON89788 Soybean (MON-89788-1)	1	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
GM Event 356043 Soybean (DP-356043-5)	0	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM Event 305423 Soybean (DP-305423-1)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM Event A5547-127 Soybean (ACS-GM006-4)	0	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0
GM Event CV127 Soybean (BPS-CV127-9)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM Event MON87701 Soybean (MON-87701-2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0
GM Event MON87769 Soybean (MON-87769-7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
GM Event MON87705 Soybean (MON-87705-6)	1	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
GM Event FG72 Soybean (MST-FG072-2)	0	0	0	0	0	0	0	0	0	0	0	2	1	2	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
GM Event MON87708 Soybean (MON-87708-9)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
GM Event DAS-68416-4 Soybean (DAS-68416-4)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0
GM Event DAS-44406-6 Soybean (DAS-44406-6)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0
GM Event SYHT0H2 Soybean	0	2	2	2	2	2	2	2	0	0	0	2	1	2	2	2	2	0	0	0	0	0	0	0	0	2	2	2	0
GM Event DAS-81419-2 Soybean (DAS-81419-2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0
GM Event MON 87751 Soybean (MON-87751-7)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0
GMB151 soybean (BCS-GM151-6)	0	1	2	2	2	2	1	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

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