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Analytes and Related PCR Primers Used for GMO Detection and Quantification

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Table of contents

Index of figures

Index of tables

Executive summary

This document provides the first general overview on PCR primers used by Member State control laboratories for the detection, identification and quantification of GMOs. The data were collected from a survey launched in 2005.

The survey aimed at reviewing the PCR primers used by members of the European Network of GMO Laboratories (ENGL) for control purposes and at gathering analytical details on the corresponding PCR procedures. It further aimed to provide a catalogue of DNA sequences as a basis for the future development of plasmid standards.

Participants to the survey were specifically requested to provide information on the genetic targets and if relevant, the GM event for which the primers were designed, to include the sequences of primers and the purpose (qualitative/quantitative and screening/identification analysis), type (i.e. single, multiplex, competitive, double-competitive, simplex real-time or multiplex real-time) and specificity (gene-specificity, construct specificity or event-specificity) of the corresponding PCR assay. They were further requested to comment on and reference their data.

As a quality and normalisation step, the collated information was checked against the corresponding data published in a GMOs method database (<http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm), in the literature or against the data from the Community Reference Laboratory for GM Food and Feed (CRL-GMFF) web site (<http://gmo-crl.jrc.it).

The results of the survey have been grouped in two sections according to the scope of the PCR analysis, namely GMO-specific application, covering primers that target transgene sequences, and taxon-specific application, covering primers that target endogenous genes. Results have been further grouped according to the purpose and specificity of the PCR assay.

The data obtained from the survey was first analysed to verify representativeness of the results and identify general trends.

The analysis revealed that the data set was representative since 55% of the ENGL laboratories participated. Furthermore, the participants were geographically widely distributed across the Member States.

The analysis also evaluated general trends such as the frequency by which published and/or validated PCR primers were used by member laboratories and the frequency by which GM events or genetic elements were targeted in the PCR assays.

The general overview revealed that 83% of the primers used by laboratories were published in peer-reviewed articles. Custom-designed primers or commercially available kits were rarely reported. The survey data further indicated that 44% of primers were used for detecting common transgenic elements and endogenous genes while the other primers were predominantly employed for identification of authorised GMOs. The general overview also revealed that 46% of reported primers had been tested in collaborative studies.

Further data analyses were performed on the sets of primers defined by the participating laboratories for a particular scope, purpose and specificity of the PCR assay.

Strikingly, these analyses revealed a great variability of primers selection for GMOs control purposes. In general, however, a single primer pair was reported by more than 30% of the ENGL members while there was a wide distribution of primer utilisation in the remaining laboratories. The primer pairs most commonly employed were construct-specific, while for quantitative identification, ENGL laboratories tended to use primers that were event-specific. In about 30% of cases, primers designed for quantitative testing were also utilised for qualitative analysis.
Primer pairs were designed predominantly for identification of GTS 40-3-2 and Event 176 which were the first GMOs approved for food use in the EU. For new transgenic events most laboratories were using primers from methods validated by the CRL-GMFF. This tendency is expected to become the dominant choice in the future.

For some GM events authorised in the EU (i.e. GT73, Falcon GS/40/90pHoe6/Ac, RF3, and MS8) no validated primers were available. Also, not all GM events that were authorised in the EU have had primers reported for corresponding detection/quantification at the time of the survey.

For identification of crops as ingredient, the results indicated that a large number of taxon-specific primers were targeting maize-specific genes. A second group of primers was specific for soybean with *lectin* as the only targeted gene. Taxon-specific primers were also reported for detection of canola, tomato, potato, wheat, cotton or chloroplast-specific sequences. In general, few of the reported taxon-specific primers have been subjected to ring-trial.

This survey has provided a significant insight into the analytical strategies exploited by the ENGL laboratories for detection, identification and quantification of GMOs. It has shown that primer pairs used in the laboratories for the same final purpose are quite diverse and commutable, even if some of the primer pairs are used more frequently than others.

Quality control of the data collected in the survey highlighted a problem of nomenclature for primers. Indeed, the same oligonucletide sequences could be reported under different formats and names by different ENGL members. The use of a common nomenclature for primers is thus a need.

In addition and line with its institutional mandate, the ENGL should drive harmonisation in the adoption of scientific and technical approaches for GMO analyses and consequently provide recommendations for primers utilisation. The lack of performance criteria about the specificity of qualitative or quantitative amplicons (the target DNA sequence for PCR assays) and methods exploiting them is certainly a priority to address.

Provision of reference amplicons and application of performance criteria to primers and probe utilisation should be conceived as an initial step in promoting coordination, standardisation and harmonisation in Member States.

1 Introduction

The practice of the last years has shown a coherent investment of all ENGL laboratories in PCR-based technologies for detection, identification and quantification of GMOs. The novelty of the analytical domain combined with the lack of available reference methods and compelling legal obligations for delivering results have favoured the adoption of different strategies for the analysis of GMOs. This has contributed on one side to the development of a wide range of detection methods for a multitude of purposes and, inevitably on the other side, to the lack of methodological coherence.
One of the statutory objectives of the European Network of GMO Laboratories (ENGL) is to promote coordination, standardization and harmonization in the adoption of scientific and technical approaches for GMO analysis. As a first step toward harmonization, the ENGL decided in June 2004 a survey on the PCR primers used by ENGL laboratories for the detection, identification and quantification of GMOs.

2 Survey

The survey was launched in January 2005 on a restricted website. It aimed at:

- reviewing the PCR primers used by ENGL members for control purposes
- gathering analytical details on the corresponding PCR procedures
- providing a catalog of DNA sequences as a basis for the future development of plasmid standards.

The ENGL members were granted password protected access to a restricted website and were provided with detailed guidelines for entering the data. A deadline of three weeks was requested for responding to the inquiry.
For those institutions encountering browsing and/or firewall problems, a second survey was launched in March 2005 using a simple Excel file. Information had to be provided within a two weeks period. All the data were stored in a relational database.

2.1 Organization of the questionnaire

Each record of the database was entitled with the appropriate administrative information such as the ENGL Lab Code, the corresponding institution, the contact person name and e-mail address.
The questionnaire itself comprised four separate sections covering transgene- or taxon-, specific genetic targets and the corresponding public- or custom-, designed primer pairs.
The following elements were asked to the institution:

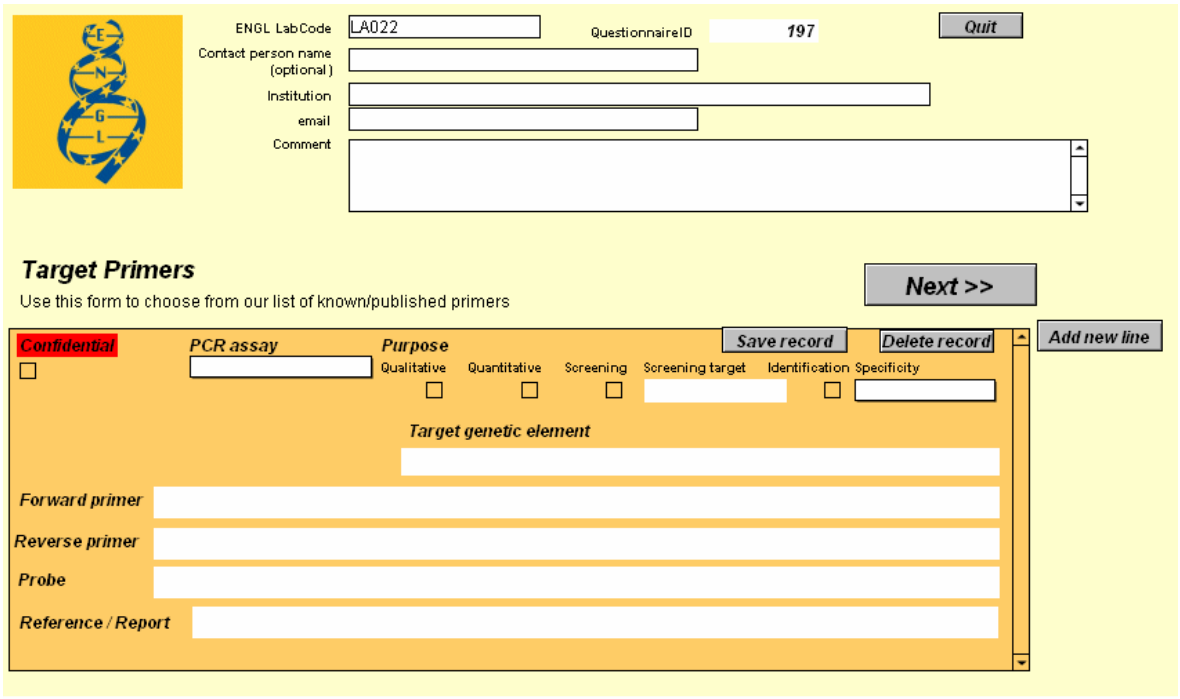
- target genetic element and, if relevant, the GM event
- corresponding sequences of primer pairs
- specificity (i.e. gene-specificity, construct-specificity or event-specificity) and purpose (i.e., qualitative, quantitative, screening, or identification purpose) of the PCR analysis
- type of PCR assay (i.e. single, multiplex, competitive, double-competitive, simplex real-time, multiplex real-time)
- references
- comments

- confidentiality status of data reported.

The four separate sections of the questionnaire are illustrated by the next four figures:

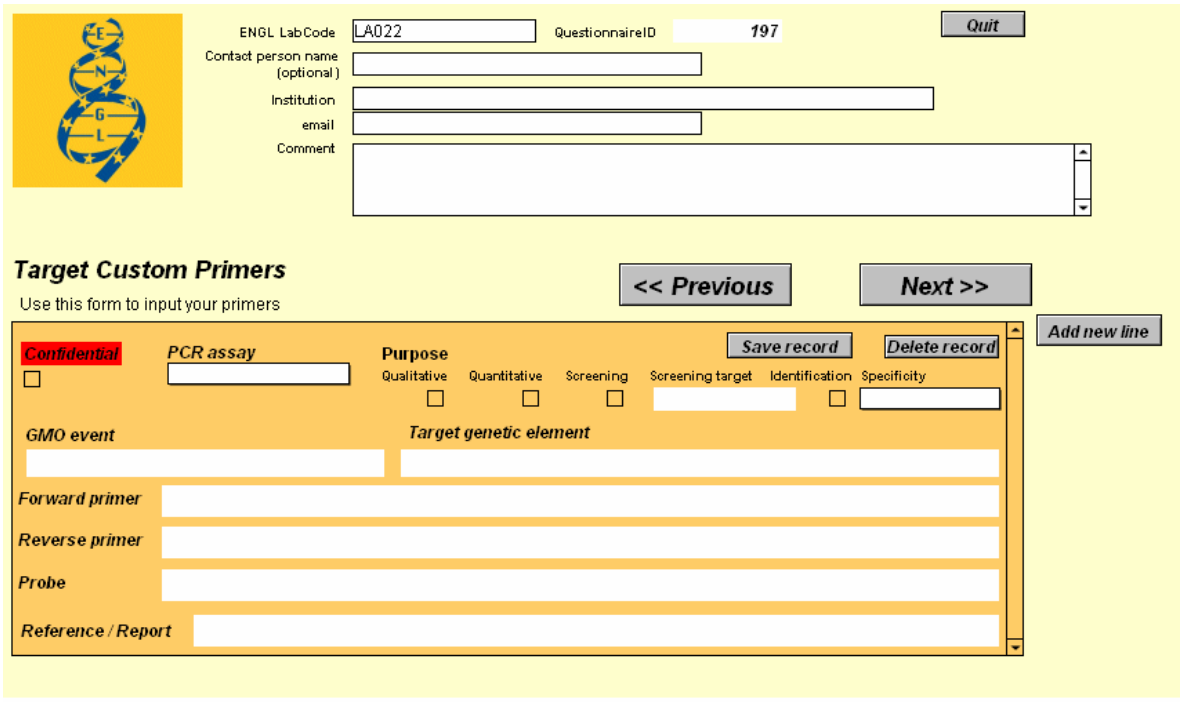
1. The first section called **Target Primers** is reproduced in Figure 1. It was designed to include transgene-specific primers that were publicly available. The sequences were mainly derived from the GMO methods database published on the IHCP-B&GMOs Unit web site (<http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm) and were provided as a popup list in the corresponding field.

Figure 1 Target primers section of the questionnaire



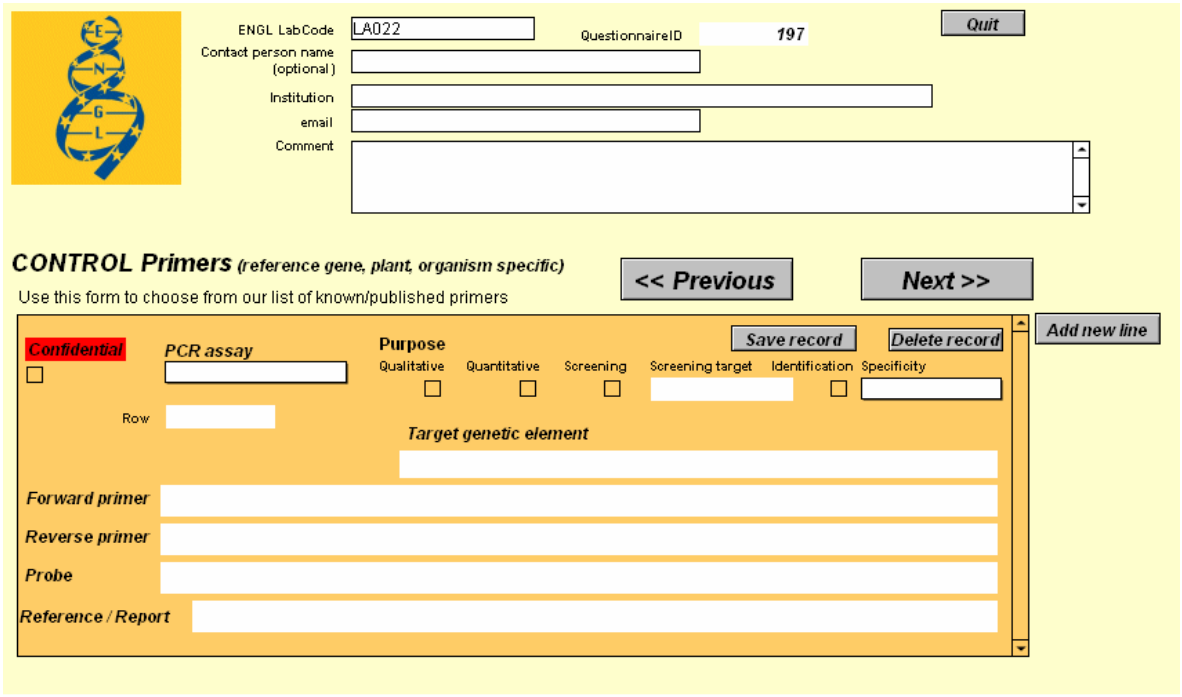
2. The second section called **Target Custom Primers** is reproduced in Figure 2. It was intended to include transgene-specific primer pairs that were custom-designed by ENGL laboratories. The ENGL members were asked to manually enter the primers and probe sequences in the corresponding fields.

Figure 2 Target custom primers section of the questionnaire



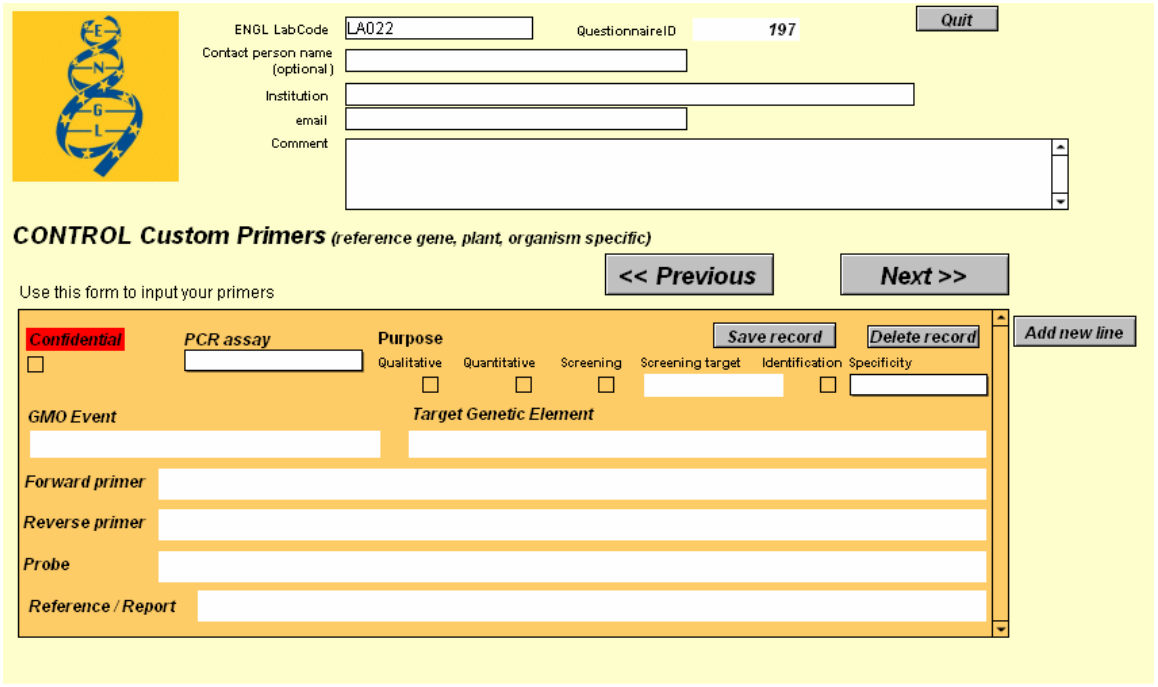
3. The third section called **Control Primers** is reproduced in Figure 3. It was designed to include primers and probes specific for endogenous DNA sequences that were publicly available. The sequences were mainly derived from the GMO methods database published on the IHCP-B&GMOs Unit web site (<http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm) and were provided as a popup list in the corresponding field.

Figure 3 Control primers section of the questionnaire



4. The fourth section called **Control Custom Primers** is reproduced in Figure 4. It was intended to include primers and probes specific for endogenous DNA sequences that were custom-designed by ENGL laboratories. The ENGL members were asked to manually enter the primer and probe sequences in the corresponding fields.

Figure 4 Control custom primers section of the questionnaire



3 Quality control of the data

The data collected were exported for further input, editing, and normalisation. As a quality control step, the collated information was checked against the corresponding data published in the GMOs methods database (http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm), in literature or on the CRL web site (http://gmo-crl.jrc.it). For the sections covering public domein primers, inconsistencies were corrected and missing data was inserted. When information on custom-made primers was incomplete, searches in the Internet were performed with the corresponding nucleotide sequences. If the search was positive, the data on transgenic events or genetic targets was inserted accordingly and included in the analysis with a non confidential status.
Unpublished confidential sequences were not included in the final report.
Nomenclature and formats of sequences were further normalized to allow comparison and analysis according to a set of descriptive statistics. The information not deriving from the survey and regarding the testing of the primers in collaborative studies or their inclusion in ISO standards was also inserted in the report. Their sources were the GMOs methods database (http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm), the CRL web site (http://gmo-crl.jrc.it) and the CEN standard documents (prEN ISO 21569 and prEN ISO 21570). Regulatory data on the status of authorisation of the related GMOs were collected from the Commission web site (http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm). Partial or lacking responses about the corresponding primers sequences were excluded of the analysis.

4 Scope and organization of the Report

Scope of this report is to review the types of primers used by ENGL laboratories in the period January - March 2005. In particular, it aims at identifying the genetic targets for which the primers were designed, the purpose (qualitative/quantitative and screening/identification analysis) and specificity of the corresponding PCR assays.
The report also aims at evaluating the frequency by which published and/or validated PCR primers are used by member laboratories for detection, identification and quantification of GMOs.

The inquiry gathered a very large amount of interconnected data. Therefore and to streamline the consultation of the report, the results of the survey have been grouped in two sections according to the scope of the PCR analysis namely GMO-specific, and Taxon-specific applications).

- The GMO section covers primers that are specific for transgene sequences. The data is derived from the “Target Primers” and “Target Custom Primers” sections of the questionnaire.

- The Taxon section covers primers that are specific for endogenous genes. The data is derived from the “Control Primers” and “Control Custom Primers” sections of the questionnaire.

The results of the survey have been further grouped according to the purpose of the PCR assay (qualitative/quantitative and screening/identification analysis) and its level of specificity.

The results are therefore reported as follows:

GMO-specific section

Qualitative analysis

- Qualitative screening
- Qualitative identification
- Qualitative event-specific identification

Quantitative analysis

- Quantitative screening
- Quantitative identification
- Quantitative event-specific identification

Taxon-specific section

- Qualitative analysis
- Quantitative analysis

For each of these analytical groups' two types of data analysis were generally performed:

- The first type of data analysis aimed at identifying the transgenic events to which the primers were mainly targeted and their frequency of use by ENGL members. Also it aimed at verifying if primers used by the laboratories had been tested in collaborative studies.

- The second type of data analysis aimed at identifying in addition the genetic elements to which the primers were targeted.

These analyses are generally reported in two separate tables. For screening and taxon-specific analytical groups however, the results are reported in one unique table were the target genetic elements and/or the species-specific genes are correspondently identified.
In each table the primer pairs are listed in decreasing order of percentage of use as reported by the ENGL laboratories. Depending on the table, primer pairs may be subdivided by the GM event, the screening genetic target, the species and the taxon-specific gene for which they were designed.

5 Results

The data obtained from the survey were first analysed to verify representativeness of the results and identify general trends. Further data analyses were next performed on the sets of primers defined by the participating laboratories for a particular scope, purpose and specificity of the PCR assay.

5.1 Representativeness of the data

The first step in the analysis of the data was to verify the representativeness of the survey for the entire ENGL network.
As illustrated in Table 1, the analysis revealed that the data set was representative since 55% of the ENGL laboratories participated to the enquiry. Furthermore, the participants were geographically distributed across the Member States as shown in

Table 2.

5.2 General trends in the uses of defined primers

The second step in the analysis of the data was aimed at evaluating general trends in primers utilisation. Particularly, it was useful to know whether the primers used by the laboratories were published, custom-designed or commercially available. Furthermore, a catalog of the GM events targeted by the set of primers could also be derived from the survey.
Equally important to know was the status of validation of the primer pairs used by the participants, in terms of collaborative studies.

The results of these analyses are illustrated in Table 3 and Table 4.

Table 3 provides the percentages of reported primers that were published, commercially available or custom-designed.
The results are expressed as "number of primers occurrences", which is equivalent to the number of times primer pairs were reported in the specified category. The "total" refers to the total number of reported primer pairs.

Table 3 provides a rough estimate of the data collected in the survey and but reveals that i) 83% of primers used by laboratories were published in peer-reviewed articles and ii) custom-designed primers or commercially available kits were rarely reported.

Table 4 provides an overall analysis on the transgenic events targeted by the primers and on the use of PCR assays that have undergone ring-trial testing. The events are listed in decreasing order of use of the corresponding primers. GMOs that are authorized for different uses in the EU (as of 20th of September) are highlighted in bold.
The results are expressed as "number of primers occurrences", which is equivalent to the number of times primer pairs were reported for identification of the corresponding GMO.
Primers that were used for detection of transgenic elements or endogenous genes are listed in a separate line. The "total" refers to the total number of reported primer pairs.

Table 4 indicates that 44% of the primers were used for detecting common transgenic elements and endogenous genes. The other primers were predominantly employed for identification of authorised GMOs.
These were mainly GTS 40-3-2 (8%), Event 176 (8%), MON810 (7%), Bt11 (7%), T25 (5%), GA21 (4%) and NK603 (2%).
Only a small proportion of primers was used for identification of GMOs that were not authorized in the EU, mainly CBH-351 (1%), tomato Nema 282F (0.7%) and tomato FLavrSavr (0.4%).

The table 4 also provides a rough estimate on the frequency of primers tested in ring-trials (% of Primers Ring-Trial Tested). The percentages refer to the ratio of reported primers that have been tested in ring trials versus the total numbers of primers reported for identification of the same GM event. These percentages do not distinguish if the reported primers had been validated for qualitative or quantitative purposes and may therefore be roughly overestimated.

In practice, 46% of reported primers have been tested in collaborative studies. In particular, approximately half of the primers used for detection of GTS 40-3-2, Event 176, Bt11, T25, and GA21 have been validated. Notably, between 80% and 100% of the primers designed for identification of NK603, MON863, and Tomato Nema 282F have been included in a collaborative-trial.
Conversely, none of the primers used for detection of the other authorized GMOs, namely GT73, Falcon GS/40/90pHoe6/Ac, Cotton1445, Cotton 531, MS8, RF3 and TC 1507 have been validated in collaborative trials. However, this does not mean that such primer pairs are not valid since they are usually published after in house validation.

The third step in the analysis of the data aimed at detailing further the genetic targets for which the primers were designed and the scope, purpose and specificity of the corresponding PCR assays.
The next results of the survey have therefore been grouped according to the scope of the PCR analysis (GMO-specific and Taxon-specific applications), purpose of the method (qualitative/quantitative, screening/identification) and its specificity (event-specific identification). Results of these analyses are reported in the sections below.

5.3 Results of the survey concerning the primer pairs targeting genetic modifications in GMOs

This section concerns the primer pairs that are specific for transgenic sequences.
It addresses qualitative and quantitative analyses that are further sub-classified in screening, identification and event-specific PCR assays.

5.3.1 Qualitative analyses

5.3.1.1 Qualitative screening

This section covers primer pairs that are used for qualitative screening of common transgenic elements.
In table five the survey reveals that the primer pairs can be subdivided according to their target genetic element (Target GE) and listed in decreasing value of use by participating laboratories.
In the table, “total” refers to the total number of primer pairs received for the qualitative screening section.
The frequency per analyte (Fpa) indicates the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative screening analyses.
The frequency within the analyte (Fwa) refers instead to the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for detection of the same genetic element.

The data in Table 5 indicate that 52% of screening primer pairs were targeting the CaMV 35S promoter, 35% the *nos* terminator and 12% the *nptII* gene.
The participating laboratories designed a great variety of primer pairs for the same genetic target; i.e. twelve and four different primer pairs respectively for detection of the CaMV 35S promoter and the *nos* terminator. However, the first two set of primer pairs covered 57% and 83% of participant’s choices while the remaining primer pairs were reported by a small number of laboratories. However such distribution may just reflect the historical development of the primers without any inference on their commutability.

Table 5 further specifies whether a primer pair has been tested in a ring-trial ("+") and/or it has been included in an ISO standard (prEN-21569. 2005) (ISO) (signs respectively in the column "Primers Ring-Trial Tested"). The numeric values listed in the same column, and in correspondence of the genetic elements, refer to the number of reported primer pairs that have been submitted to ring-trial evaluation. The survey revealed that 67% of primer pairs reported for screening purposes had been tested in collaborative studies.

5.3.1.2 Qualitative identification

This section covers assays for qualitative identification of transgenic events. It includes primer pairs targeting event-specific and construct-specific amplicons, or gene-specific amplicons which can identify more that one transgenic event. In the last case, they are reported together in the table under the column "GMO".
The results of the analyses are represented in Table 6 and Table 7. In the tables the transgenic events that are authorised in the EU for different intended uses are highlighted in yellow. The target genetic elements for event specific primer pairs are listed with the format "5'plant genome / insert" and "3' insert/ plant genome". When the target sequences are identified the word "insert" is replaced by the name of the genetic element.

In Table 6, primer pairs are classified according to their target GMO and are sorted in the decreasing order of use (N. of Primers Occurrences). The "total" indicates the total number of primer pairs received for the qualitative identification section. The frequency per analyte (Fpa) refers to the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative identification analyses. The frequency within the analyte (Fwa) indicates instead the ratio between the numbers of times the specific primer pair was reported and the total number of primer pairs received for identification of the same transgenic event.

Table 6 gathers the primer pairs that were predominantly designed for the identification of Event 176 (17%), GTS 40-3-2 (15%), MON810 (15%), T25 (12%), Bt11 (12%), GA21 (7%), CBH-351 (3%) and NK603 (3%). A variety of primer pairs was reported by the participants for the same transgenic event i.e. fifteen and twelve different primer pairs respectively for identification of Event 176 and GTS 40-3-2. However, a single set of primer pairs was reported by approximately 30% of ENGL members while the remaining primer pairs were reported by a small number of laboratories. Again, this might just reflect the historical non coordinated development of primers pairs without inference about their commutability.

Table 6 further informs that 30%-50% of reported primer pairs had been tested in collaborative studies. However, no trial reports were available for the primer pairs used for the identification of the authorised events GA21, NK603, MON863 GT73, Falcon GS/40/90pHoe6/Ac, Cotton, 1445 and Cotton 531. However all these primers were validated in house validated without assumption made in this report on their appropriate trueness and repeatability.

After analysis of the occurrence of use and status of validation, questions have been asked related to the choice of genetic elements targeted for the identification of GMO events.

Table 7 identifies the genetic elements that were targeted for identification of the transgenic events. Primer pairs are first sorted according to the GMO event concerned and next to the targeted genetic elements. The primer pairs are listed in the decreasing order of use by the participant laboratories (N. of Primers Occur.).

The data reveals -as expected- that several analytes were chosen in various genetic elements to target the same GMO event. Particularly, seven and six different genetic elements were targeted for identification of Bt176 and GTS 40-3-2 respectively, the two first commercial GMOs having been placed on the European Market..
In these examples, the primer pairs most commonly designed were construct-specific. With a pretention of identification of Bt 176 however, a substantial number of laboratories was exploiting primer pairs targeting the cry1A (b) gene alone, a genetic element that can only be considered as indicative of a family of different GMOs events in various crops. However, BT176 was the only GM Cry1A-containing maize placed on the EU market at the time of primer development, which made them historically event specific in those years. Today, Bt176 is redrawn of the market and replaced by similar GMOs for which CRL-GMFF validated methods are available.
Finally, about 30% of the reported primer airs were defined as event-specific.

AS a general observation, there was an available report of collaborative trial for the most frequently primer pairs used by ENGL participants.

5.3.1.3 Qualitative event-specific identification

This section concerns primers targeting amplicons chosen at the junction region between the host genome and the transgenic construct, the so-called “edge-fragment”, and that are used for qualitative event-specific identification of GMOs. The results of the survey are organized in Table 8 and Table 9.

In these tables, the GMO events authorised in the EU for different intended purposes are highlighted in yellow.
The target genetic elements of event-specific primer pairs are listed with the format “5’plant genome / insert” and “3’ insert/ plant genome”. When the transgenic target sequences are identified the word “insert” is replaced by the name of the genetic element.

In Table 8, primer pairs are subdivided according to their target GMO and are listed in decreasing order of use (N. of Primers Occur.). The “total” refers to the total number of primer pairs received for the qualitative event-specific section. The frequency per analyte (Fpa) indicates the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative event-specific analyses. The frequency within the analyte (Fwa) refers to the ratio between the numbers of times a specific primer pair was reported and the total number of primer pairs received for event-specific identification of the same transgenic event.

Table 8 indicates that event-specific primer pairs were predominantly employed for identification of MON810 (28%), NK603 (11%), GTS 40-3-2 (9%), MON863 (9%), Bt11 (9%), T25 (9%), and CBH-351(7%). The number of primer pairs reported for event-specific identification was much lower than for screening or construct-specific analyses. Yet, it was confirmed the tendency where for the same transgenic event a single primer pair was used by more than 30% of the laboratories.
Table 8 reveals that event-specific primer pairs have been only validated for qualitative detection of MON810.

Table 9 identifies the genetic elements that were targeted for event-specific identification of the transgenic events. Primer pairs are sorted first by the transgenic event and then by the targeted genetic

elements. The primer pairs are listed in the decreasing order of use by the participant laboratories (N. of Primers Occur.).

5.3.2 Quantitative analyses

5.3.2.1 Quantitative screening

This section covers primer pairs that are used for quantitative screening of common transgenic elements. The results of the analysis are represented in Table 10.

The primer pairs are subdivided according to their target genetic element (Target GE) and listed by decreasing value of use. In the table, "total" refers to the total number of primer pairs received for the quantitative screening section. The frequency per analyte (Fpa) indicates the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative screening analyses. The frequency within the analyte (Fwa) refers instead to the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative detection of the same genetic element.

Table 10 shows that 73% and 27% of primer pairs were designed for detection of the CaMV 35S promoter and the *nos* terminator respectively. Moreover, the number of primer pairs employed for quantitative screening analyses was lower than for qualitative applications. One primer pair targeting the CaMV 35S promoter had been tested in collaborative studies. Such a primer pair was used by more than 30% of laboratories. Whether or not the corresponding amplicons are commutable or not in terms of specificity remains to be seen..

5.3.2.2 Quantitative identification

This section covers assays for quantitative identification of transgenic events. It includes primer pairs targeting event-specific, construct-specific amplicons and gene-specific amplicons which can identify more that one transgenic event. In that case they are reported together in the table under the column "GMO". The results of the analyses are represented in Table 11 and Table 12.

In these tables, the GMOs authorized in the EU for different intended uses are highlighted in yellow. The target genetic elements for event specific primer pairs are listed with the format "5'plant genome / insert" and "3' insert/ plant genome". When the transgenic target sequences are identified the word "insert" is replaced by the name of the genetic element.

In Table 11 primer pairs are subdivided according to their target GMO and are listed in decreasing order of use by the participant laboratories. The "total" refers to the total number of primer pairs received for the quantitative identification section. The frequency per analyte (Fpa) indicates the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative identification analyses. The frequency within the analyte (Fwa) refers instead to the ratio between the numbers of times the specific primer pair was reported and the total number of primer received for quantitative identification of the same transgenic event.

The data in Table 11 indicate that primer pairs were predominantly used for quantitative identification of GTS 40-3-2 (20%), Bt11 (18%), MON810 (17%), Event 176 (14%), GA21 (9%), T25 (7%) and NK603 (6%). A great variety of primer pairs was again used, although at a lower degree than for qualitative analyses. As previously seen, one single set of primer pairs was generally used by more than 30% of the laboratories. The majority of primer pairs was reported by one or few ENGL members.
Table 11 further shows that almost all reported primers had been validated for the quantitative identification of Bt11, GA21, NK603, and MON863. Whereas, a significant proportion of the primer pairs (36%-55%) have been tested in ring-trial for Event 176, MON810, GTS 40-3-2 and T25. For some GM events authorised in EU (i.e. GT73, Falcon GS/40/90pHoe6/Ac, RF3 and MS8) no reports of collaborative validation of these primer pairs were available.

Table 12 identifies the genetic elements that were targeted for identification of the GMOs. In the table, primer pairs are classified first by the GMO event and next by the targeted genetic elements. The primers are listed in decreasing order of use by the participant laboratories (N. of Primers Occur.).

The data reveals as expected that several analytes were chosen in various genetic elements to target the same GMO event Indeed, seven and six different combinations of genetic elements were targeted respectively for identification of GTS 40-3-2 and Bt11. For quantitative identification analyses, ENGL laboratories tended to use event-specific primer pairs.
Table 12 indicates that even primer pairs frequently reported were only in house validated.

5.3.2.3 Quantitative event-specific identification

Next to 5.3.2.1, this section also covers edge fragments as the amplicon for PCR primers but assayed by quantitative PCR. The analysis of the data is represented in Table 13 and Table 14.

In these tables the GMO events authorised in the EU for different intended uses are highlighted in yellow. The target genetic elements for event specific primer pairs are listed with the format “5’plant genome / insert” and “3’ insert/ plant genome”. When the transgenic target sequence is identified the word “insert” is replaced by the name of the genetic element.

Table 13 indicates that event-specific primer pairs were predominantly used for identification of Bt11 (23%), MON810 (21%), GTS 40-3-2 (19%), NK603 (11%), GA21 (9%), T25 (5%), GT73 (4%) and MON863 (4%). The number of primer pairs reported for event-specific identification was lower than for the other quantitative analyses. As it has been noticed before however, a single set of primer pairs was used by 43%-100% of the laboratories.
Like elsewhere, certain primer pairs were validated in collaborative trials (NK603, GA21 and MON863) whereas as other were only in house validated (GTS 40-3-2, T25, GT73 and Event176).

Table 14 identifies the genetic elements that were targeted for identification of the GMO. In the table primer pairs are subdivided first by the transgenic event and then by the targeted genetic elements. The primer pairs are listed in decreasing order of use in quantitative PCR by the ENGL laboratories (N. of Primers Occur.).

5.3.2.4 Quantitative and qualitative identification

This section covers primer pairs that are used both for quantitative and qualitative identification. It includes event-specific, construct-specific and gene-specific primer pairs which can identify more that one GMO. In the latter case the corresponding transgenic events are reported together in the table under

the column “GMO”. The transgenic events that are authorized in the EU for different intended uses are highlighted in yellow.

Table 15 provides the frequency at which primer pairs used for quantitative PCR are also used in qualitative PCR. Primer pairs are subdivided according to their target GMO and are listed in decreasing order of use for quantitative purposes. The “total” refers to the total number of primer pairs received for the quantitative identification section. The quantitative frequency per analyte (quantFpa) indicates the ratio between the number of times the specific primer pair was reported for quantitative identification of the GMO and the total number of primer pairs received for the quantitative identification section. The qualitative frequency per analyte (qualFpa) refers to the ratio between the number of times the specific primer pair was reported for qualitative identification of the GMO and the total number of primer pairs received for the quantitative identification section.

Table 15 reveals that in approximately 30% of cases, primer pairs designed for quantitative determination were also employed for qualitative detection.

5.4 Results of the survey concerning the primers pairs targeting the crop as ingredient (taxon-specific amplicon in genes)

This section includes primer pairs targeting endogenous sequences also named “reference gene” and “taxon-specific genes” following the context. It is divided in qualitative and quantitative analyses.

5.4.1 Qualitative analyses

This section covers primer pairs use in qualitative PCR for the identification of endogenous sequences (i.e. taxon-specific genes). The results of the analysis are represented in Table 16.

Primer pairs are grouped by crop name and further subdivided by target taxon-specific gene or genetic element. Primers are listed in decreasing order of use by ENGL laboratories. In the table “total” refers to the total number of primer pairs received for the qualitative taxon-specific section. The frequency per analyte (Fpa) refers to the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative taxon-specific analyses. The frequency within the analyte (Fwa) refers to the ratio between the numbers of times a specific primer pair was reported and the total number of primer pairs received for qualitative identification of the same taxon.

Table 16 indicates that 41% of reported primer pairs were targeting maize-specific sequences, precisely the *zein* (13%), *ivr1* (11%), *adh1* (8%), *hmg* (6%) and *zSSIIb* (3%) genes. The second principle reported group of primer pairs (29%) was specific for soybean with *lectin* being the only targeted gene. Taxon-specific primer pairs were also designed for detection of tomato (8%) and canola (7%) while 9% of primer pairs were employed for detection of plant-specific sequences. Only one or two laboratories reported primer pairs targeting endogenous sequences in potato, papaya, wheat, cotton and eukaryotic-specific genes.
Few of reported primer pairs have been subjected to ring-trial evaluation.

5.4.2 Quantitative analyses

This section covers primer pairs that are used for quantitative determination of endogenous sequences. The results of the analysis are represented in Table 17.

The primer pairs are grouped by crop name and further subdivided by target taxon-specific gene or genetic element. Primer pairs are listed in decreasing order of use by ENGL laboratories. In the table "total" refers to the total number of primer pairs received for the quantitative taxon-specific section. The frequency per analyte (Fpa) indicates the ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative taxon-specific analyses. The frequency within the analyte (Fwa) refers to the ratio between the numbers of times the specific primer pair was reported and the total number of primer pairs received for quantitative identification of the same taxon.

As for qualitative analyses, a large number (56%) of primer pairs were targeting maize-specific sequences. The *adh1* gene was the principle target (24%) whiles the *zein* (9%), *zSSIIb* (8%), *ivr1* (8%) and *hmg* (8%) genes shared the remaining preferences. The second principle reported group of primer pairs (35%) was specific for soybean with *lectin* being the only targeted gene. Remaining primer pairs were employed for detection of canola (5%), tomato (1%), potato (1%) or plant-specific genes (1%).
Only few primer pairs specific for maize and soybean had been tested in collaborative studies.

5.5 GMOs authorized in EU for which identification primers were reported by ENGL laboratories

The GMOs authorised in EU under the different Regulations and EC Directives (as of 20th of September 2006) are listed in **Error! Reference source not found.**. In the same table are highlighted in yellow the transgenic events for which qualitative and quantitative identification analyses were reported by ENGL laboratories. Two additional columns further specify if the reported qualitative and quantitative assays were event-specific. The GMO listed were authorized as such or as derived products at the time of the survey (January-April 2005). In particular they were authorised under the Regulation (EC) N. 1829/2003, the Novel Food Regulation (EC) N. 258/97 or Directive N. 2001/18/EC.

The table reveals that at the time of the survey ENGL laboratories were using primer pairs for identification/quantification of fourteen out of the twenty-three GMOs listed.

6 Conclusions

The data colleted from the survey was first analysed to verify representativeness of the results and identify general trends.
The analysis revealed that the data set was representative since 55% of the ENGL members participated. Furthermore, the laboratories were geographically widely distributed across the Member States.
The analysis also evaluated general trends such as the frequency by which published and/or validated PCR primers were used by member laboratories and the frequency by which GM events or genetic elements were targeted in the PCR assays.

The general overview revealed that 83% of primer pairs employed by laboratories were published in peer-reviewed articles. Custom-designed primer pairs or commercially available kits were rarely reported. The survey data further indicated that 44% of primer pairs were used for detecting common transgenic elements and endogenous genes while the other primer pairs were predominantly employed for identification of authorised GMOs. The general overview also revealed that 46% of reported primer pairs had been tested in collaborative studies.

Further data analyses were performed on the sets of primer pairs defined by the participating laboratories for a particular scope, purpose and specificity of the PCR assay.

Strikingly, these analyses revealed a great variability of primer pairs selection for GMOs control purposes. In general, however, a single primer pair was reported by more than 30% of the ENGL members while there was a wide distribution of primer utilisation in the remaining laboratories.

The primer pairs most commonly employed were construct-specific, while for quantitative identification, ENGL laboratories tended to use primer pairs that were event-specific. In approximately 30% of cases primers designed for quantitative determination were also utilised for qualitative analyses.

Primer pairs were designed predominantly for identification of GTS 40-3-2 and Event 176 which were the first GMOs approved for food in the EU. For new transgenic events most laboratories were using primer pairs validated by the CRL-GMFF. This tendency is expected to continue in the future.

For identification of endogenous sequences the results indicated that a large number of primer pairs were targeting maize-specific genes. The second group of primers was specific for soybean with *lectin* being the only targeted gene. Taxon-specific primers were also reported for detection of canola, tomato, potato, wheat, cotton or chloroplast-specific sequences. In general, few of the endogenous primers used by the laboratories have been subjected to collaborative trial.

Quality control of the data collected in the survey highlighted a nomenclature problem for primer pairs. Indeed, the same oligonucletide sequences had different formats and names in different ENGL laboratories. Standardisation of primer pairs nomenclature should be promoted at the ENGL level. Criteria for assigning names to primer pairs could be the followings:

1. The name of the primer/probe should be the one given by the author in the first corresponding article published in literature.
2. If it is not reported in literature the name should correspond to the one given in the first protocol/validation report made publicly available.
3. Genetic targets of event-specific primers should be indicated with the format “5’plant genome / transgenic element name” and “3’ transgenic element name/plant genome”.

The survey has provided a significant insight into the analytical strategies exploited by the ENGL laboratories for detection, identification and quantification of GMOs. It has shown that primer pairs used in the laboratories for the same final purpose are quite variable and not harmonised, even if some of the primer pairs are used more frequently than others.

The ENGL could drive harmonisation in the adoption of scientific and technical approaches for GMO analyses and provide recommendations for primers uses. The lack of performance criteria for qualitative amplicons and methods exploiting them is certainly a weakness to address.

Provision of reference amplicons and application of performance criteria to primers and probes utilisation should be conceived as an initial step in promoting coordination, standardisation and harmonisation in Member States. This should also facilitate enhanced implementation of the modular approach proposed by Holst-Jensen, A. and Berdal, K.G. (Journal of AOAC International 87: 927-936 (2004)).

7 Acknowledgements

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8 Glossary

Definitions used in this report:

Construct-specific primers: primers that cover a junction region between two genetic elements of the transgenic construct
Control primers (in the questionnaire): primers specific for the crop-specific endogenous DNA sequences.
Control custom primers (in the questionnaire): primers specific for endogenous DNA sequences that were custom-designed by ENGL laboratories.
CRL-GMFF: Community Reference Laboratory for GM Food and Feed as instituted by Regulation (EC) 1829/2003
Event-specific primers or edge fragment primers: primers that cover a junction region between the host genome and the transgenic construct.
Frequency of the analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs reported in the survey or the specific analytical section
Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs reported for the specific analysis of the same transgenic event, genetic element or taxon.
Gene-specific primers: primers that cover a region within one coding genetic element of a trans-gene.
Identification primers: primers that are used for the identification of a GMO. They can be gene-specific, construct-specific or event- specific.
Ring trial or collaborative trial: a study in which several laboratories detect/ identify/ quantify an analyte in one or more "identical" portions of homogenous, stable materials under documented conditions, the results of which are compiled into a single document.

> **ISO ring-trial or ISO collaborative trial**: is a ring trial performed according to ISO 5725 norms or ISO/AOAC/IUPAC harmonised protocols. For qualitative studies it refers to a ring trial of a method that has been accepted and included in the CEN standards.

Screening primers: primers specific for genetic elements common to several GMOs such as promoters, terminators, or antibiotic resistance genes.
Target primers (in the questionnaire): transgene-specific primers that are publicly available.
Target custom primers (in the questionnaire): transgene-specific primers custom-designed by ENGL laboratories.
Taxon-specific primers: primers that cover sequences consistently present in the target taxon and absent in other taxa. There at least two types of target taxon-specific sequences:

- Variable number /multicopy sequences that can be used e.g. to assess the presence of nucleic acid from the target taxon
- Low copy/single copy sequences that can be used e.g. as a reference sequence to establish the background of target taxon genome equivalents in a quantitative analysis

Total: total number of reported primers re in the survey or the specific analytical section

Table 1 ENGL survey response

Category	ENGL Members	Frequency of Replay
Total ENGL members	74	
ENGL members that did not have a lab yet	3	
ENGL members that were invited to participate in the survey	71	100%
Respondents	39	55%
Non respondents	32	45%

Table 2 ENGL survey response by country

Country	ENGL Members	Respondents	Frequency of Replay per Country
AT	3	3	100%
BE	3	3	100%
CY	2	0	0%
CZ	5	3	60%
DE	5	1	20%
DK	3	2	67%
EE	2	1	50%
ES	3	0	0%
FI	3	1	33%
FR	4	4	100%
GR	3	0	0%
HU	3	2	67%
IE	2	0	0%
IT	3	3	100%
LT	1	1	100%
LU	1	1	100%
LV	1	1	100%
MT	1	0	0%
NL	4	3	75%
NO	2	1	50%
PL	5	2	40%
PT	3	1	33%
SE	2	2	100%
SI	2	2	100%
SK	3	1	33%
UK	5	1	20%

Table 3 Overall analyses on primers types

Primer Type	N. of Primers Occurrences[1]	% of Tot.[2]
Published	564	82,5%
Custom	58	8,5%
Commercial	48	7,0%
Not defined[3] by the respondent	14	2,0%
Total[4]	**684**	**100,0%**

[1] Number of times primer pairs were reported in the specified category.
[2] The ratio between the number of times primer pairs were reported in the specified category and the total number of reported primers.
[3] No relevant information was provided by the respondent.
[4] Total number of primer pairs reported in the survey.

Table 4 Overall analyses on target transgenic events and corresponding use of primers tested in ring-trials

Target	N. of Primers Occur.[1]	Fpa[2]	N. of Primers Ring-Trial Tested[3]	% of Primers Ring-Trial Tested[4]
Screening or taxon-specific primers	339	50,4%	148	43,7%
Primers for GMO identification				
GTS 40-3-2[5]	56	8,3%	26	46,4 %
Event 176	53	7,9%	24	45,3%
MON810	49	7,3%	19	38,8%
Bt11	46	6,8%	30	65,2%
T25	33	4,9%	18	54,5%
GA21	24	3,6%	14	58,3%
NK603	15	2,2%	12	80,0%
CBH-351	8	1,2%	2	25,0%
MON863	8	1,2%	7	87,5%
GT73 (RT73)	6	0,9%		0,0%
Tomato Nema 282F	5	0,7%	5	100,0%
Bt11 and/or T25	5	0,7%		0,0%
Falcon GS/40/90pHoe6/Ac	5	0,7%		0,0%
FlavrSavr	3	0,4%		0,0%
Event 176 and/or DBT418	2	0,3%		0,0%
Papaya 55-1 and/or Papaya 63-1	2	0,3%		0,0%
PHW 99-429	2	0,3%		0,0%
Tomato Zeneca	2	0,3%		0,0%
Bt10	1	0,1%		0,0%
B33-inv potato	1	0,1%	1	100,0%
Bt11 and/or MON810	1	0,1%		0,0%
Cotton 1445	1	0,1%		0,0%
Cotton 531	1	0,1%		0,0%
GA21 and/or NK603	1	0,1%		0,0%
MS8	1	0,1%		0,0%
RF3	1	0,1%		0,0%
TC 1507	1	0,1%		0,0%
Total[6]	**672**	**100,0%**	**306**	**45,5 %**

[1] Number of times a specific primer pair was reported in the survey.
[2] Frequency per analyte (Fpa): The ratio between the number of times primer pairs were reported for the specific purpose and the total number of primers received in the survey.
[3] Number of reported primers that have been tested in ring trials.
[4] Frequency of primers validation: Ratio of primers that have been tested in ring trials versus the total numbers of primers reported for the same GM event or type of analysis.
[5] GMOs authorized in the EU are highlighted in bold.
[6] Total number of primer pairs reported in the survey.

Table 5 GMO qualitative screening: analysis on "universal" target genetic elements and corresponding use of primers tested in ring-trials

Target GE	Primer Pair (Forward @ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
CaMV 35 S promoter		**53**	**52,0%**	**100,0%**	**30**
	5'-CCACGTCTTCAAAGCAAGTGG-3' @@@ 5'-TCCTCTCCAAATGAAATGAACTTCC-3'	18	17,6%	34,0%	ISO*
	5'-GCTCCTACAAATGCCATCA-3' @@@ 5'-GATAGTGGGATTGTGCGTCA-3'	12	11,8%	22,6%	ISO*
	5'-GACAGTGGTCCCAAAGATGG-3' @@@ 5'-GTCTTGCGAAGGATAGTGGG-3'	7	6,9%	13,2%	
	5'-CGTCTTCAAAGCAAGTGGATTG-3' @@@ 5'-TCTTGCGAAGGATAGTGGGATT-3'	3	2,9%	5,7%	
	5'-ATTGATGTGATATCTCCACTGACGT-3' @@@ 5'-CCTCTCCAAATGAAATGAACTTCCT-3'	3	2,9%	5,7%	
	5'-CCTACAAATGCCATCATTGCG-3' @@@ 5'-GGGTCTTGCGAAGGATAGTG-3'	3	2,9%	5,7%	
	5'-GCCTCTGCCGACAGTGGT-3' @@@ 5'-AAGACGTGGTTGGAACGTCTTC-3'	2	2,0%	3,8%	
	5'-CACCTACAAATGCCATCATTGC-3' @@@ 5'-GGGTCTTGCGAAGGATAGTG-3'	1	1,0%	1,9%	
	5'-CACTACAAATGCCATCATTGCGATA-3' @@@ 5'-CTTATATAGAGGAAGGGTCTTGCGA-3'	1	1,0%	1,9%	
	5'-GACATTGCGATAAAGGAAAGGC-3' @@@ 5'-GGGTCCATCTTTGGGACCA-3'	1	1,0%	1,9%	
	5'-GCCATCATTGCGATAAAGG-3' @@@ 5'-CCTCTCCAAATGAAATGAAC-3'	1	1,0%	1,9%	
	5'-GGGTCTTGCGAAGGATAGTG-3' @@@ 5'-CCTACAAATGCCATCATTGCG-3'	1	1,0%	1,9%	
nopaline synthase (nos) terminator		**36**	**35,3%**	**100,0%**	**30**
	5'-GCATGACGTTATTTATGAGATGGG-3' @@@ 5'-GACACCGCGCGCGATAATTTATCC-3'	18	17,6%	50,0%	ISO*
	5'-GAATCCTGTTGCCGGTCTTG-3' @@@ 5'-TTATCCTAGTTTGCGCGCTA-3'	12	11,8%	33,3%	+
	5'-GTCTTGCGATGATTATCATATAATTTCTG-3' @@@ 5'-CGCTATATTTTGTTTTCTATCGCGT-3'	4	3,9%	11,1%	
	5'-GTAATGCATGACGTTATTTATGAGA-3' @@@ 5'-TAATTTATCCTAGTTTGCGCGC-3'	2	2,0%	5,6%	
neomycin-phosphotransferase II (nptII) gene		**12**	**11,8%**	**100,0%**	**8**
	5'-GGATCTCCTGTCATCT-3' @@@ 5'-GATCATCCTGATCGAC-3'	4	3,9%	33,3%	+
	5'-CTCACCTTGCTCCTGCCGAGA-3' @@@ 5'-CGCCTTGAGCCTGGCGAACAG-3'	4	3,9%	33,3%	ISO*
	5'-CTCGACGTTGTCACTGAAG-3' @@@ 5'-GATGGATACTTTCTCGGCAG-3'	2	2,0%	16,7%	
	5'-ACCTGTCGGGTGCCCTGAATGAACTGC-3' @@@ 5'-GCCATGATGGATACTTTCTCGGCAGGAGC-3'	1	1,0%	8,3%	
	5'-GACAGGTCGGTCTTGACAAAAAG-3' @@@ 5'-GAACAAGATGGATTGCACGC-3'	1	1,0%	8,3%	
ampicillin (amp)gene		**1**	**1,0%**	**100,0%**	
	5'-CATTTCCGTGTCGCCCTTATTCC-3' @@@ 5'-GGCACCTATCTCAGCGATCTGTCTA-3'	1	1,0%	100,0%	
Total[5]		**102**	**100,0%**		**68**

[1] Number of times a primer pair was reported for qualitative screening analyses.
[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative screening analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative detection of the same genetic element (GE).
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial; ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).
[5] Total number of primer pairs received for qualitative screening analyses.

Table 6 GMO qualitative identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
Event 176[5]		**31**	**16,7%**	**100,0%**	**13**
	5'-CTCTCGCCGTTCATGTCCGT-3' @@@ 5'-GGTCAGGCTCAGGCTGATGT-3'	11	5,9%	35,5%	ISO*
	5'-GCACAATCCCACTATCCTTCGC-3' @@@ 5'-TCCGTCCACTCCTGCGGTTC-3'	2	1,1%	6,5%	
	5'-TGTTCACCAGCAGCAACCAG-3' @@@ 5'-ACTCCACTTTGTGCAGAACAGATCT-3'	2	1,1%	6,5%	
	5'-GTGGACAGCCTGGACGAGAT-3' @@@ 5'-TGCTGAAGCCACTGCGGAAC-3'	2	1,1%	6,5%	
	5'-GTAGCAGACACCCCTCTCCACA-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	2	1,1%	6,5%	
	5'-AGATTCTTCACTCCGATGCAGCCTA-3' @@@ 5'-GATGTTTGGGTTGTTGTCCAT-3'	2	1,1%	6,5%	
	5'-CCGCAGCCGATCCAACAATG-3' @@@ 5'-GCTGATGTCGATGGGGGTGTAG-3'	2	1,1%	6,5%	+
	5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	0,5%	3,2%	
	5'-ACCATCAACAGCCGCTACAACGACC-3' @@@ 5'-TGGGGAACAGGCTCACGATGTCCAG-3'	1	0,5%	3,2%	
	5'-AGATTCTTCACTCCGATGCAGCCTA-3' @@@ 5'-AGATCATCAATCCACTCTTGTGGTG-3'	1	0,5%	3,2%	
	5'-ATGGACAACAACCCCAACATC-3' @@@ 5'-GGTCTTCAGGCCGATCTGGTT-3'	1	0,5%	3,2%	
	5'-CCGCACCCTGAGCAGCAC-3' @@@ 5'-GGTGGCACGTTGTTGTTCTGA-3'	1	0,5%	3,2%	
	5'-CGGCCCCGAGTTCACCTT-3' @@@ 5'-CTGCTGGGGATGATGTTGTTG-3'	1	0,5%	3,2%	
	5'-GCAGGAACCGCAGGAGTGGA-3' @@@ 5'-AGCCCGATGACAGCGACCAC-3'	1	0,5%	3,2%	
	5'-TGGGGAACAGGCTCACGATGTCCAG-3' @@@ 5'-AACATCAACAGCCGCTACAACGACC-3'	1	0,5%	3,2%	
GTS 40-3-2		**28**	**15,1%**	**100,0%**	**14**
	5'-TGATGTGATATCTCCACTGACG-3' @@@ 5'-TGTATCCCTTGAGCCATGTTGT-3'	9	4,8%	32,1%	ISO*
	5'-ATCCCACTATCCTTCGCAAGA-3' @@@ 5'-TGGGGTTTATGGAAATTGGAA-3'	5	2,7%	17,9%	+
	5'-CCACTGACGTAAGGGATGACG-3' @@@ 5'-CATGAAGGACCGGTGGGAGAT-3'	3	1,6%	10,7%	
	5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	2	1,1%	7,1%	
	5'-CCTTTAGGATTTCAGCATCAGTGG-3' @@@ 5'-GACTTGTCGCCGGGAATG-3'	2	1,1%	7,1%	
	5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	1	0,5%	3,6%	
	5'-GCAAATCCTCTGGCCTTTCC-3' @@@ 5'-CTTGCCCGTATTGATGACGTC-3'	1	0,5%	3,6%	
	5'-GGCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	1	0,5%	3,6%	
	5'-CCACTATCCTTCGCAAGACCCTTCC-3' @@@ 5'-CTTCTGTGCTGTAGCCACTGATGC-3'	1	0,5%	3,6%	
	5'-CCCCAAGTTCCTAAATCTTCAAGT-3' @@@ 5'-TGCGGGCCGGCTGCTTGCA-3'	1	0,5%	3,6%	
	5'-GATAGTGGGATTGTGCGTCA-3' @@@ 5'-CCTTCAATTTAACCGATGC-3'	1	0,5%	3,6%	
	5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	1	0,5%	3,6%	
MON810		**28**	**15,1%**	**100,0%**	**10**
	5'-TCGAAGGACGAAGGACTCTAACG-3' @@@ 5'-TCCATCTTTGGGACCACTGTCG-3'	10	5,4%	35,7%	ISO*
	5'-AGTTTCCTTTTTGTTGCTCTCCT-3' @@@ 5'-GATGTTTGGGTTGTTGTCCAT-3'	3	1,6%	10,7%	
	5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	3	1,6%	10,7%	
	5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	2	1,1%	7,1%	
	5'-GATGCCTTCTCCCTAGTGTTGA-3' @@@ 5'-GGATGCACTCGTTGATGTTTG-3'	2	1,1%	7,1%	
	5'-GAGTTTCCTTTTTGTTGCTCTC-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	2	1,1%	7,1%	
	5'-TATCTCCACTGACGTAAGGGATGAC-3' @@@ 5'-GCATTCAGAGAAACGTGGCAGTAAC-3'	2	1,1%	7,1%	
	5'-GCTCTCCTTACCTCCTGATGG-3' @@@ 5'-GTTGATGTTTGGGTTGTTGTCC-3'	1	0,5%	3,6%	
	5'-ACTATCCTTCGCAAGACCCTTCCTC-3' @@@ 5'-GCATTCAGAGAAACGTGGCAGTAAC-3'	1	0,5%	3,6%	
	5'-TATCTCCACTGACGTAAGGGATGAC-3' @@@ 5'-TGCCCTATAACACCAACATGTGCTT-3'	1	0,5%	3,6%	
	5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	0,5%	3,6%	
Bt11		**22**	**11,8%**	**100,0%**	**9**
	5'-CTGGGAGGCCAAGGTATCTAAT-3' @@@ 5'-GCTGCTGTAGCTGGCCTAATCT-3'	9	4,8%	40,9%	ISO*
	5'-AAAAGACCACAACAAGCCGC-3' @@@ 5'-CAATGCGTTCTCCACCAAGTACT-3'	3	1,6%	13,6%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	3	1,6%	13,6%	
	5'-GGTACAGTACACACACATGTAT-3' @@@ 5'-GATGTTTGGGTTGTTGTCCAT-3'	2	1,1%	9,1%	
	5'-CCATTTTTCAGCTAGGAAGTTC-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	2	1,1%	9,1%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-AGCCAGTTACCTTCGGAAAA-3'	1	0,5%	4,5%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	0,5%	4,5%	
	5'-GCTGCTGTAGCTGGCCTAATCT-3' @@@ 5'-CTGGGAGGCCAAGGTATCTAAT-3'	1	0,5%	4,5%	
T25		**22**	**11,8%**	**100,0%**	**7**
	5'-ATGGTGGATGGCATGATGTTG-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCC-3'	7	3,8%	31,8%	ISO*
	5'-GCCAGTTAGGCCAGTTACCA-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCCT-3'	6	3,2%	27,3%	
	5'-CCTTCGCAAGACCCTTCCTCTATA-3' @@@ 5'-AGATCATCAATCCACTCTTGTGGTG-3'	4	2,2%	18,2%	

[1] Number of times a primer pair was reported for qualitative identification analyses.
[2] Frequency per analyte (Fpa.): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative identification analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative identification of the same transgenic event (GMO).
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).
[5] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 6 GMO qualitative identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
	5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	3	1,6%	13,6%	
	5'-ACAAGCGTGTCGTGCTCCAC-3' @@@ 5'-GACATGATACTCCTTCCACCG-3'	1	0,5%	4,5%	
	5'-CTGCGTTTGGTATGGCTTCATTCAG-3' @@@ 5'-CTTCAAAGCAAGTGGATGATGAGC-3'	1	0,5%	4,5%	
GA21		**12**	**6,5%**	**100,0%**	
	5'-ACGGTGGAAGAGTTCAATGTATG-3' @@@ 5'-TCTCCTTGATGGGCTGCA-3'	4	2,2%	33,3%	
	5'-GAAGCCTCGGCAACGTCA-3' @@@ 5'-ATCCGGTTGGAAAGCGACTT-3'	2	1,1%	16,7%	
	5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	2	1,1%	16,7%	
	5'-TGTGCTGAGCACTTTCGTCAA-3' @@@ 5'-CCGGCAACAGGATTCAATCT-3'	1	0,5%	8,3%	
	5'-AGAGCTGTAGTTGTTGGCTGTG-3' @@@ 5'-GCTGGGGGATCCACTAGTTCT-3'	1	0,5%	8,3%	
	5'-AAGAAGCTCGAGACGCTGTCGT-3' @@@ 5'-ATCCGGTTGGAAAGCGACTTGG-3'	1	0,5%	8,3%	
	5'-GACAAATGCAGCCTCGTGCGGAGT-3' @@@ 5'-TGCTGGGAAGGGTGGAGAAGTCGT-3'	1	0,5%	8,3%	
CBH-351		**6**	**3,2%**	**100,0%**	**2**
	5'-GCGGTGTCATCTATGTTACTAG-3' @@@ 5'-TCTGCCCATCGGAGTTATTTCC-3'	2	1,1%	33,3%	
	5'-CCTTCGCAAGACCCTTCCTCTATA-3' @@@ 5'-GTAGCTGTCGGTGTAGTCCTCGT-3'	2	1,1%	33,3%	+
	5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	0,5%	16,7%	
	5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	0,5%	16,7%	
NK603		**6**	**3,2%**	**100,0%**	
	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	6	3,2%	100,0%	
Tomato Nema 282F		**5**	**2,7%**	**100,0%**	**5**
	5'-GGATCCTTAGAAGCATCTAGT-3' @@@ 5'-CATCGCAAGACCGGCAACAG-3'	5	2,7%	100,0%	ISO*
MON863		**5**	**2,7%**	**100,0%**	
	5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	5	2,7%	100,0%	
Bt11 and/or T25		**4**	**2,2%**	**100,0%**	
	5'-CCTTCGCAAGACCCTTCCTCTATA-3' @@@ 5'-AGATCATCAATCCACTCTTGTGGTG-3'	2	1,1%	50,0%	
	5'-CAAGTGGATTGATGTGATATCTCC-3' @@@ 5'-TGGTTAACGATATCACAAACCG-3'	1	0,5%	25,0%	
	5'-GAAGGCTAGGAACGCTTACG-3' @@@ 5'-GCCAAAAACCAACATCATGC-3'	1	0,5%	25,0%	
GT73 (RT73)		**3**	**1,6%**	**100,0%**	
	5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	0,5%	33,3%	
	5'-CATATTGACCATCATATCCATTGC-3' @@@ 5'-CACATGTGGAATGTTCAATACC-3'	1	0,5%	33,3%	
	5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	1	0,5%	33,3%	
FlavrSavr		**3**	**1,6%**	**100,0%**	
	5'-CCACTGACGTAAGGGATGACG-3' @@@ 5'-AGGGGAAAGTGGAAAACCATC-3'	2	1,1%	66,7%	
	5'-AGTTCATTTCATTTGGAGAGGACA-3' @@@ 5'-GAAGATCTGCATGGACCTGAAAA-3'	1	0,5%	33,3%	
Papaya 55-1 and/or Papaya 63-1		**2**	**1,1%**	**100,0%**	
	5'-TTCATTTGGAGAGGACAGGGTAC-3' @@@ 5'-TCATTCTTGGACTGACGACGT-3'	2	1,1%	100,0%	
Falcon GS/40/90pHoe6/Ac		**2**	**1,1%**	**100,0%**	
	5'-CGCGGTTTGTGATATCGTTAAC-3' @@@ 5'-TCTTGCAACCTCTCTAGATCATCAA-3'	1	0,5%	50,0%	
	5'-CGCAACAAGTCACGGATATTC-3' @@@ 5'-ATGAGTGTGTTCACATGAGACC-3'	1	0,5%	50,0%	
Event 176 and/or DBT418		**2**	**1,1%**	**100,0%**	
	5'-GCAGGAACCGCAGGAGTGGA-3' @@@ 5'-AGCCCGATGACAGCGACCAC-3'	1	0,5%	50,0%	
	5'-GCACAATCCCACTATCCTTCGC-3' @@@ 5'-TCCGTCCACTCCTGCGGTTC-3'	1	0,5%	50,0%	
Bt10		**1**	**0,5%**	**100,0%**	
	5'-CACACAGGAGATTATTATAGGG-3' @@@ 5'-GGGAATAAGGGCGACACGG-3'	1	0,5%	100,0%	
Cotton 1445		**1**	**0,5%**	**100,0%**	
	5'-GGAGTAAGACGATTCAGATCAAACAC-3' @@@ 5'-ATCGACCTGCAGCCCAAGCT-3'	1	0,5%	100,0%	
GA21 and/or NK603		**1**	**0,5%**	**100,0%**	
	5'-CCTCAGCATTGTTCATCGGTAG-3' @@@ 5'-ATGCTGCTGCGTCTTCAGAG-3'	1	0,5%	100,0%	
B33-inv potato		**1**	**0,5%**	**100,0%**	**1**
	5'-CGCCGATGGTTTCTACAA-3' @@@ 5'-GGCGTCGGTTTCCACTAT-3'	1	0,5%	100,0%	1
Cotton 531		**1**	**0,5%**	**100,0%**	
	5'-TCCCATTCGATTTCTCACGT-3' @@@ 5'-AACCAATGCCACCCCACTGA-3'	1	0,5%	100,0%	

Table 6 GMO qualitative identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
Total[6]		202	100,0%		61

[6] Total number of primer pairs received for qualitative identification analyses.

Table 7 GMO qualitative identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
Event 176[3]			**31**	**13**
	CDPK pro / cryIA(b) gene		13	13
		5'-CTCTCGCCGTTCATGTCCGT-3' @@@ 5'-GGTCAGGCTCAGGCTGATGT-3'	11	ISO*
		5'-CCGCAGCCGATCCAACAATG-3' @@@ 5'-GCTGATGTCGATGGGGGTGTAG-3'	2	+
	cryIA(b) gene		7	
		5'-GTGGACAGCCTGGACGAGAT-3' @@@ 5'-TGCTGAAGCCACTGCGGAAC-3'	2	
		5'-TGGGGAACAGGCTCACGATGTCCAG-3' @@@ 5'-AACATCAACAGCCGCTACAACGACC-3'	1	
		5'-ACCATCAACAGCCGCTACAACGACC-3' @@@ 5'-TGGGGAACAGGCTCACGATGTCCAG-3'	1	
		5'-ATGGACAACAACCCCAACATC-3' @@@ 5'-GGTCTTCAGGCCGATCTGGTT-3'	1	
		5'-CCGCACCCTGAGCAGCAC-3' @@@ 5'-GGTGGCACGTTGTTGTTCTGA-3'	1	
		5'-CGGCCCCGAGTTCACCTT-3' @@@ 5'-CTGCTGGGGATGATGTTGTTG-3'	1	
	PEPC pro / cryIA(b) gene		5	
		5'-AGATTCTTCACTCCGATGCAGCCTA-3' @@@ 5'-GATGTTTGGGTTGTTGTCCAT-3'	2	
		5'-GTAGCAGACACCCCTCTCCACA-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	2	
		5'-AGATTCTTCACTCCGATGCAGCCTA-3' @@@ 5'-AGATCATCAATCCACTCTTGTGGTG-3'	1	
	CaMV 35S pro / bar gene		2	
		5'-GCACAATCCCACTATCCTTCGC-3' @@@ 5'-TCCGTCCACTCCTGCGGTTC-3'	2	
	cryIA(b) gene / PEPC IVS 9		2	
		5'-TGTTCACCAGCAGCAACCAG-3' @@@ 5'-ACTCCACTTTGTGCAGAACAGATCT-3'	2	
	3' truncated bar gene / maize genome		1	
		5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	
	bar gene		1	
		5'-GCAGGAACCGCAGGAGTGGA-3' @@@ 5'-AGCCCGATGACAGCGACCAC-3'	1	
GTS 40-3-2			**28**	**14**
	CaMV 35S pro / CTP		9	9
		5'-TGATGTGATATCTCCACTGACG-3' @@@ 5'-TGTATCCCTTGAGCCATGTTGT-3'	9	ISO*
	CaMV 35S pro / CP4 epsps gene		9	5
		5'-ATCCCACTATCCTTCGCAAGA-3' @@@ 5'-TGGGGTTTATGGAAATTGGAA-3'	5	+
		5'-CCACTGACGTAAGGGATGACG-3' @@@ 5'-CATGAAGGACCGGTGGGAGAT-3'	3	
		5'-CCACTATCCTTCGCAAGACCCTTCC-3' @@@ 5'-CTTCTGTGCTGTAGCCACTGATGC-3'	1	
	5' soybean genome / CaMV 35S pro		4	
		5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	2	
		5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	1	
		5'-GATAGTGGGATTGTGCGTCA-3' @@@ 5'-CCTTCAATTTAACCGATGC-3'	1	
	CTP / CP4 epsps gene		3	
		5'-CCTTTAGGATTTCAGCATCAGTGG-3' @@@ 5'-GACTTGTCGCCGGGAATG-3'	2	
		5'-CCCCAAGTTCCTAAATCTTCAAGT-3' @@@ 5'-TGCGGGCCGGCTGCTTGCA-3'	1	
	CP4 epsps gene		2	
		5'-GGCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	1	
		5'-GCAAATCCTCTGGCCTTTCC-3' @@@ 5'-CTTGCCCGTATTGATGACGTC-3'	1	
	3' nos ter / soybean genome		1	
		5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	1	
MON810			**28**	**10**
	5' maize genome / CaMV 35S pro		13	10
		5'-TCGAAGGACGAAGGACTCTAACG-3' @@@ 5'-TCCATCTTTGGGACCACTGTCG-3'	10	ISO*
		5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	2	
		5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	

[1] Number of times a primer pair was reported for qualitative identification analyses.
[2] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).
[3] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 7 GMO qualitative identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
	hsp 70 IVS1 / cryIA(b) gene		8	
		5'-AGTTTCCTTTTTGTTGCTCTCCT-3' @@@ 5'-GATGTTTGGGTTGTTGTCCAT-3'	3	
		5'-GAGTTTCCTTTTTGTTGCTCTC-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	2	
		5'-GATGCCTTCTCCCTAGTGTTGA-3' @@@ 5'-GGATGCACTCGTTGATGTTTG-3'	2	
		5'-GCTCTCCTTACCTCCTGATGG-3' @@@ 5'-GTTGATGTTTGGGTTGTTGTCC-3'	1	
	CaMV 35S pro / hsp 70 IVS1		4	
		5'-TATCTCCACTGACGTAAGGGATGAC-3' @@@ 5'-GCATTCAGAGAAACGTGGCAGTAAC-3'	2	
		5'-TATCTCCACTGACGTAAGGGATGAC-3' @@@ 5'-TGCCCTATAACACCAACATGTGCTT-3'	1	
		5'-ACTATCCTTCGCAAGACCCTTCCTC-3' @@@ 5'-GCATTCAGAGAAACGTGGCAGTAAC-3'	1	
	5' maize genome / insert		3	
		5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	3	
Bt11			**22**	**9**
	adh1-1S IVS2 / pat gene		10	9
		5'-CTGGGAGGCCAAGGTATCTAAT-3' @@@ 5'-GCTGCTGTAGCTGGCCTAATCT-3'	9	ISO*+
		5'-GCTGCTGTAGCTGGCCTAATCT-3' @@@ 5'-CTGGGAGGCCAAGGTATCTAAT-3'	1	
	adh1-1S IVS6 / cryIA(b) gene		7	
		5'-AAAAGACCACAACAAGCCGC-3' @@@ 5'-CAATGCGTTCTCCACCAAGTACT-3'	3	
		5'-GGTACAGTACACACACATGTAT-3' @@@ 5'-GATGTTTGGGTTGTTGTCCAT-3'	2	
		5'-CCATTTTTCAGCTAGGAAGTTC-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	2	
	3' pUC18 / pUC18-maize genome		3	
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	3	
	3' pUC18 / maize genome		1	
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	
	5' maize genome / insert		1	
		5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-AGCCAGTTACCTTCGGAAAA-3'	1	
T25			**22**	**7**
	pat gene / CaMV 35S ter		13	7
		5'-ATGGTGGATGGCATGATGTTG-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCC-3'	7	ISO*
		5'-GCCAGTTAGGCCAGTTACCCA-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCCT-3'	6	
	CaMV 35S pro / pat gene		4	
		5'-CCTTCGCAAGACCCTTCCTCTATA-3' @@@ 5'-AGATCATCAATCCACTCTTGTGGTG-3'	4	
	5' maize genome / CaMV 35S pro		3	
		5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	3	
	5' maize genome / insert		2	
		5'-ACAAGCGTGTCGTGCTCCAC-3' @@@ 5'-GACATGATACTCCTTCCACCG-3'	1	
		5'-CTGCGTTTGGTATGGCTTCATTCAG-3' @@@ 5'-CTTCAAAGCAAGTGGATGATGAGC-3'	1	
GA21			**12**	
	OTP / m-epsps gene		7	
		5'-ACGGTGGAAGAGTTCAATGTATG-3' @@@ 5'-TCTCCTTGATGGGCTGCA-3'	4	
		5'-GAAGCCTCGGCAACGTCA-3' @@@ 5'-ATCCGGTTGGAAAGCGACTT-3'	2	
		5'-AAGAAGCTCGAGACGCTGTCGT-3' @@@ 5'-ATCCGGTTGGAAAGCGACTTGG-3'	1	
	5' maize genome / insert		2	
		5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	2	
	rice actin pro / OTP		1	
		5'-GACAAATGCAGCCTCGTGCGGAGT-3' @@@ 5'-TGCTGGGAAGGGTGGAGAAGTCGT-3'	1	
	truncated m-epsps gene / truncated actin pro		1	
		5'-AGAGCTGTAGTTGTTGGCTGTG-3' @@@ 5'-GCTGGGGGATCCACTAGTTCT-3'	1	
	m-epsps gene / nos ter		1	
		5'-TGTGCTGAGCACTTTCGTCAA-3' @@@ 5'-CCGGCAACAGGATTCAATCT-3'	1	
CBH-351			**6**	**2**
	3' nos ter / maize genome		4	

Table 7 GMO qualitative identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
		5'-GCGGTGTCATCTATGTTACTAG-3' @@@ 5'-TCTGCCCATCGGAGTTATTTCC-3'	2	
		5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	
		5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	
	CaMV 35S pro / cry9C gene		2	2
		5'-CCTTCGCAAGACCCTTCCTCTATA-3' @@@ 5'-GTAGCTGTCGGTGTAGTCCTCGT-3'	2	+
NK603			**6**	
	3' insert / maize genome		6	
		5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	6	
Tomato Nema 282F			**5**	**5**
	PG gene / nos ter		5	5
		5'-GGATCCTTAGAAGCATCTAGT-3' @@@ 5'-CATCGCAAGACCGGCAACAG-3'	5	ISO*
MON863			**5**	
	5' maize genome / insert		5	
		5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	5	
Bt11 and/or T25			**4**	
	CaMV 35S pro / pat gene		3	
		5'-CCTTCGCAAGACCCTTCCTCTATA-3' @@@ 5'-AGATCATCAATCCACTCTTGTGGTG-3'	2	
		5'-CAAGTGGATTGATGTGATATCTCC-3' @@@ 5'-TGGTTAACGATATCACAAACCG-3'	1	
	pat gene		1	
		5'-GAAGGCTAGGAACGCTTACG-3' @@@ 5'-GCCAAAAACCAACATCATGC-3'	1	
GT73 (RT73)			**3**	
	3' insert / canola genome		1	
		5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	1	
	Ti-plasmid / canola genome		1	
		5'-CATATTGACCATCATATCCATTGC-3' @@@ 5'-CACATGTGGAATGTTCAATACC-3'	1	
	3' pTi15955 plasmid / canola genome		1	
		5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	
FlavrSavr			**3**	
	CaMV 35S pro / anti-PG gene		2	
		5'-CCACTGACGTAAGGGATGACG-3' @@@ 5'-AGGGGAAAGTGGAAAACCATC-3'	2	
	CaMV 35S pro / PG gene		1	
		5'-AGTTCATTTCATTTGGAGAGGACA-3' @@@ 5'-GAAGATCTGCATGGACCTGAAAA-3'	1	
Event 176 and/or DBT418			**2**	
	bar gene		1	
		5'-GCAGGAACCGCAGGAGTGGA-3' @@@ 5'-AGCCCGATGACAGCGACCAC-3'	1	
	CaMV 35S pro / bar gene		1	
		5'-GCACAATCCCACTATCCTTCGC-3' @@@ 5'-TCCGTCCACTCCTGCGGTTC-3'	1	
B33-inv potato			**1**	**1**
	hygromycin phosphotransferase (hph) gene		1	1
		5'-CGCCGATGGTTTCTACAA-3' @@@ 5'-GGCGTCGGTTTCCACTAT-3'	1	+
Falcon GS/40/90pHoe6/Ac			**1**	
	pat gene		1	
		5'-CGCGGTTTGTGATATCGTTAAC-3' @@@ 5'-TCTTGCAACCTCTCTAGATCATCAA-3'	1	
Cotton 531			**1**	
	5' cotton genome / insert		1	
		5'-TCCCATTCGATTTCTCACGT-3' @@@ 5'-AACCAATGCCACCCCACTGA-3'	1	
GA21 and/or NK603			**1**	
	rice actin pro / CTP2		1	
		5'-CCTCAGCATTGTTCATCGGTAG-3' @@@ 5'-ATGCTGCTGCGTCTTCAGAG-3'	1	
Cotton 1445			**1**	
	5' cotton genome / insert		1	

Table 7 GMO qualitative identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
		5'-GGAGTAAGACGATTCAGATCAAACAC-3' @@@ 5'-ATCGACCTGCAGCCCAAGCT-3'	1	
Total[4]			**182**	**61**

[4] Total number of primer pairs received for qualitative identification analyses.

Table 8 GMO qualitative event-specific identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
MON810[5]		**16**	**28,1%**	**100,0%**	**10**
	5'-TCGAAGGACGAAGGACTCTAACG-3' @@@ 5'-TCCATCTTTGGGACCACTGTCG-3'	10	17,5%	62,5%	ISO*
	5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	3	5,3%	18,8%	
	5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	2	3,5%	12,5%	
	5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	1,8%	6,3%	
NK603		**6**	**10,5%**	**100,0%**	
	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	6	10,5%	100,0%	
GTS 40-3-2		**5**	**8,8%**	**100,0%**	
	5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	2	3,5%	40,0%	
	5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	1	1,8%	20,0%	
	5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	1	1,8%	20,0%	
	5'-GATAGTGGGATTGTGCGTCA-3' @@@ 5'-CCTTCAATTTAACCGATGC-3'	1	1,8%	20,0%	
MON863		**5**	**8,8%**	**100,0%**	
	5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	5	8,8%	100,0%	
Bt11		**5**	**8,8%**	**100,0%**	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	3	5,3%	60,0%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-AGCCAGTTACCTTCGGAAAA-3'	1	1,8%	20,0%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	1,8%	20,0%	
T25		**5**	**8,8%**	**100,0%**	
	5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	3	5,3%	60,0%	
	5'-ACAAGCGTGTCGTGCTCCAC-3' @@@ 5'-GACATGATACTCCTTCCACCG-3'	1	1,8%	20,0%	
	5'-CTGCGTTTGGTATGGCTTCATTCAG-3' @@@ 5'-CTTCAAAGCAAGTGGATGATGAGC-3'	1	1,8%	20,0%	
CBH-351		**4**	**7,0%**	**100,0%**	
	5'-GCGGTGTCATCTATGTTACTAG-3' @@@ 5'-TCTGCCCATCGGAGTTATTTCC-3'	2	3,5%	50,0%	
	5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	1,8%	25,0%	
	5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	1,8%	25,0%	
GT73 (RT73)		**3**	**5,3%**	**100,0%**	
	5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	1,8%	33,3%	
	5'-CATATTGACCATCATATCCATTGC-3' @@@ 5'-CACATGTGGAATGTTCAATACC-3'	1	1,8%	33,3%	
	5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	1	1,8%	33,3%	
GA21		**2**	**3,5%**	**100,0%**	
	5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	2	3,5%	100,0%	
Papaya 55-1 and/or Papaya 63-1		**2**	**3,5%**	**100,0%**	
	5'-TTCATTTGGAGAGGACAGGGTAC-3' @@@ 5'-TCATTCTTGGACTGACGACGT-3'	2	3,5%	100,0%	
Event 176		**1**	**1,8%**	**100,0%**	
	5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	1,8%	100,0%	
Cotton 1445		**1**	**1,8%**	**100,0%**	
	5'-GGAGTAAGACGATTCAGATCAAACAC-3' @@@ 5'-ATCGACCTGCAGCCCAAGCT-3'	1	1,8%	100,0%	
Cotton 531		**1**	**1,8%**	**100,0%**	
	5'-TCCCATTCGATTTCTCACGT-3' @@@ 5'-AACCAATGCCACCCCACTGA-3'	1	1,8%	100,0%	
Bt10		**1**	**1,8%**	**100,0%**	
	5'-CACACAGGAGATTATTATAGGG-3' @@@ 5'-GGGAATAAGGGCGACACGG-3'	1	1,8%	100,0%	
Total[6]		**57**	**100,0%**		**10**

[1] Number of times a primer pair was reported for qualitative event-specific identification analyses.
[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative event-specific identification analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative event-specific identification of the same transgenic event (GMO).
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).
[5] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.
[6] Total number of primer pairs received for qualitative event-specific identification analyses.

Table 9 GMO qualitative event-specific identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
MON810[3]			16	10
	5' maize genome / CaMV 35S pro		13	10
		5'-TCGAAGGACGAAGGACTCTAACG-3' @@@ 5'-TCCATCTTTGGGACCACTGTCG-3'	10	ISO*
		5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	2	
		5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	
	5' maize genome / insert		3	
		5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	3	
NK603			6	
	3' insert / maize genome		6	
		5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	6	
GTS 40-3-2			5	
	5' soybean genome / CaMV 35S pro		4	
		5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	2	
		5'-GATAGTGGGATTGTGCGTCA-3' @@@ 5'-CCTTCAATTTAACCGATGC-3'	1	
		5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	1	
	3' nos ter / soybean genome		1	
		5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	1	
Bt11			5	
	3' pUC18 / pUC18-maize genome		3	
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	3	
	5' maize genome / insert		1	
		5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-AGCCAGTTACCTTCGGAAAA-3'	1	
	3' pUC18 / maize genome		1	
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	
T25			5	
	5' maize genome / CaMV 35S pro		3	
		5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	3	
	5' maize genome / insert		2	
		5'-ACAAGCGTGTCGTGCTCCAC-3' @@@ 5'-GACATGATACTCCTTCCACCG-3'	1	
		5'-CTGCGTTTGGTATGGCTTCATTCAG-3' @@@ 5'-CTTCAAAGCAAGTGGATGATGAGC-3'	1	
MON863			5	
	5' maize genome / insert		5	
		5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	5	
CBH-351			4	
	3' nos ter / maize genome		4	
		5'-GCGGTGTCATCTATGTTACTAG-3' @@@ 5'-TCTGCCCATCGGAGTTATTTCC-3'	2	
		5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	
		5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	
GT73 (RT73)			3	
	Ti-plasmid / canola genome		1	
		5'-CATATTGACCATCATATCCATTGC-3' @@@ 5'-CACATGTGGAATGTTCAATACC-3'	1	
	3' insert / canola genome		1	
		5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	1	
	3' pTi15955 plasmid / canola genome		1	
		5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	

[1] Number of times a primer pair was reported for qualitative event-specific identification analyses
[2] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).
[3] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 9 TGMO qualitative event-specific identification: Tanalysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
GA21			**2**	
	5' maize genome / insert		2	
		5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	2	
Event 176			**1**	
	3' truncated bar gene / maize genome		1	
		5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	
Cotton 1445			**1**	
	5' cotton genome / insert		1	
		5'-GGAGTAAGACGATTCAGATCAAACAC-3' @@@ 5'-ATCGACCTGCAGCCCAAGCT-3'	1	
Cotton 531			**1**	
	5' cotton genome / insert		1	
		5'-TCCCATTCGATTTCTCACGT-3' @@@ 5'-AACCAATGCCACCCCACTGA-3'	1	
Total[4]			**54**	**10**

[4] Total number of primer pairs received for qualitative event-specific identification analyses.

Table 10 GMO quantitative screening: analysis on "universal" target genetic elements and corresponding use of primers tested in ring-trials

Target GE	Primer Pair (Forward @@@ Reverse)	N. of Prim Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
CaMV 35 S promoter		**16**	**72,7%**	**100,0%**	**5**
	5'-GCCTCTGCCGACAGTGGT-3' @@@ 5'-AAGACGTGGTTGGAACGTCTTC-3'	5	22,7%	31,3%	ISO*
	5'-CGTCTTCAAAGCAAGTGGATTG-3' @@@ 5'-TCTTGCGAAGGATAGTGGGATT-3'	4	18,2%	25,0%	
	5'-ATTGATGTGATATCTCCACTGACGT-3' @@@ 5'-CCTCTCCAAATGAAATGAACTTCCT-3'	3	13,6%	18,8%	
	5'-GCTCCTACAAATGCCATCA-3' @@@ 5'-GGAACGTCTTCTTTTTCCACG-3'	1	4,5%	6,3%	
	5'-GACAGTGGTCCCAAAGATGG-3' @@@ 5'-GTCTTGCGAAGGATAGTGGG-3'	1	4,5%	6,3%	
	5'-GACATTGCGATAAAGGAAAGGC-3' @@@ 5'-GGGTCCATCTTTGGGACCA-3'	1	4,5%	6,3%	
	5'-CACGTCTTCAAAGCAAGTGGA-3' @@@ 5'-GTCTTGCGAAGGATAGTGGGA-3'	1	4,5%	6,3%	
nopaline synthase (nos) terminator		**6**	**27,3%**	**100,0%**	
	5'-GTCTTGCGATGATTATCATATAATTTCTG-3' @@@ 5'-CGCTATATTTTGTTTTCTATCGCGT-3'	4	18,2%	66,7%	
	5'-GTAATGCATGACGTTATTTATGAGA-3' @@@ 5'-TAATTTATCCTAGTTTGCGCGC-3'	2	9,1%	33,3%	
Total[5]		**22**	**100,0%**		**5**

[1] Number of times a primer pair was reported for quantitative screening analyses

[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative screening analyses.

[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative detection of the same genetic element (GE)

[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the trial followed criteria specified in ISO 5725-2.

[5] Total number of primer pairs received for quantitative screening analyses.

Table 11 GMO quantitative identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
GTS 40-3-2[5]		**28**	**20,1%**	**100,0%**	**10**
	5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	6	4,3%	21,4%	
	5'-GCCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	4	2,9%	14,3%	ISO*
	5'-CCTTTAGGATTTCAGCATCAGTGG-3' @@@ 5'-GACTTGTCGCCGGGAATG-3'	4	2,9%	14,3%	ISO*
	5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	4	2,9%	14,3%	
	5'-TGATGTGATATCTCCACTGACG-3' @@@ 5'-TGTATCCCTTGAGCCATGTTGT-3'	2	1,4%	7,1%	
	5'-CATTTGGAGAGGACACGCTGA-3' @@@ 5'-GAGCCATGTTGTTAATTTGTGCC-3'	2	1,4%	7,1%	ISO*
	5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	2	1,4%	7,1%	
	5'-CCGCGGAAAATAAACATAGGGAACCCGCGG-MR-HG-GAGCCACCTTCCTTTTCCATTT-3' @@@ 5'-ACCCTTCAATTTAACCGATGCT-3'	1	0,7%	3,6%	
	5'-GGCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	1	0,7%	3,6%	
	5'-CCTTTCCTTTATCGCAATGAT-3' @@@ 5'-GGTTAAAATAAACATAGGGAACCCA-3'	1	0,7%	3,6%	
	5'-GCAAATCCTCTGGCCTTTCC-3' @@@ 5'-CTTGCCCGTATTGATGACGTC-3'	1	0,7%	3,6%	
Bt11		**25**	**18,0%**	**100,0%**	**20**
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	14	10,1%	56,0%	ISO*
	5'-AAAAGACCACAACAAGCCGC-3' @@@ 5'-CAATGCGTTCTCCACCAAGTACT-3'	6	4,3%	24,0%	ISO*
	5'-GCGGCTTATCTGTCTCAGGG-3' @@@ 5'-CAACTGGTCTCCTCTCCGGA-3'	2	1,4%	8,0%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-CTTTGATCTTTTCTACGGGGTCT-3'	1	0,7%	4,0%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	0,7%	4,0%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-GCAGATTACGCGCAGAAA-3'	1	0,7%	4,0%	
MON810		**24**	**17,3%**	**100,0%**	**9**
	5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	8	5,8%	33,3%	
	5'-GATGCCTTCTCCCTAGTGTTGA-3' @@@ 5'-GGATGCACTCGTTGATGTTTG-3'	6	4,3%	25,0%	ISO*
	5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	3	2,2%	12,5%	ISO*
	5'-CAAGTGTGCCCACCACAGC-3' @@@ 5'-GCAAGCAAATTCGGAAATGAA-3'	2	1,4%	8,3%	
	5'-AGCCACCACTTCTCCTTGGA-3' @@@ 5'-AGGCTACCGAAAGTCCTCGTT-3'	2	1,4%	8,3%	
	5'-CATTTGGAGAGGACACGCTGA-3' @@@ 5'-CGTGACAGTAACAAAGGCAGA-3'	1	0,7%	4,2%	
	5'-ACTATCCTTCGCAAGACCCTTC-3' @@@ 5'-TTCAGAGAAACGTGGCAGTAACA-3'	1	0,7%	4,2%	
	5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	0,7%	4,2%	
Event 176		**20**	**14,4%**	**100,0%**	**11**
	5'-TGTTCACCAGCAGCAACCAG-3' @@@ 5'-ACTCCACTTTGTGCAGAACAGATCT-3'	8	5,8%	40,0%	ISO*
	5'-GTGGACAGCCTGGACGAGAT-3' @@@ 5'-TGCTGAAGCCACTGCGGAAC-3'	3	2,2%	15,0%	
	5'-CTCGCTTCCGTGCTTAGCTT-3' @@@ 5'-ATGCACTCGTTGATGTTGGG-3'	3	2,2%	15,0%	
	5'-CCCATCGACATCAGCCTGAGC-3' @@@ 5'-CAGGAAGGCGTCCCACTGGC-3'	3	2,2%	15,0%	ISO*
	5'-GCGACTGGATCAGGTACAA-3' @@@ 5'-ACGGGGTTGGTGTAAATCT-3'	1	0,7%	5,0%	
	5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	0,7%	5,0%	
	5'-GTAGCAGACACCCCTCTCCACA-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	1	0,7%	5,0%	
GA21		**13**	**9,4%**	**100,0%**	**11**
	5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	7	5,0%	53,8%	ISO*
	5'-GAAGCCTCGGCAACGTCA-3' @@@ 5'-ATCCGGTTGGAAAGCGACTT-3'	4	2,9%	30,8%	ISO*
	5'-AGAGCTGTAGTTGTTGGCTGTG-3' @@@ 5'-GCTGGGGGATCCACTAGTTCT-3'	1	0,7%	7,7%	
	5'-ACGGTGGAAGAGTTCAATGTATG-3' @@@ 5'-TCTCCTTGATGGGCTGCA-3'	1	0,7%	7,7%	
T25		**10**	**7,2%**	**100,0%**	**4**
	5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	4	2,9%	40,0%	
	5'-GCCAGTTAGGCCAGTTACCCA-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCCT-3'	4	2,9%	40,0%	ISO*
	5'-GGAAGTTCATTTCATTTGGAGAGG-3' @@@ 5'-GGCCATATCAGCTGCTGTAGC-3'	1	0,7%	10,0%	
	5'-ATGGTGGATGGCATGATGTTG-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCC-3'	1	0,7%	10,0%	
NK603		**8**	**5,8%**	**100,0%**	**8**
	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	8	5,8%	100,0%	ISO*
GT73 (RT73)		**3**	**2,2%**	**100,0%**	
	5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	2	1,4%	66,7%	

[1] Number of times a primer pair was reported for quantitative identification analyses.
[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative identification analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative identification of the same transgenic event (GMO).
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the trial followed criteria specified in ISO 5725-2.
[5] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 11 TGMO quantitative identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
	5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	0,7%	33,3%	
MON863		**3**	**2,2%**	**100,0%**	**3**
	5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	3	2,2%	100,0%	ISO*
Falcon GS/40/90pHoe6/Ac		**3**	**2,2%**	**100,0%**	
	5'-TAGCTGGCCTAATCTCAACTGGTC-3' @@@ 5'-ATTTCATTTGGAGAGGACAGGGTAC-3'	3	2,2%	100,0%	
CBH-351		**2**	**1,4%**	**100,0%**	
	5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	0,7%	50,0%	
	5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	0,7%	50,0%	
Total[6]		**139**	**100,0%**		**76**

[6] Total number of primer pairs received for quantitative identification analyses.

Table 12 GMO quantitative identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
GTS 40-3-2[3]			28	10
	5' soybean genome / CaMV 35S pro		9	
		5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	6	
		5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	2	
		5'-CCTTTCCTTTATCGCAATGAT-3' @@@ 5'-GGTTAAAATAAACATAGGGAACCCA-3'	1	
	3' nos ter / soybean genome		4	
		5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	4	
	CaMV 35S pro / CTP		4	2
		5'-TGATGTGATATCTCCACTGACG-3' @@@ 5'-TGTATCCCTTGAGCCATGTTGT-3'	2	
		5'-CATTTGGAGAGGACACGCTGA-3' @@@ 5'-GAGCCATGTTGTTAATTTGTGCC-3'	2	ISO*
	CTP / CaMV 35 S pro		4	
		5'-GCCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	4	ISO*
	CTP / CP4 epsps gene		4	4
		5'-CCTTTAGGATTTCAGCATCAGTGG-3' @@@ 5'-GACTTGTCGCCGGGAATG-3'	4	ISO*
	CP4 epsps gene		2	1
		5'-GGCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	1	
		5'-GCAAATCCTCTGGCCTTTCC-3' @@@ 5'-CTTGCCCGTATTGATGACGTC-3'	1	
	5' soybean genome / insert		1	
		5'-CCGCGGAAAATAAACATAGGGAACCCGCGG-MR-HG-GAGCCACCTTCCTTTTCCATTT-3' @@@ 5'-ACCCTTCAATTTAACCGATGCT-3'	1	
Bt11			25	20
	3' pUC18 / pUC18-maize genome		14	14
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	14	ISO*
	adh1-1S IVS6 / cryIA(b) gene		6	6
		5'-AAAAGACCACAACAAGCCGC-3' @@@ 5'-CAATGCGTTCTCCACCAAGTACT-3'	6	ISO*
	adh1-1S IVS2 / pat gene		2	
		5'-GCGGCTTATCTGTCTCAGGG-3' @@@ 5'-CAACTGGTCTCCTCTCCGGA-3'	2	
	5' maize genome / insert		1	
		5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-GCAGATTACGCGCAGAAA-3'	1	
	5' maize genome / CaMV 35S pro		1	
		5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-CTTTGATCTTTTCTACGGGGTCT-3'	1	
	3' pUC18 / maize genome		1	
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	
MON810			24	9
	5' maize genome / insert		7	
		5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	7	
	hsp 70 IVS1 / cryIA(b) gene		6	6
		5'-GATGCCTTCTCCCTAGTGTTGA-3' @@@ 5'-GGATGCACTCGTTGATGTTTG-3'	6	ISO*
	5' maize genome / CaMV 35S pro		5	3
		5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	3	ISO*
		5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	
		5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	1	
	CaMV 35S pro / hsp 70 IVS1		2	
		5'-CATTTGGAGAGGACACGCTGA-3' @@@ 5'-CGTGACAGTAACAAAGGCAGA-3'	1	

[1] Number of times a primer pair was reported for quantitative identification analyses.
[2] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the trial followed criteria specified in ISO 5725-2.
[3] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 12 TGMO quantitative identification: Tanalysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
		5'-ACTATCCTTCGCAAGACCCTTC-3' @@@ 5'-TTCAGAGAAACGTGGCAGTAACA-3'	1	
	3' insert / maize genome		2	
		5'-AGCCACCACTTCTCCTTGGA-3' @@@ 5'-AGGCTACCGAAAGTCCTCGTT-3'	2	
	3' cryIA(b) gene / maize genome		2	
		5'-CAAGTGTGCCCACCACAGC-3' @@@ 5'-GCAAGCAAATTCGGAAATGAA-3'	2	
Event 176			**20**	**11**
	cryIA(b) gene / PEPC IVS 9		8	8
		5'-TGTTCACCAGCAGCAACCAG-3' @@@ 5'-ACTCCACTTTGTGCAGAACAGATCT-3'	8	ISO*
	cryIA(b) gene		7	3
		5'-GTGGACAGCCTGGACGAGAT-3' @@@ 5'-TGCTGAAGCCACTGCGGAAC-3'	3	
		5'-CCCATCGACATCAGCCTGAGC-3' @@@ 5'-CAGGAAGGCGTCCCACTGGC-3'	3	ISO*
		5'-GCGACTGGATCAGGTACAA-3' @@@ 5'-ACGGGGTTGGTGTAAATCT-3'	1	
	PEPC pro / cryIA(b) gene		4	
		5'-CTCGCTTCCGTGCTTAGCTT-3' @@@ 5'-ATGCACTCGTTGATGTTGGG-3'	3	
		5'-GTAGCAGACACCCCTCTCCACA-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	1	
	3' truncated bar gene / maize genome		1	
		5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	
GA21			**13**	**11**
	5' maize genome / insert		7	7
		5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	7	ISO*
	OTP / m-epsps gene		5	4
		5'-GAAGCCTCGGCAACGTCA-3' @@@ 5'-ATCCGGTTGGAAAGCGACTT-3'	4	ISO*
		5'-ACGGTGGAAGAGTTCAATGTATG-3' @@@ 5'-TCTCCTTGATGGGCTGCA-3'	1	
	truncated m-epsps gene / truncated actin pro		1	
		5'-AGAGCTGTAGTTGTTGGCTGTG-3' @@@ 5'-GCTGGGGGATCCACTAGTTCT-3'	1	
T25			**10**	**4**
	pat gene / CaMV 35S ter		5	4
		5'-GCCAGTTAGGCCAGTTACCCA-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCCT-3'	4	ISO*
		5'-ATGGTGGATGGCATGATGTTG-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCC-3'	1	
	5' maize genome / CaMV 35S pro		4	
		5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	4	
	CaMV 35 S pro / pat gene		1	
		5'-GGAAGTTCATTTCATTTGGAGAGG-3' @@@ 5'-GGCCATATCAGCTGCTGTAGC-3'	1	
NK603			**8**	**8**
	3' insert / maize genome		8	8
		5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	8	ISO*
GT73 (RT73)			**3**	
	3' insert / canola genome		2	
		5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	2	
	3' pTi15955 plasmid / canola genome		1	
		5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	
MON863			**3**	**3**
	5' maize genome / CaMV 35 S pro		3	3
		5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	3	ISO*
Falcon GS/40/90pHoe6/Ac			**3**	
	CaMV 35S pro / pat gene		3	
		5'-TAGCTGGCCTAATCTCAACTGGTC-3' @@@ 5'-ATTTCATTTGGAGAGGACAGGGTAC-3'	3	
CBH-351			**2**	
	3' nos ter / maize genome		2	
		5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	
		5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	

Table 12 TGMO quantitative identification: Tanalysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
Total[4]			139	76

[4] Total number of primer pairs received for quantitative identification analyses.

Table 13 GMO quantitative event-specific identification: analysis on target transgenic events and corresponding use of primers tested in ring-trials

GMO	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
Bt11[5]		**17**	**22,7%**	**100,0%**	**14**
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	14	18,7%	82,4%	ISO*
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-CTTTGATCTTTTCTACGGGGTCT-3'	1	1,3%	5,9%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	1,3%	5,9%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-GCAGATTACGCGCAGAAA-3'	1	1,3%	5,9%	
MON810		**16**	**21,3%**	**100,0%**	**3**
	5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	8	10,7%	50,0%	
	5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	3	4,0%	18,8%	ISO*
	5'-CAAGTGTGCCCACCACAGC-3' @@@ 5'-GCAAGCAAATTCGGAAATGAA-3'	2	2,7%	12,5%	
	5'-AGCCACCACTTCTCCTTGGA-3' @@@ 5'-AGGCTACCGAAAGTCCTCGTT-3'	2	2,7%	12,5%	
	5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	1,3%	6,3%	
GTS 40-3-2		**14**	**18,7%**	**100,0%**	
	5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	6	8,0%	42,9%	
	5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	4	5,3%	28,6%	
	5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	2	2,7%	14,3%	
	5'-CCGCGGAAAATAAACATAGGGAACCCGCGG-MR-HG-GAGCCACCTTCCTTTTCCATTT-3' @@@ 5'-ACCCTTCAATTTAACCGATGCT-3'	1	1,3%	7,1%	
	5'-CCTTTCCTTTATCGCAATGAT-3' @@@ 5'-GGTTAAAATAAACATAGGGAACCCA-3'	1	1,3%	7,1%	
NK603		**8**	**10,7%**	**100,0%**	**8**
	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	8	10,7%	100,0%	ISO*
GA21		**7**	**9,3%**	**100,0%**	**7**
	5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	7	9,3%	100,0%	ISO*
T25		**4**	**5,3%**	**100,0%**	
	5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	4	5,3%	100,0%	
GT73 (RT73)		**3**	**4,0%**	**100,0%**	
	5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	2	2,7%	66,7%	
	5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	1,3%	33,3%	
MON863		**3**	**4,0%**	**100,0%**	**3**
	5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	3	4,0%	100,0%	ISO*
CBH-351		**2**	**2,7%**	**100,0%**	
	5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	1,3%	50,0%	
	5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	1,3%	50,0%	
Event 176		**1**	**1,3%**	**100,0%**	
	5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	1,3%	100,0%	
Total[6]		**75**	**100,0%**		**35**

[1] Number of times a primer pair was reported for quantitative event-specific identification analyses.
[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative event-specific identification analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative event-specific identification of the same transgenic event (GMO).
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the trial followed criteria specified in ISO 5725-2.
[5] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.
[6] Total number of primer pairs received for quantitative event-specific identification analyses.

Table 14 GMO quantitative event-specific identification: analysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
Bt11[3]			**17**	**14**
	3' pUC18 / pUC18-maize genome		14	14
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	14	ISO*
	5' maize genome / insert		1	
		5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-GCAGATTACGCGCAGAAA-3'	1	
	5' maize genome / CaMV 35S pro		1	
		5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-CTTTGATCTTTTCTACGGGGTCT-3'	1	
	3' pUC18 / maize genome		1	
		5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	
MON810			**16**	**3**
	5' maize genome / insert		7	
		5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	7	
	5' maize genome / CaMV 35S pro		5	3
		5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	3	ISO*
		5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	
		5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	1	
	3' insert / maize genome		2	
		5'-AGCCACCACTTCTCCTTGGA-3' @@@ 5'-AGGCTACCGAAAGTCCTCGTT-3'	2	
	3' cryIA(b) gene / maize genome		2	
		5'-CAAGTGTGCCCACCACAGC-3' @@@ 5'-GCAAGCAAATTCGGAAATGAA-3'	2	
GTS 40-3-2			**14**	
	5' soybean genome / CaMV 35S pro		9	
		5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	6	
		5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	2	
		5'-CCTTTCCTTTATCGCAATGAT-3' @@@ 5'-GGTTAAAATAAACATAGGGAACCCA-3'	1	
	3' nos ter / soybean genome		4	
		5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	4	
	5' soybean genome / insert		1	
		5'-CCGCGGAAAATAAACATAGGGAACCCGCGG-MR-HG-GAGCCACCTTCCTTTTCCATTT-3' @@@ 5'-ACCCTTCAATTTAACCGATGCT-3'	1	
NK603			**8**	**8**
	3' insert / maize genome		8	8
		5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	8	ISO*
GA21			**7**	**7**
	5' maize genome / insert		7	7
		5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	7	ISO*
T25			**4**	
	5' maize genome / CaMV 35S pro		4	
		5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	4	
GT73 (RT73)			**3**	
	3' insert / canola genome		2	
		5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	2	
	3' pTi15955 plasmid / canola genome		1	
		5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	

[1] Number of times a primer pair was reported for quantitative event-specific identification analyses.
[2] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the trial followed criteria specified in ISO 5725-2.
[3] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 14 TGMO quantitative event-specific identification: Tanalysis on target genetic elements and corresponding use of primers tested in ring-trials

GMO	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Primers Ring-Trial Tested[2]
MON863			3	3
	5' maize genome / insert		3	3
		5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	3	ISO*
CBH-351			2	
	3' nos ter / maize genome		2	
		5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	
		5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	
Event 176			1	
	3' truncated bar gene / maize genome		1	
		5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	
Total[4]			75	35

[4] Total number of primer pairs received for quantitative event-specific identification analyses.

Table 15 GMO quantitative identification: analysis on target transgenic events and corresponding use of primers for qualitative purposes

GMO	Primer Pair (Forward @@@ Reverse)	N. of Quant Primers Occur.[1]	Quant Fpa[2]	N. of Qual. Primers Occur.[3]	Qual. Fpa[4]	Primers Ring-Trial Tested[5]
GTS 40-3-2[6]		28	20,1%	7	5,0%	
	5'-CGCAATGATGGCATTTGTAGG-3' @@@ 5'-TTTCATTCAAAATAAGATCATACATACAGGTTA-3'	6	4,3%	2	1,4%	
	5'-GCCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	4	2,9%		0,0%	ISO*
	5'-CCTTTAGGATTTCAGCATCAGTGG-3' @@@ 5'-GACTTGTCGCCGGGAATG-3'	4	2,9%	2	1,4%	ISO*
	5'-TAGCATCTACATATAGCTTC-3' @@@ 5'-GACCAGGCCATTCGCCTCA-3'	4	2,9%	1	0,7%	
	5'-TGATGTGATATCTCCACTGACG-3' @@@ 5'-TGTATCCCTTGAGCCATGTTGT-3'	2	1,4%		0,0%	
	5'-CATTTGGAGAGGACACGCTGA-3' @@@ 5'-GAGCCATGTTGTTAATTTGTGCC-3'	2	1,4%		0,0%	ISO*
	5'-CATCTTTGGGACCACTGTCG-3' @@@ 5'-ACAGGTTAAAATAAACATAGGGAACC-3'	2	1,4%	1	0,7%	
	5'-CCGCGGAAAATAAACATAGGGAACCCGCGG-MR-HG-GAGCCACCTTCCTTTTCCATTT-3' @@@ 5'-ACCCTTCAATTTAACCGATGCT-3'	1	0,7%		0,0%	
	5'-GGCATGTTGTTAATTTGTGCCAT-3' @@@ 5'-GAAGTTCATTTCATTTGGAGAGGAC-3'	1	0,7%		0,0%	
	5'-CCTTTCCTTTATCGCAATGAT-3' @@@ 5'-GGTTAAAATAAACATAGGGAACCCA-3'	1	0,7%		0,0%	
	5'-GCAAATCCTCTGGCCTTTCC-3' @@@ 5'-CTTGCCCGTATTGATGACGTC-3'	1	0,7%	1	0,7%	
Bt11		25	18,0%	6	4,3%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-TCCAAGAATCCCTCCATGAG-3'	14	10,1%	3	2,2%	ISO*
	5'-AAAAGACCACAACAAGCCGC-3' @@@ 5'-CAATGCGTTCTCCACCAAGTACT-3'	6	4,3%	2	1,4%	ISO*
	5'-GCGGCTTATCTGTCTCAGGG-3' @@@ 5'-CAACTGGTCTCCTCTCCGGA-3'	2	1,4%		0,0%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-CTTTGATCTTTTCTACGGGGTCT-3'	1	0,7%		0,0%	
	5'-GCGGAACCCCTATTTGTTTA-3' @@@ 5'-CAAGAAATGGTCTCCACCAAA-3'	1	0,7%	1	0,7%	
	5'-TATCATCGACTTCCATGACCA-3' @@@ 5'-GCAGATTACGCGCAGAAA-3'	1	0,7%		0,0%	
MON810		24	17,3%	8	5,8%	
	5'-CCTTCATAACCTTCGCCCG-3' @@@ 5'-AATAAAGTGACAGATAGCTGGGCA-3'	8	5,8%	3	2,2%	
	5'-GATGCCTTCTCCCTAGTGTTGA-3' @@@ 5'-GGATGCACTCGTTGATGTTTG-3'	6	4,3%	2	1,4%	ISO*
	5'-TCGAAGGACGAAGGACTCTAACGT-3' @@@ 5'-GCCACCTTCCTTTTCCACTATCTT-3'	3	2,2%	2	1,4%	ISO*
	5'-CAAGTGTGCCCACCACAGC-3' @@@ 5'-GCAAGCAAATTCGGAAATGAA-3'	2	1,4%		0,0%	
	5'-AGCCACCACTTCTCCTTGGA-3' @@@ 5'-AGGCTACCGAAAGTCCTCGTT-3'	2	1,4%		0,0%	
	5'-CATTTGGAGAGGACACGCTGA-3' @@@ 5'-CGTGACAGTAACAAAGGCAGA-3'	1	0,7%		0,0%	
	5'-ACTATCCTTCGCAAGACCCTTC-3' @@@ 5'-TTCAGAGAAACGTGGCAGTAACA-3'	1	0,7%		0,0%	
	5'-ATAACCTTCGCCCGAAAATC-3' @@@ 5'-CAACGATGGCCTTTCCTTTA-3'	1	0,7%	1	0,7%	
Event 176		20	14,4%	6	4,3%	
	5'-TGTTCACCAGCAGCAACCAG-3' @@@ 5'-ACTCCACTTTGTGCAGAACAGATCT-3'	8	5,8%	2	1,4%	ISO*
	5'-GTGGACAGCCTGGACGAGAT-3' @@@ 5'-TGCTGAAGCCACTGCGGAAC-3'	3	2,2%	2	1,4%	
	5'-CTCGCTTCCGTGCTTAGCTT-3' @@@ 5'-ATGCACTCGTTGATGTTGGG-3'	3	2,2%		0,0%	
	5'-CCCATCGACATCAGCCTGAGC-3' @@@ 5'-CAGGAAGGCGTCCCACTGGC-3'	3	2,2%		0,0%	ISO*
	5'-GCGACTGGATCAGGTACAA-3' @@@ 5'-ACGGGGTTGGTGTAAATCT-3'	1	0,7%		0,0%	
	5'-GACTTCAGCCTGCCGGTACT-3' @@@ 5'-GTGCATCAATGGAGGAGAGAAC-3'	1	0,7%	1	0,7%	
	5'-GTAGCAGACACCCCTCTCCACA-3' @@@ 5'-TCGTTGATGTTKGGGTTGTTGTCC-3'	1	0,7%	1	0,7%	
GA21		13	9,4%	3	2,2%	
	5'-CTTATCGTTATGCTATTTGCAACTTTAGA-3' @@@ 5'-TGGCTCGCGATCCTCCT-3'	7	5,0%		0,0%	ISO*
	5'-GAAGCCTCGGCAACGTCA-3' @@@ 5'-ATCCGGTTGGAAAGCGACTT-3'	4	2,9%	1	0,7%	ISO*
	5'-AGAGCTGTAGTTGTTGGCTGTG-3' @@@ 5'-GCTGGGGGATCCACTAGTTCT-3'	1	0,7%	1	0,7%	
	5'-ACGGTGGAAGAGTTCAATGTATG-3' @@@ 5'-TCTCCTTGATGGGCTGCA-3'	1	0,7%	1	0,7%	
T25		10	7,2%	5	3,6%	
	5'-TCAATTGCCCTTTGGTCTTCTGA-3' @@@ 5'-TACGACATGATACTCCTTCCAC-3'	4	2,9%	3	2,2%	
	5'-GCCAGTTAGGCCAGTTACCCA-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCCT-3'	4	2,9%	2	1,4%	ISO*
	5'-GGAAGTTCATTTCATTTGGAGAGG-3' @@@ 5'-GGCCATATCAGCTGCTGTAGC-3'	1	0,7%		0,0%	
	5'-ATGGTGGATGGCATGATGTTG-3' @@@ 5'-TGAGCGAAACCCTATAAGAACCC-3'	1	0,7%		0,0%	
NK603		8	5,8%	2	1,4%	

[1] Number of times a primer pair was reported for quantitative identification analyses.
[2] Quantitative frequency per analyte (QuantFpa): ratio between the number of times the specific primer pair was reported for quantitative identification of the GMO and the total number of primer pairs received for the quantitative identification section.
[3] Number of times a primer pair was also reported for qualitative identification analyses.
[4] Qualitative frequency per analyte (QualFpa): ratio between the number of times the specific primer pair was reported for qualitative identification of the GMO and the total number of primer pairs received for the quantitative identification section.
[5] Primers that have been submitted to ring-trial evaluation for a quantitative purpose: a + sign indicates an inter-laboratory trial, ISO indicates that the trial followed criteria specified in ISO 5725-2.
[6] GMOs that are authorized in the EU for different intended purposes are highlighted in yellow.

Table 15 TGMO quantitative identification: analysis on target transgenic events and corresponding use of primers for qualitative purposes

GMO	Primer Pair (Forward @@@ Reverse)	N. of Quant Primers Occur.[1]	Quant Fpa[2]	N. of Qual. Primers Occur.[3]	Qual. Fpa[4]	Primers Ring-Trial Tested[5]
	5'-ATGAATGACCTCGAGTAAGCTTGTTAA-3' @@@ 5'-AAGAGATAACAGGATCCACTCAAACACT-3'	8	5,8%	2	1,4%	ISO*
GT73 (RT73)		**3**	**2,2%**	**1**	**0,7%**	
	5'-CCATATTGACCATCATACTCATTGCT-3' @@@ 5'-GCTTATACGAAGGCAAGAAAAGGA-3'	2	1,4%		0,0%	
	5'-TAGATTTCCCGGACATGAAGAT-3' @@@ 5'-TCAGCAAGATTCTCTGTCAACAA-3'	1	0,7%	1	0,7%	
MON863		**3**	**2,2%**	**1**	**0,7%**	
	5'-GTAGGATCGGAAAGCTTGGTAC-3' @@@ 5'-TGTTACGGCCTAAATGCTGAACT-3'	3	2,2%	1	0,7%	ISO*
Falcon GS/40/90pHoe6/Ac		**3**	**2,2%**		**0,0%**	
	5'-TAGCTGGCCTAATCTCAACTGGTC-3' @@@ 5'-ATTTCATTTGGAGAGGACAGGGTAC-3'	3	2,2%		0,0%	
CBH-351		**2**	**1,4%**	**1**	**0,7%**	
	5'-GCGCGGTGTCATCTATGTTA-3' @@@ 5'-ACAACGGGGAGTTGTATGCT-3'	1	0,7%		0,0%	
	5'-AGCGCGCAAACTAGGATAAA-3' @@@ 5'-CGTTCTGGGAAGGATAGAATCGTC-3'	1	0,7%	1	0,7%	
Total[7]		**139**	**100%**	**40**	**28,8%**	

[7] Total number of primer pairs received for quantitative identification analyses.

Table 16 Taxon-specific qualitative identification: analysis on target species-specific genes and use of the corresponding primers in ring-trials

Taxon	Target GE(Forward/ Reverse)	Primer Pair (Forward. @@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
maize			**49**	**40,5%**	**100,0 %**	**12**
	zein gene		16	13,2%	32,7%	
		5'-AGTGCGACCCATATTCCAG-3' @@@ 5'-GACATTGTGGCATCATCATTT-3'	7	5,8%	14,3%	
		5'-TGCTTGCATTGTTCGCTCTCCTAG-3' @@@ 5'-GTCGCAGTGACATTGTGGCAT-3'	5	4,1%	10,2%	
		5'-GCTTGCATTGTTCGCTCTC-3' @@@ 5'-CGATGGCATGTCAACTCATTA-3'	1	0,8%	2,0%	
		5'-GCATTGTTCGCTCTCCTAGC-3' @@@ 5'-TACTGCATGCATGGGTTCAT-3'	1	0,8%	2,0%	
		5'-CCTCAGTCGCACATATCTACTATACT-3' @@@ 5'-CTAGAATGCAGCACCAACAAA-3'	1	0,8%	2,0%	
		5'-AGTGCGACCCATATTCCAG-3' @@@ 5'-GCTACATAGGGAGCCTTGTCCT-3'	1	0,8%	2,0%	
	invertase (ivr1) gene		13	10,7%	26,5%	12
		5'-CCGCTGTATCACAAGGGCTGGTACC-3' @@@ 5'-GGAGCCCGTGTAGAGCATGACGATC-3'	12	9,9%	24,5%	ISO*
		5'-CTCTAACTTGGTTCCGCGTTT-3' @@@ 5'-CGCCGGCGCCGGGTCGTACCG-3'	1	0,8%	2,0%	
	alcohol dehydrogenase (adh1) gene		10	8,3%	20,4%	
		5'-CGTCGTTTCCCATCTCTTCCTCC-3' @@@ 5'-CCACTCCGAGACCCTCAGTC-3'	8	6,6%	16,3%	
		5'-CGTCGTTTCCCATCTCTTCCTCC-3' @@@ 5'-TCGATTTCTCTCTTGGTGACAGG-3'	1	0,8%	2,0%	
		5'-CCAGCCTCATGGCCAAAG-3' @@@ 5'-CCTTCTTGGCGGCTTATCTG-3'	1	0,8%	2,0%	
	high-mobility-group (hmg) gene		7	5,8%	14,3%	
		5'-GAAATCCCTGAGCGAGTCGGTA-3' @@@ 5'-GCGATGGCCTTGTTGTACTCGA-3'	4	3,3%	8,2%	
		5'-TTGGACTAGAAATCTCGTGCTGA-3' @@@ 5'-GCTACATAGGGAGCCTTGTCCT-3'	3	2,5%	6,1%	
	starch synthase IIb (zSSIIb) gene		3	2,5%	6,1%	
		5'-CTCCCAATCCTTTGACATCTGC-3' @@@ 5'-TCGATTTCTCTCTTGGTGACAGG-3'	3	2,5%	6,1%	
soybean			**35**	**28,9%**	**100,0 %**	**14**
	lectin gene		35	28,9%	100,0 %	14
		5'-GCCCTCTACTCCACCCCCATCC-3' @@@ 5'-GCCCATCTGCAAGCCTTTTTGTG-3'	14	11,6%	40,0%	ISO*
		5'-CATTACCTATGATGCCTCCACC-3' @@@ 5'-AAGCACGTCATGCGATTCC-3'	5	4,1%	14,3%	
		5'-TGCCGAAGCAACCAAACATGATCCT-3' @@@ 5'-TGATGGATCTGATAGAATTGACGTT-3'	3	2,5%	8,6%	
		5'-AACCGGTAGCGTTGCCAG-3' @@@ 5'-AGCCCATCTGCAAGCCTTT-3'	2	1,7%	5,7%	
		5'-GCCCTCTACTCCACCCCCA-3' @@@ 5'-GCCCATCTGCAAGCCTTTTT-3'	2	1,7%	5,7%	
		5'-GATGGATCTGATAGAATTGAC-3' @@@ 5'-GCCGAAGCAACCAAACATG-3'	1	0,8%	2,9%	
		5'-GACGCTATTGTGACCTCCTC-3' @@@ 5'-TCTTTGTCCCAAATGTGGATG-3'	1	0,8%	2,9%	
		5'-GACGCTATTGTGACCTCCTC-3' @@@ 5'-CGAAGCTGGCAACGCTACC-3'	1	0,8%	2,9%	
		5'-GAAGCAACCAAACATGATCCTC-3' @@@ 5'-ATGGATCTGATAGAATTGACGTTA-3'	1	0,8%	2,9%	
		5'-CTCTACTCCACCCCCATCC-3' @@@ 5'-TGATGGATCTGATAGAATTGACGTT-3'	1	0,8%	2,9%	
		5'-CTCTACTCCACCCCCATC-3' @@@ 5'-GATGGATCTGATAGAATTGAC-3'	1	0,8%	2,9%	
		5'-CCATGCATCACAGTGCAATTTAGC-3' @@@ 5'-CGATCGAGTAGTGAGAGTCGTCTT-3'	1	0,8%	2,9%	
		5'-TCCACCCCCATCCACATTT-3' @@@ 5'-GGCATAGAAGGTGAAGTTGAAGGA-3'	1	0,8%	2,9%	
		5'-CATCCACATTTGGGACAAAG-3' @@@ 5'-TCTGCAAGCCTTTTTGTGTC-3'	1	0,8%	2,9%	
plant specific			**11**	**9,1%**	**100,0 %**	**8**
	plant chloroplast genome		8	6,6%	72,7%	8
		5'-CGAAATCGGTAGACGCTACG-3' @@@ 5'-GGGGATAGAGGGACTTGAAC-3'	8	6,6%	72,7%	ISO*
	ribulose-1,5-bisphosphate carboxylase (RuBPC) large subunit gene		1	0,8%	9,1%	
		5'-CTTACCAGyCTTGATCGTTACAAAGG-3' @@@ 5'-CAAAAAGGTCTAAiGGyTAAGCTACA-3'	1	0,8%	9,1%	
	plant 18S rRNA gene		1	0,8%	9,1%	
		5'-ATTCCAGCTCCATAGCGTATA-3‘ @@@ 5‘-TTCCATGCTAATGTATTCAGAG-3‘	1	0,8%	9,1%	

[1] Number of times a primer pair was reported for qualitative taxon-specific analyses.
[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative taxon-specific analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for qualitative identification of the same taxon.
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).

Table 16 TTaxon-specific qualitative identification: analysis on target species-specific genes and use of the corresponding primers in ring-trials

Taxon	Target GE(Forward/ Reverse)	Primer Pair (Forward. @@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
	plant 26S rRNA gene		1	0,8%	9,1%	
		5'-CCTGATCTTCTGTGAAGGGTTCGAGT-3' @@@ 5'-CCTATACCCAAGTCAGACGAACGAT-3'	1	0,8%	9,1%	
tomato			**10**	**8,3%**	**100,0 %**	**8**
	polygalacturonase (PG) gene		8	6,6%	80,0%	8
		5'-GGATCCTTAGAAGCATCTAGT-3' @@@ 5'-CGTTGGTGCATCCCTGCATGG-3'	8	6,6%	80,0%	ISO*
	tomato 25S-18S rRNA intergenic spacer		1	0,8%	10,0%	
		5'-GTGAGGTTCGCGTGCTGCCTG-3' @@@ 5'-CGCACCCGACATCCCGAAAAC-3'	1	0,8%	10,0%	
	tomato metallo-carboxypeptidase inhibitor (mcpi) gene		1	0,8%	10,0%	
		5'-TTGCTGCTCAAGATGTGATGG-3' @@@ 5'-ACGTACCACCAGAACAATCGTCT-3'	1	0,8%	10,0%	
canola			**8**	**6,6%**	**100,0 %**	
	phosphoenolpyruvate carboxylase (pepC) gene		4	3,3%	50,0%	
		5'-GCTAGTGTAGACCAGTTCTTG-3' @@@ 5'-CACTCTTGTCTCTTGTCCTC-3'	4	3,3%	50,0%	
	cruciferin gene		2	1,7%	25,0%	
		5'-TGTTAGGACAGCGCAACAAC-3' @@@ 5'-AACTTCCTCCTGCGGTCTCT-3'	1	0,8%	12,5%	
		5'-TTCAGAACCAGCAAGACAACC-3' @@@ 5'-AGAGACGAAGGAAGCGAAGG-3'	1	0,8%	12,5%	
	FatA gene		1	0,8%	12,5%	
		5'-GGTCTCTCAGCAAGTGGGTGAT-3' @@@ 5'-TCGTCCCGAACTTCATCTGTAA-3'	1	0,8%	12,5%	
	Acetyl-CoA carboxylase (BnACCg8) gene		1	0,8%	12,5%	
		5'-GAGAATGAGGAGGACCAAGCTC-3' @@@ 5'-GGCGCAGCATCGGCT-3'	1	0,8%	12,5%	
potato			**3**	**2,5%**	**100,0 %**	
	potato metallo-carboxypeptidase inhibitor (pci) gene		2	1,7%	66,7%	
		5'-TGACAATTCATTCTACTCCACGAAA-3' @@@ 5'-TGTTACAAATTGGATCTGCGTGTT-3'	2	1,7%	66,7%	
	patatin gene		1	0,8%	33,3%	
		5'-GGATCCAGCATTTTCTTCA-3' @@@ 5'-TAGCTAACATCCATCGTAGAGG-3'	1	0,8%	33,3%	
papaya			**2**	**1,7%**	**100,0 %**	
	papain gene		2	1,7%	100,0 %	
		5'-GGGCATTCTCAGCTGTTGTA-3' @@@ 5'-CGACAATAACGTTGCACTCC-3'	2	1,7%	100,0 %	
eukaryotic specific			**1**	**0,8%**	**100,0 %**	
	eukaryotic highly conserved 18S-rRNA gene		1	0,8%	100,0 %	
		5'-TCTGCCCTATCAACTTTCGATGGTA-3' @@@ 5'-AATTTGCGCGCCTGCTGCCTTCCTT-3'	1	0,8%	100,0 %	
wheat			**1**	**0,8%**	**100,0 %**	
	wheat rDNA intergenic spacer		1	0,8%	100,0 %	
		5'-GCGGCGTGTGCCACGTACGTGGTTT-3' @@@ 5'-GAACGGGCGTTACGTGGACACGGGA-3'	1	0,8%	100,0 %	
cotton			**1**	**0,8%**	**100,0 %**	
	acyl carrier protein (acp1) gene		1	0,8%	100,0 %	
		5'-ATTGTGATGGGACTTGAGGAAGA-3' @@@ 5'-CTTGAACAGTTGTGATGGATTGTG-3'	1	0,8%	100,0 %	

Table 16 TTaxon-specific qualitative identification: analysis on target species-specific genes and use of the corresponding primers in ring-trials

Taxon	Target GE(Forward/ Reverse)	Primer Pair (Forward. @@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
Total[5]			121	100,0 %		42

[5] Total number of primer pairs received for qualitative taxon-specific analyses.

Table 17 Taxon-specific quantitative identification: analysis on target species-specific genes and use of the corresponding primers in ring-trials

Taxon	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
maize			**44**	**56,4%**	**100,0%**	**20**
	alcohol dehydrogenase (adh1) gene		19	24,4%	43,2%	14
		5'-CGTCGTTTCCCATCTCTTCCTCC-3' @@@ 5'-CCACTCCGAGACCCTCAGTC-3'	14	17,9%	31,8%	ISO*
		5'-CCAGCCTCATGGCCAAAG-3' @@@ 5'-CCTTCTTGGCGGCTTATCTG-3'	5	6,4%	11,4%	
	zein gene		7	9,0%	15,9%	
		5'-GCATGATGCAACAAGGGCTT-3' @@@ 5'-AGGCCAACAGTTGCTGCAG-3'	3	3,8%	6,8%	
		5'-TGCAGCAACTGTTGGCCTTAC-3' @@@ 5'-TGTTAGGCGTCATCATCTGTGG-3'	2	2,6%	4,5%	
		5'-CGTGTCCGTCCCTGATGC-3' @@@ 5'-AGGCGTCATCATCTGTGGC-3'	1	1,3%	2,3%	
		5'-GCATTGTTCGCTCTCCTAGC-3' @@@ 5'-TACTGCATGCATGGGTTCAT-3'	1	1,3%	2,3%	
	starch synthase IIb (zSSIIb) gene		6	7,7%	13,6%	
		5'-CTCCCAATCCTTTGACATCTGC-3' @@@ 5'-TCGATTTCTCTCTTGGTGACAGG-3'	6	7,7%	13,6%	
	invertase (ivr1) gene		6	7,7%	13,6%	
		5'-TGGCGGACGACGACTTGT-3' @@@ 5'-AAAGTTTGGAGGCTGCCGT-3'	4	5,1%	9,1%	
		5'-CACTCCATCGTGGAGAGCTT-3' @@@ 5'-GGCGTTGTTGAAGAGGAAGA-3'	2	2,6%	4,5%	
	high-mobility-group (hmg) gene		6	7,7%	13,6%	6
		5'-TTGGACTAGAAATCTCGTGCTGA-3' @@@ 5'-GCTACATAGGGAGCCTTGTCCT-3'	6	7,7%	13,6%	ISO*
soybean			**27**	**34,6%**	**100,0%**	**7**
	lectin gene		27	34,6%	100,0%	7
		5'-CCAGCTTCGCCGCTTCCTTC-3' @@@ 5'-GAAGGCAAGCCCATCTGCAAGCC-3'	7	9,0%	25,9%	
		5'-TCCACCCCCATCCACATTT-3' @@@ 5'-GGCATAGAAGGTGAAGTTGAAGGA-3'	7	9,0%	25,9%	
		5'-AACCGGTAGCGTTGCCAG-3' @@@ 5'-AGCCCATCTGCAAGCCTTT-3'	4	5,1%	14,8%	
		5'-GCCCTCTACTCCACCCCCA-3' @@@ 5'-GCCCATCTGCAAGCCTTTTT-3'	2	2,6%	7,4%	
		5'-GACGCTATTGTGACCTCCTC-3' @@@ 5'-TGTCAGGGGCATAGAAGGTG-3'	2	2,6%	7,4%	
		5'-CTTTCTCGCACCAATTGACA-3' @@@ 5'-TCAAACTCAACAGCGACGAC-3'	2	2,6%	7,4%	
		5'-GCGGCCAACGCTACCGGTTTCTTTGTCCCAAATGGCCGC-MR-HG-GCCCTCTACTCCACCCCCATCC-3' @@@ 5'-GCCCATCTGCAAGCCTTTTTGTG-3'	1	1,3%	3,7%	
		5'-CATCCACATTTGGGACAAAG-3' @@@ 5'-TCTGCAAGCCTTTTTGTGTC-3'	1	1,3%	3,7%	
		5'-GCCCTCTACTCCACCCCCATCC-3' @@@ 5'-GCCCATCTGCAAGCCTTTTTGTG-3'	1	1,3%	3,7%	
canola			**4**	**5,1%**	**100,0%**	
	cruciferin gene		2	2,6%	50,0%	
		5'-TGGCTAAAGGTACGTGAATCTG-3' @@@ 5'-CTCTCCCCATAAGACCTTCTCC-3'	1	1,3%	25,0%	
		5'-TGTTAGGACAGCGCAACAAC-3' @@@ 5'-AACTTCCTCCTGCGGTCTCT-3'	1	1,3%	25,0%	
	Acetyl-CoA carboxylase (BnACCg8) gene		1	1,3%	25,0%	
		5'-GGTGAGCTGTATAATCGAGCGA-3' @@@ 5'-GGCGCAGCATCGGCT-3'	1	1,3%	25,0%	
	FatA gene		1	1,3%	25,0%	
		5'-GGTCTCTCAGCAAGTGGGTGAT-3' @@@ 5'-TCGTCCCGAACTTCATCTGTAA-3'	1	1,3%	25,0%	
plant specific			**1**	**1,3%**	**100,0%**	
	plant chloroplast genome		1	1,3%	100,0%	
		5'-GCAAAAATRTTCTGATAAAGACG-3' @@@ 5'-GCCAAGGCTTAGCTCAGGAC-3'	1	1,3%	100,0%	
tomato			**1**	**1,3%**	**100,0%**	
	tomato metallo-carboxypeptidase inhibitor (mcpi) gene		1	1,3%	100,0%	
		5'-TTGCTGCTCAAGATGTGATGG-3' @@@ 5'-ACGTACCACCAGAACAATCGTCT-3'	1	1,3%	100,0%	
potato			**1**	**1,3%**	**100,0%**	
	potato metallo-carboxypeptidase inhibitor (pci) gene		1	1,3%	100,0%	
		5'-TGACAATTCATTCTACTCCACGAAA-3' @@@ 5'-TGTTACAAATTGGATCTGCGTGTT-3'	1	1,3%	100,0%	

[1] Number of times a primer pair was reported for quantitative taxon-specific analyses.
[2] Frequency per analyte (Fpa): ratio between the number of times the specific primer pair was reported and the total number of primer pairs received for quantitative taxon-specific analyses.
[3] Frequency within the analyte (Fwa): ratio between the number of times the specific primer pair was reported and the total. number of primer pairs received for quantitative identification of the same taxon.
[4] Primers that have been submitted to ring-trial evaluation: a + sign indicates an inter-laboratory trial, ISO indicates that the primers have been included in an ISO standard method (prEN-21569. 2005).

Table 17 TTaxon-specific quantitative identification: analysis on target species-specific genes and use of the corresponding primers in ring-trials

Taxon	Target GE (Forward/ Reverse)	Primer Pair (Forward @@@ Reverse)	N. of Primers Occur.[1]	Fpa[2]	Fwa[3]	Primers Ring-Trial Tested[4]
Total[5]			**78**	**100,0%**		**27**

[5] Total number of primer pairs received for quantitative taxon-specific analyses.

9 Annex1: ENGL members that participated to the survey

Country	Organisation	Acronym	City
AT	AGES- Austrian Agency for Health and Food Safety Ltd. Food Control and Research (LUVIE) Wien	AGES-LUVIE	Wien
AT	AGES - Austrian Agency for Health and Food Safety Agricultural Inspection Service and Research Centre Vienna Institut für Pflanzenschutzmittelprüfung	AGES	Vienna
AT	Umweltbundesamt (UBA)	UBA	Wien
BE	Instituut voor Landbouw en Visserij Onderzoek - Institute for Agricultural and Fisheries Research	ILVO	Merelbeke
BE	CRA-Département Qualité des productions agricoles	CRA	Gembloux
BE	Institut Scientifique de la Santé Publique - Section de Biosecurité et Biotechnologie	IPH	Bruxelles
CZ	Institute of Chemical Technology		Prague 6
CZ	National Institute of Public Health		Brno
CZ	Czech Agriculture and Food Inspection		Brno
DE	Landesamt für Gesundheit und Lebensmittelsicherheit	LGL	Oberschleißheim
DK	Danish Institute for Food and Veterinary Research		Søborg
DK	Danish Plant Directorate - Fødevareministeriet - Plantedirektoratet		Lyngby
EE	National Institute of Chemical Physics and Biophysics		Tallinn
FI	Finnish Customs Laboratory - Tullilaboratorio	FCL	Espoo
FR	Institut National de la Recherche Agronomique - Centre de Versailles - Laboratoire de Phytopathologie et Méthodologie de la Détection Végétale (PMDV)	INRA	Versailles Cedex
FR	Laboratoire Interrégional de la Direction Générale de la Concurrence - Consommation et Répression des Fraudes de Strasbourg	DGCCRF	Illkirch Graffenstaden
FR	Laboratoire National de la Protection des Végétaux (LNPV) d'Orleans	LNPV	Fleury les Aubrais Cedex
FR	BioGEVES		Surgeres
HU	Godollo Agricultural Biotechnology Centre - Environmental Biosafety Research Institute		Godollo
HU	National Institute for Food Safety and Nutrition	NIFSN	
IT	Istituto Zooprofilattico Sperimentale Lazio e Toscana - Dipartimento di Virologia e Biotecnologie	IZSLT	Roma
IT	Istituto Superiore di Sanità (ISS) - Laboratorio di chimica dei cereali	ISS	Roma
IT	ENSE Laboratorio Analisi Sementi	ENSE	Tavazzano (LO)
LT	National Veterinary Laboratory		Vilnius
LU	Laboratoire National de Santé - Division du Contrôle des Denrées Alimentaires		Luxembourg
LV	State Veterinary Medicine Diagnostic Center (SVMDC) of Food and Veterinary Service	SVMDC	Riga
NL	Rijks-Kwaliteitsinstituut voor Land- en Tuinbouwproducten	RIKILT	AE Wageningen
NL	Keuringsdienst van Waren - Inspectorate for Health Protection and Veterinary Public Health	KvW	BK Amsterdam
NL	NAK - Nederlandse Algemene Keuringsdienst	NAK	BC Emmeloord
NO	Veterinærinstituttet Seksjon for fôr- og næringsmiddelmikrobiologi		Oslo
PL	Plant Breeding and Acclimatisation Institute Radzikow		Blonie
PL	The National Veterinary Research Institute		Pulawy
PT	Instituto de Biologia Experimental e Tecnólogica (IBET)	IBET	Oeiras
SE	National Food Administration	SLV	Uppsala
SE	National Food Administration	SLV	Uppsala
SI	National Institute of Biology		Ljubljana
SI	Agricultural Institute of Slovenia		Ljubljana
SK	State Veterinary and Food Institute		Dolny Kubin
UK	Laboratory of Government Chemists	LGC	Teddington Middlesex

European Commission

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Abstract
This document provides the first general overview on PCR primers used by Member State control laboratories for the detection, identification and quantification of GMOs. The data has been collected from a survey launched in 2005.

The survey aimed at reviewing the PCR primers used by ENGL members for control purposes and at gathering analytical details on the corresponding PCR procedures. It further aimed to provide a catalogue of DNA sequences as a basis for the future development of plasmid standards.

Participants to the survey were specifically requested to provide information on the genetic targets and if relevant, the GM event for which the primers were designed, to include the sequences of primers and the purpose (qualitative/quantitative and screening/identification analysis), type (i.e. single, multiplex, competitive, double-competitive, simplex real-time or multiplex real-time) and specificity (gene-specificity, construct specificity or event-specificity) of the corresponding PCR assay. They were further requested to provide comments and references, as well as to specify the confidentiality status of data provided.

As a quality control step, the collated information was checked against the corresponding data published in the GMOs methods database (<http://bgmo.jrc.ec.europa.eu/home/ict/methodsdatabase.htm), in literature or on the CRL web site (<http://gmo-crl.jrc.it) and further edited and normalised.

The survey has provided a very large amount of interconnected data. To streamline the consultation of the report, the results of the survey have been grouped in two sections according to the scope of the PCR analysis namely, GMO-specific, and Taxon-specific applications. The GMO section covers primers that are specific for transgene sequences while the Taxon section covers PCR primers that are specific for endogenous genes. The results of the survey have been further grouped according to the purpose of the PCR assay (qualitative/quantitative and screening/identification analysis) and its corresponding specificity.

The data obtained from the survey was first analysed to verify representativeness of the results and identify general trends.

The analysis revealed that the data set was representative since 55% of the ENGL laboratories participated. Furthermore, the participants were geographically widely distributed across the Member States.

The analysis also evaluated general trends such as the frequency by which published and/or validated PCR primers were used by member laboratories and the frequency by which GM events or genetic elements were targeted in the PCR assays.

The general overview revealed that 83% of the primers used by laboratories were published in peer-reviewed articles. Custom-designed primers or commercially available kits were rarely reported. The survey data further indicated that 44% of primers were used for detecting common transgenic elements and endogenous genes while the other primers were predominantly employed for identification of authorised GMO. The general overview also revealed that 46% of reported primers had been tested in collaborative studies.
Further data analyses were performed on the sets of primers defined by the participating laboratories for a particular scope, purpose and specificity of the PCR assay.

Strikingly, these analyses revealed a great variability of primers selection for GMOs control purposes. In general, however, a single primer pair was reported by more than 30% of the ENGL members while there was a wide distribution of primer utilisation in the remaining laboratories. The primer pairs most commonly employed were construct-specific, while for quantitative identification, ENGL laboratories tended to use primers that were event-specific. In approximately 30% of cases, primers designed for quantitative determination were also utilised for qualitative analysis.

Primer pairs were designed predominantly for identification of GTS 40-3-2 and Event 176 which were the first GMOs approved for food in the EU. For new transgenic events most laboratories were using primers validated by the Community Reference Laboratory for GM Food and Feed (CRL-GMFF). This tendency is expected to continue in the future.

For some GM events authorised in the EU (i.e. GT73, Falcon GS/40/90pHoe6/Ac, RF3, and MS8) no validated primers were available. Also, not all GM events that were authorised in the EU have had primers reported for corresponding detection/quantification.

For identification of crops as ingredient the results indicated that a large number of taxon-specific primers were targeting maize genes. A second group of primers was specific for soybean with lectin as the only targeted gene. Taxon-specific primers were also reported for detection of canola, tomato, potato, wheat, cotton or chloroplast-specific sequences. In general, few of the reported taxon-specific primers have been subjected to ring-trial evaluation.

This survey has provided a significant insight into the analytical strategies exploited by the ENGL laboratories for detection, identification and quantification of GMOs. It has shown that primer pairs used in the laboratories for the same final purpose are quite diverse, even if some of the primer pairs are used more frequently than others.

Quality control of the data collected in the survey highlighted a problem of nomenclature for primers. Indeed, the same oligonucletide sequences had different formats and names in different ENGL laboratories. Use of the same nomenclature for primers should be promoted at ENGL level.

In addition, the ENGL could drive harmonisation in the adoption of scientific and technical approaches for GMO analyses and provide recommendations for primers utilisation. The lack of performance criteria for the specificity of qualitative amplicons and methods exploiting them is certainly a weakness to address.

Provision of reference amplicons and application of performance criteria to primers and probe utilisation should be conceived as an initial step in promoting coordination, standardisation and harmonisation in Member States.

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