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Report on the Verification of the Performance of Bt11, MIR162, MIR604 and GA21 Event-specific PCR-based Methods Applied to DNA Extracted from Stack Maize Bt11 x MIR162 x MIR604 x GA21

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Report on the Verification of the Performance of Bt11, MIR162, MIR604 and GA21 Event-specific PCR-based Methods Applied to DNA Extracted from Stack Maize Bt11 x MIR162 x MIR604 x GA21

11 December 2013

European Union Reference Laboratory for GM Food and Feed

Executive Summary

An application was submitted by Syngenta Crop Protection AG to request the authorisation of genetically modified Bt11 x MIR162 x MIR604 x GA21 maize (resistant to lepidopteran and coleopteran pests, able to utilise mannose as the only primary carbon source and tolerant to glufosinate ammonium and glyphosate) and all sub-combinations of the individual events as present in the segregating progeny, for food and feed uses, and import and processing, in accordance with articles 5 and 17 of Regulation (EC) No 1829/2003 GM Food and GM Feed. The unique identifier assigned to Bt11 x MIR162 x MIR604 x GA21 maize is SYN-BTØ11-1x YN-IR162-4xSYN-IR6Ø4-5xMON-ØØØ21-9.

The genetically modified maize line Bt11 x MIR162 x MIR604 x GA21 maize has been obtained by conventional crossing of four genetically modified maize events: Bt11, MIR162, MIR604, and GA21 without any new genetic modification.

The EU-RL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events Bt11, MIR162, MIR604 and GA21 (see <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>). In line with the approach defined by the ENGL (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EU-RL GMFF therefore has carried out only an in-house verification of the performance of each validated method when applied to DNA extracted from Bt11 x MIR162 x MIR604 x GA21.

The hereby reported the *in-house* verification study lead to the conclusion that the individual methods meet the ENGL requirements also when applied to DNA extracted from the GM maize stack Bt11 x MIR162 x MIR604 x GA21.

This report is published at <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>.

Quality assurance

The EU-RL GMFF is ISO 17025:2005 accredited [certificate number: ACCREDIA 1172 (Flexible Scope for DNA extraction and qualitative/quantitative PCR) - Accredited tests are available at http://www.accredia.it/accredia_labsearch.jsp?ID_LINK=293&area=7].

The original version of the document containing evidence of internal checks and authorisation for publication is archived within the EU-RL GMFF quality system.

The EU-RL GMFF is also ISO 17043:2010 accredited (proficiency test provider) and applies the corresponding procedures and processes for the management of ring trials during the method validation.

The EU-RL GMFF conducts its activities under the certification ISO 9001:2008 of the Institute for Health and Consumer Protection (IHCP) provided by CERMET.

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1. Introduction

The EU legislative system ^(1, 2) for genetically modified food and feed provides that any GMO for food and feed use shall undergo the authorisation process before it can be placed on the market. This holds true also for a GMO containing more than one single GM event obtained by conventional crossing, co-transformation or re-transformation (genetically modified stack).

Consequently, the application for authorisation of a GMO shall be accompanied, among others, by an event-specific method for detection, identification and quantification for each GM event composing the stack, and by samples of the stack and food and feed derived from it. The EU-RL GMFF shall validate the event specific methods of detection proposed by the applicant with regard to their performance when applied to DNA extracted from the stack, and shall report to the European Food Safety Authority, who will include the EU-RL GMFF report in the overall opinion concerning the risk assessment and potential authorization of the assessed stack.

Upon reception of methods, samples and related data (step 1), the EU-RL GMFF carried out the assessment of the documentation (step 2) and the in-house verification of the methods (step 3) according to the requirements of Regulation (EC) No 641/2004 (Annex I).

The results of the in-house verification study were evaluated with reference to ENGL method performance requirements and to the validation results on the individual events.

2. Step 1 (dossier reception and acceptance)

Syngenta Crop Protection AG submitted the detection methods, data demonstrating their adequate performance, and the corresponding control samples DNA extracted from the GM maize stack Bt11 x MIR162 x MIR604 x GA21 maize.

The dossier was found to be complete and thus was moved to step 2.

3. Step 2 (dossier scientific assessment)

The data provided by the applicant were assessed against the method acceptance criteria set out by the ENGL ⁽³⁾ and with regard to their documentation and reliability.

Table 1 shows values of trueness (expressed as bias %) and precision (expressed as RSDr %) calculated by the applicant for the four methods on the stack DNA. Means are the average of sixteen replicates obtained through four runs on ABI7900. Percentages are expressed as GM DNA / total DNA x 100.

Table 1. Trueness (expressed as bias %) and precision (expressed as relative repeatability standard deviation, RSD_r %) for the Bt11, MIR162, MIR604 and GA21 methods applied to stack maize Bt11 x MIR162 x MIR604 x GA21 DNA.

Bt11			
Unknown sample GM%	Expected value (GMO %)		
	0.08	0.90	5.00
Mean	0.096	0.92	5.4
RSD _r (%)	14.3	12	6.7
Bias (%)	20	2.2	8.0
MIR162			
Unknown sample GM%	Expected value (GMO %)		
	0.08	0.90	5.00
Mean	0.075	0.95	5.2
RSD _r (%)	14	8.2	9.4
Bias (%)	-6.3	5.6	4.0
MIR604			
Unknown sample GM%	Expected value (GMO %)		
	0.08	0.90	5.00
Mean	0.074	0.88	5.2
RSD _r (%)	18	7.6	8.3
Bias (%)	-7.5	-2.2	4.0
GA21			
Unknown sample GM%	Expected value (GMO %)		
	0.08	0.90	5.00
Mean	0.076	0.92	5.1
RSD _r (%)	9.5	9.9	12
Bias (%)	-5.0	2.2	2.0

The EU-RL GMFF verified the data and concluded that they were reliable and seemed to confirm that the methods meet the ENGL performance criteria ⁽³⁾.

No further requests of complementary information were addressed to the applicant; therefore the dossier was moved to step 3.

4. Step 3 (EU-RL GMFF experimental testing)

In step 3 the EU-RL GMFF implemented all four methods in its own laboratory and performed a verification of their performance when applied to DNA extracted from GM maize stack Bt11 x MIR162 x MIR604 x GA21 maize.

4.1 Materials

The following control samples were provided by the applicant:

- genomic DNA extracted from homogenised seeds of Bt11 x MIR162 x MIR604 x GA21 maize
- genomic DNA extracted from homogenised seeds of non-GM maize.

Test samples containing mixtures of GM maize stack Bt11 x MIR162 x MIR604 x GA21 and non-GM maize genomic DNA at different GMO concentrations were prepared by the EU-RL GMFF in a constant amount of background total maize DNA.

The protocols (reagents, concentrations, primer/probe sequences) described by the applicant were implemented precisely in the EU-RL GMFF laboratory. The *in-house* verification followed exactly the protocols already published as validated methods for the individual Bt11, MIR162, MIR604, and GA21 single line events (available at <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>), with the small modifications described in section 4.5. (deviations from the single-line validated methods).

Table 2 shows the five GM levels used in the verification of the Bt11, MIR162, MIR604, and GA21 methods.

Table 2. Percentage of Bt11, MIR162, MIR604, and GA21 in Bt11 x MIR162 x MIR604 x GA21 in the verification samples.

Bt11 GM % (GM DNA / total DNA x 100)	MIR162 GM % (GM DNA / total DNA x 100)	MIR604 GM % (GM DNA / total DNA x 100)	GA21 GM % (GM DNA / total DNA x 100)
0.045	0.10	0.10	0.09
0.20	0.40	0.40	0.50
0.45	0.90	0.90	0.90
2.50	2.00	2.50	5.00
4.00	5.00	6.00	8.00

4.2 DNA extraction

A method for DNA extraction from maize seeds and grains was previously evaluated by the EU-RL GMFF with regard to its performance characteristics and was considered valid i.e. fit for the purpose of providing maize DNA of appropriate quality and amount for being used in subsequent PCR experiments. The protocol for the DNA extraction method is available at http://gmo-crl.jrc.ec.europa.eu/summaries/GA21%20Syng_DNAExtr_report.pdf.

Consequently, the EU-RL GMFF did not verify the DNA extraction method proposed by the applicant.

4.3 Experimental design

Eight runs for each method were carried out. In each run, samples were analysed in parallel with both the GM specific system and the reference system *Adh1* (*alcohol dehydrogenase 1*). Five GM levels were examined per run, for each GM level in duplicate. In total, for each method (Bt11, MIR162, MIR604, and GA21), the quantification of the five GM levels was calculated as an average of sixteen replicates per GM level (8 runs x 2 replicated levels per run). An Excel spreadsheet was used for determination of GM%.

4.4 PCR methods

During the verification study the EU-RL GMFF carried out tests on DNA extracted from GM maize stack Bt11 x MIR162 x MIR604 x GA21 maize using the methods previously validated for the respective single GM events Bt11, MIR162, MIR604, and GA21.

For the detection of GM maize events Bt11, MIR162, MIR604, and GA21, DNA fragments of 68-bp, 92-bp, 76-bp and 101-bp respectively are amplified using specific primers. PCR products are measured during each cycle (real-time) by means of target-specific oligonucleotide probes labelled with two fluorescent dyes: FAM (6-carboxyfluorescein) is used as reporter dye at its 5'-end and TAMRA (6-carboxytetramethylrhodamine) as a quencher dye at its 3'-end.

For quantification of GM maize events Bt11, MIR162, MIR604, and GA21, a taxon-specific reference system amplifies a 135-bp fragment of *Adh1* (*alcohol dehydrogenase 1*) maize endogenous gene (GenBank N. X04050), using two *Adh1* gene-specific primers and one *Adh1* gene-specific probe labelled with VIC and TAMRA.

For quantification of GM maize events Bt11, MIR162, MIR604, and GA21 DNA, respectively in a separate test samples, the normalised ΔCt values of calibration samples are used to calculate, by linear regression, standard curves (plotting ΔCt values against the logarithm of the amounts of Bt11, MIR162, MIR604, and GA21 events DNA, respectively). The normalised ΔCt values of the unknown samples are measured and, by means of the regression formula, the relative amount of Bt11, MIR162, MIR604, and GA21 events, respectively, are estimated.

For detailed information on the preparation of the respective standard curve calibration samples please refer to the protocols of the validated methods at <http://gmo-crl.jrc.ec.europa.eu/>.

4.5 Deviations from the validated methods

As indicated in the validated methods for Bt11, MIR162, MIR604 and GA21 (single events), sulforhodamine was added to the Sigma JumpStart Taq Ready Mix. The final sulforhodamine concentration specified in the validated protocols for the MIR604 and GA21 event-specific methods (i.e. 150 nM) was doubled by the applicant in order to get passive reference

fluorescence values clearly above the background for the ABI PRISM® 7900 HT sequence detection system used in the testing.

The final sulforhodamine concentration (i.e. 300 nM) used by the applicant for the detection of MIR604 and GA21 events in stack maize Bt11 x MIR162 x MIR604 x GA21 corresponds to the one specified in the Bt11 and MIR162 protocols.

4.6 Results

The values of the slopes of the different standard curves performed by the EU-RL GMFF, from which the PCR efficiency is calculated using the formula $[10(-1/\text{slope}) - 1] \times 100$, and of the R^2 (expressing the linearity of the regression) reported for all PCR systems in the eight runs, are presented in Tables 3, 4, 5, and 6 for GM maize events Bt11, MIR162, MIR604, and GA21, respectively.

Table 3. Values of standard curve slope, PCR efficiency and linearity (R^2) for the Bt11 method on Bt11 x MIR162 x MIR604 x GA21.

Run	Bt11		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.48	94	1.00
2	-3.33	100	1.00
3	-3.37	98	1.00
4	-3.29	101	1.00
5	-3.42	96	1.00
6	-3.46	95	1.00
7	-3.29	101	1.00
8	-3.47	94	1.00
Mean	-3.39	97	1.00

Table 4. Values of standard curve slope, PCR efficiency and linearity (R^2) for the MIR162 method on Bt11 x MIR162 x MIR604 x GA21.

Run	MIR162		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.17	107	1.00
2	-3.24	104	1.00
3	-3.35	99	1.00
4	-3.27	102	1.00
5	-3.2	105	1.00
6	-3.24	104	1.00
7	-3.15	108	1.00
8	-3.2	105	1.00
Mean	-3.23	104	1.00

Table 5. Values of standard curve slope, PCR efficiency and linearity (R^2) for the MIR604 method on Bt11 x MIR162 x MIR604 x GA21.

Run	MIR604		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.24	104	1.00
2	-3.35	99	1.00
3	-3.26	103	1.00
4	-3.14	108	1.00
5	-3.21	105	1.00
6	-3.26	103	1.00
7	-3.22	104	1.00
8	-3.46	95	1.00
Mean	-3.27	102	1.00

Table 6. Values of standard curve slope, PCR efficiency and linearity (R^2) for the GA21 method on Bt11 x MIR162 x MIR604 x GA21.

Run	GA21		
	Slope	PCR Efficiency (%)	Linearity (R^2)
1	-3.39	97	1.00
2	-3.28	102	1.00
3	-3.43	96	1.00
4	-3.16	107	1.00
5	-3.35	99	1.00
6	-3.36	98	1.00
7	-3.32	100	1.00
8	-3.33	100	1.00
Mean	-3.33	100	1.00

The mean PCR efficiencies of the calibration curves for each of the four event-specific methods were above 90% (97% for Bt11, 104% for MIR162, 102% for MIR604 and 100% for GA21, respectively). The linearity of the methods (R^2) was 1.00 for all four methods. The data presented in Tables 3, 4, 5 and 6 are in line with the data presented by the applicant and confirm the appropriate performance characteristics of the four methods when tested on DNA extracted from the stacked GMO Bt11 x MIR162 x MIR604 x GA21 in terms of PCR efficiency and linearity.

The EU-RL GMFF also assessed the values of trueness and precision (expressed as RSD_r %, relative repeatability standard deviation) of the four methods applied to samples of DNA extracted from GM maize stack Bt11 x MIR162 x MIR604 x GA21.

Tables 7 to 10 report the trueness and precision for each GM level for each of the four methods.

Table 7. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the Bt11 method applied to Bt11 x MIR162 x MIR604 x GA21 maize DNA.

Bt11					
Unknown sample GM%	Expected value (GMO%)				
	0.045	0.20	0.45	2.50	4.00
Mean	0.043	0.19	0.43	2.42	3.90
SD	0.006	0.02	0.02	0.07	0.18
RSD _r (%)	14.5	10.0	4.7	3.0	4.7
Bias (%)	-4.8	-4.5	-3.6	-3.1	-2.5

Table 8. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation of the MIR162 method applied to Bt11 x MIR162 x MIR604 x GA21 maize DNA.

MIR162					
Unknown sample GM%	Expected value (GMO%)				
	0.10	0.40	0.90	2.00	5.00
Mean	0.09	0.39	0.86	1.94	5.13
SD	0.008	0.04	0.04	0.11	0.32
RSD _r (%)	9.4	10.9	4.2	5.8	6.2
Bias (%)	-8.4	-2.7	-4.2	-3.0	2.6

Table 9. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the MIR604 method applied to Bt11 x MIR162 x MIR604 x GA21 maize DNA.

MIR604					
Unknown sample GM%	Expected value (GMO%)				
	0.10	0.40	0.90	2.50	6.00
Mean	0.09	0.39	0.86	2.51	6.12
SD	0.015	0.04	0.05	0.14	0.30
RSD _r (%)	16.3	9.0	6.3	5.7	5.0
Bias (%)	-5.8	-1.4	-4.6	0.4	2.1

Table 10. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSD_r %) of the GA21 method applied to Bt11 x MIR162 x MIR604 x GA21 maize DNA.

GA21					
Unknown sample GM%	Expected value (GMO%)				
	0.09	0.50	0.90	5.00	8.00
Mean	0.09	0.50	0.90	4.89	7.51
SD	0.010	0.03	0.06	0.31	0.58
RSD _r (%)	10.8	6.6	7.1	6.4	7.8
Bias (%)	0.04	-0.5	0.1	-2.2	-6.2

The trueness of the method is estimated using the measurements of the method bias for each GM level. According to the ENGL acceptance criteria and method performance requirements, the trueness of the method, measured as bias from the expected value, should be $\pm 25\%$ across the entire dynamic range. As shown in Tables 7, 8, 9 and 10, the values range from -4.8% to -2.5% for Bt11, from -8.4% to 2.6% for MIR162, from -5.8% to 2.1% for MIR604 and from -6.2% to 0.1% for GA21. Therefore, the four methods satisfy the above mentioned requirement throughout their respective dynamic ranges.

Tables 7, 8, 9 and 10 also document the relative repeatability standard deviation (RSD_r) for each GM level. As indicated by the ENGL, the EU-RL GMFF requires RSD_r values to be below 25%. As it can be observed from Tables 7 to 10, the values range between 3.0% and 14.5% for Bt11, between 4.2% and 10.9% for MIR162, between 5.0% and 16.3% for MIR604 and between 6.4% and 10.8% for GA21. Therefore, the four methods satisfy this requirement throughout their respective dynamic ranges when applied to stack-derived DNA.

5. Comparison of method performance on Bt11 x MIR162 x MIR604 x GA21 and on the single events

An indicative comparison of the performance of the four methods applied to GM maize stack Bt11 x MIR162 x MIR604 x GA21 and on the single events is shown in Tables 11, 12, 13 and 14. The performance of the methods on the single lines was previously validated through international collaborative trials (<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>).

The comparison of data generated in different testing conditions and different times is intended to be only of qualitative nature; differences in the figures reported are not necessarily statistically significant.

Table 11. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the Bt11 detection method applied to Bt11 x MIR162 x MIR604 x GA21 and to the single event Bt11.

Trueness and repeatability of Bt11 quantification on Bt11 x MIR162 x MIR604 x GA21			Trueness and repeatability of Bt11 quantification on single event Bt11*		
GM%	Bias (%)	RSD_r (%)	GM%	Bias (%)	RSD_r (%)
0.09	-4.8	14.5	0.09	2.2	17
0.40	-4.5	10.0	0.40	-1.9	13
0.90	-3.6	4.7	0.90	1.8	11
5.00	-3.1	3.0	5.00	-5.2	13
8.00	-2.5	4.7	8.00	-1.2	9

*method validated in inter-laboratory study (<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>)

Table 12. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the MIR162 detection method applied to Bt11 x MIR162 x MIR604 x GA21 and to the single event MIR162.

Trueness and repeatability of MIR162 quantification on Bt11 x MIR162 x MIR604 x GA21			Trueness and repeatability of MIR162 quantification on single event MIR162*		
GM%	Bias (%)	RSD _r (%)	GM%	Bias (%)	RSD _r (%)
0.10	-8.4	9.4	0.10	0.2	13
0.40	-2.7	10.9	0.40	2.9	12
0.90	-4.2	4.2	0.90	-1.7	12
2.00	-3.0	5.8	2.00	1.4	10
5.00	2.6	6.2	5.00	4.3	8

*method validated in inter-laboratory study (<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>)

Table 13. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the MIR604 detection method applied to Bt11 x MIR162 x MIR604 x GA21 and to the single event MIR604.

Trueness and repeatability of MIR604 quantification on Bt11 x MIR162 x MIR604 x GA21			Trueness and repeatability of MIR604 quantification on single event MIR604*		
GM%	Bias (%)	RSD _r (%)	GM%	Bias (%)	RSD _r (%)
0.10	-5.8	16.3	0.10	3.6	24
0.40	-1.4	9.0	0.40	3.1	17
0.90	-4.6	6.3	0.90	-1.0	12
2.00	0.4	5.7	2.00	0.7	16
5.00	2.1	5.0	5.00	-3.6	14

*method validated in inter-laboratory study (<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>)

Table 14. Trueness (bias %) and relative repeatability standard deviation (RSD_r %) of the GA21 detection method applied to Bt11 x MIR162 x MIR604 x GA21 and to the single event GA21.

Trueness and repeatability of GA21 quantification on Bt11 x MIR162 x MIR604 x GA21			Trueness and repeatability of GA21 quantification on single event GA21*		
GM%	Bias (%)	RSD_r (%)	GM%	Bias (%)	RSD_r (%)
0.09	0.04	10.8	0.09	-8.7	23
0.50	-0.5	6.6	0.50	0.8	17
0.90	0.1	7.1	0.90	1.6	20
5.00	-2.2	6.4	5.00	-5.6	20
8.00	-6.2	7.8	8.00	-8.5	17

*method validated in inter-laboratory study (<http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>)

The trueness of the four event-specific methods when applied to GM maize stack Bt11 x MIR162 x MIR604 x GA21 maize is within the acceptance range set by ENGL ($\pm 25\%$) for the whole dynamic ranges studied.

The relative repeatability standard deviation (RSD_r %) of the four event-specific methods when applied to GM maize stack Bt11 x MIR162 x MIR604 x GA21 maize are below the ENGL acceptance level established at maximum 25%.

The data presented in Tables 11, 12, 13 and 14 show that the methods perform according to the ENGL performance criteria when applied to DNA extracted from the maize single events Bt11, MIR162, MIR604 and GA21 and to the DNA extracted from GM maize stack Bt11 x MIR162 x MIR604 x GA21 maize.

6. Conclusions

The performance of the four event-specific methods for the detection and quantification of maize events Bt11, MIR162, MIR604 and GA21, when applied to DNA extracted from GM maize stack Bt11 x MIR162 x MIR604 x GA21, meets the ENGL performance requirements, as assessed on the control samples provided by the applicant.

The method verification has demonstrated that the PCR efficiency, linearity, trueness and repeatability of the methods were within the limits established by the ENGL.

In conclusion, the verification study confirmed that the four methods are capable to detect, identify and quantify each of the GM events when applied to genomic DNA of suitable quality, extracted from GM maize stack Bt11 x MIR162 x MIR604 x GA21.

Therefore these methods, developed and validated to detect and quantify the single events, can be equally applied for the quantification of the respective events combined in GM maize stack Bt11 x MIR162 x MIR604 x GA21.

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Title: Report on the Verification of the Performance of Bt11, MIR162, MIR604 and GA21 Event-specific PCR-based Methods Applied to DNA Extracted from Stack Maize Bt11 x MIR162 x MIR604 x GA21

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Abstract

An application was submitted by Syngenta Crop Protection AG to request the authorisation of genetically modified Bt11 x MIR162 x MIR604 x GA21 maize (resistant to lepidopteran and coleopteran pests, able to utilise mannose as the only primary carbon source and tolerant to glufosinate ammonium and glyphosate) and all sub-combinations of the individual events as present in the segregating progeny, for food and feed uses, and import and processing, in accordance with articles 5 and 17 of Regulation (EC) No 1829/2003 GM Food and GM Feed. The unique identifier assigned to Bt11 x MIR162 x MIR604 x GA21 maize is SYN-BT011-1x YN-IR162-4xSYN-IR604-5xMON-00021-9.

The genetically modified maize line Bt11 x MIR162 x MIR604 x GA21 maize has been obtained by conventional crossing of four genetically modified maize events: Bt11, MIR162, MIR604, and GA21 without any new genetic modification.

The EU-RL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events Bt11, MIR162, MIR604 and GA21 (see <http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx>). In line with the approach defined by the ENGL (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf) the EU-RL GMFF therefore has carried out only an in-house verification of the performance of each validated method when applied to DNA extracted from Bt11 x MIR162 x MIR604 x GA21.

The hereby reported the in-house verification study lead to the conclusion that the individual methods meet the ENGL requirements also when applied to DNA extracted from the GM maize stack Bt11 x MIR162 x MIR604 x GA21.

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