

JRC VALIDATED METHODS, REFERENCE METHODS AND MEASUREMENTS REPORT

Report on the In-house Validation of a DNA Extraction Method from Maize Grains and Validated Method

Validation report

European Union Reference Laboratory for
Genetically Modified Food and Feed

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Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit



Report on the In-house Validation of a DNA Extraction Method from Maize Grains and Validated Method

21 June 2016

European Union Reference Laboratory for GM Food and Feed

Executive Summary

In accordance with relevant EU legislation^a, Genective SA provided to the European Union Reference Laboratory for GM Food and Feed (EURL GMFF) a DNA extraction method for maize and the relevant samples (maize seeds).

In line with its mandate^b, the EURL GMFF has conducted an in-house validation of this DNA extraction method. To this end, it tested the DNA extraction method on the samples provided and evaluated its performance in terms of DNA yield, integrity and quality.

The in-house validation study confirmed that the method meets the method performance requirements as established by the EURL GMFF and the ENGL^c, and that it satisfies the provisions of Annex I-2.C.2 to Regulation (EC) No 641/2004 and of Annex III to Regulation (EU) No 503/2013.

The method is therefore fit for the purpose of producing maize DNA of suitable quantity and quality for subsequent PCR-based analysis when applied to maize seeds, grain, or flour. In case of other, more complex or difficult samples, a thorough verification of the DNA quality is recommended.

^a Regulation (EC) No 641/2004 of 6 April 2004 "on detailed rules for the implementation of Regulation (EC) No 1829/2003".

^b Regulation (EC) No 1829/2003 of 22 September 2003 "on genetically modified food and feed".

^c Definition of minimum performance requirements for analytical methods of GMO testing. http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf

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

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 SGS.

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1. Introduction

This report describes the validation of a 'CTAB-based' DNA extraction method ⁽¹⁻³⁾ followed by further purification of the extracted DNA by applying the commercially available Wizard[®] mini column DNA clean-up system (Promega). Its applicability on the samples of food and feed provided by the applicant was studied. This protocol can be used for the extraction of DNA from maize seeds, grain and flour.

The purpose of the DNA extraction method described is to provide DNA with purity and quantity suitable for real-time PCR based detection methods.

This protocol is recommended to be executed only by skilled laboratory personnel as the procedures comprise the use of hazardous chemicals and materials. It is strongly advised to take particular notice of products safety recommendations and guidelines.

2. Materials (Equipment/Chemicals/Plastic ware)

2.1. Equipment

The equipment used in the DNA extraction procedure is reported here below (equivalents may be used).

1. Centrifuges (EURL: Eppendorf 5810R and 5415D; notifier: Cooled centrifuge Sigma 4K15C combined with rotor 1150, bucket 11350 and rotor 12169 and microcentrifuge Eppendorf 5451C)
2. Incubator (EURL: thermomixer: Eppendorf Thermo-mixer Comfort 5355; notifier: Dry incubator FED 53 WTB Binder Labtechnic)
3. Shaker (EURL: LabLine Enviro 3527 and Vortex shaker MS1 Minishaker IKA; notifier: Vortex shaker, any model appropriate)
4. Water bath (EURL: Bibby Scientific Ltd, Stuart SWB3D; notifier: Julabo MWB)
5. Thermometer (EURL: VWR 61222-504; notifier: Fisher Emergo 5-8-822)
6. Balances (EURL: Mettler Toledo XS2002S; notifier: Sartorius DP4100S)
7. Pipettes (Gilson P1000 and P200)
8. Vacuum (EURL: Vacufuge Eppendorf 5301 22 82 010-9; notifier: Accu- Jet[®] any appropriate model and vacuum pump: Welch EB06/1560)
9. Freezer -20°C and Fridge 4°C (any model appropriate)
10. Fume hood (any model appropriate)
11. Vacuum manifold (EURL: Vac-Man[®] Promega; notifier: Qiagen Qiavac 24)

2.2. Plastic ware

The following plastic ware was used:

1. 50 mL conical polypropylene tubes (EURL: Corning 430290; notifier: Greiner Cat. No 227261)
2. Other conical polypropylene tubes (EURL: 13 mL Sarstedt tubes Cat. No 60.540; notifier: 12 mL Greiner tubes Cat. No 227261)
3. 1.5 microcentrifuge tubes (Eppendorf Cat. No 0030 120.086)
4. Filter tips (Greiner P200 Cat. No 739288 and Greiner P1000 Cat. No 740288)
5. 10 mL pipettes (Greiner Cat. No 607180)
6. 25 mL pipettes (Greiner Cat. No 760180)

Note: all plastic ware should be sterile and free of DNases, RNases and nucleic acids.

2.3. Chemicals

The following chemicals were used in the DNA extraction procedure described (equivalents may be used):

1. 25:24:1 phenol:chloroform:isoamylalcohol (Sigma P-3803)
2. 24:1 chloroform isoamylalcohol (Sigma C-0549)
3. Ammonium acetate 7.5 M (Sigma A2706)
4. Hexadecyltrimethylammonium bromide (CTAB Sigma H6269)
5. Sodium Chloride 5 M (5 M NaCl Sigma S5150)
6. 0.5M EDTA Buffer, pH 8.0 (Invitrogen 15575020)
7. Tris HCl 1M stock solution pH 8.0 (EURL: FLUKA 93283; notifier: Sigma T3038)
8. 2-mercaptoethanol (EURL: Sigma M3148; notifier: Bio-Rad 161-0710)
9. RNase A (EURL: Sigma R6513; notifier: Roche 10109169001)
10. Proteinase K (EURL: Sigma P2308; notifier: Roche 03115836001)
11. Isopropanol (2-propanol. EURL: Sigma I9516; notifier: Merck 1009634)
12. Ethanol absolute (EURL: FLUKA 02860; notifier: Merck 100983)
13. Distilled water DNase/RNase free (EURL: Promega P119C; notifier: Gibco/Invitrogen 10977)
14. Wizard[®] DNA Clean-Up System (Promega A7280)

2.4. Solutions

The following buffers and solutions are suggested for use in the DNA extraction procedure by the notifier:

1. **CTAB Extraction Buffer (2%)** (prepare fresh before use)
 - 2% w/v CTAB
 - 100 mM Tris HCl pH 8.0
 - 20 mM EDTA pH 8.0
 - 1.4 M NaCl
2. **Tris-EDTA buffer (TE 1X)**
 - 10 mM Tris HCl pH 8.0
 - 1 mM EDTA pH 8.0

3. **Proteinase K (10 mg/mL), store at -20°C**
4. **RNase A (10 mg/mL), store at -20°C**
5. **Isopropanol 80% (v/v), store at room temperature**
6. **Ethanol 70% (v/v), store at room temperature**

2.5. Precautions

- The protocol is recommended for use by skilled personnel only because of the use of hazardous chemicals and materials
- Consideration of notice of products and operating instructions safety recommendations and guidelines is strongly recommended.
- Phenol, Chloroform, isoamyl alcohol and isopropanol are hazardous chemicals; therefore, all manipulations have to be performed according to safety guidelines, under a fume hood.
- Maintain strictly separate working areas for DNA extraction, PCR set-up and amplifications.
- All equipment and lab benches should be free of DNA residues.
- It is recommended to use clean containers for Waring blenders for grinding the seed bulk samples.
- All tubes and pipette tips have to be discarded as biological hazardous material.

2.6 Abbreviations

EDTA	ethylenediaminetetraacetic acid
PCR	polymerase chain reaction
RNase A	ribonuclease A
TE	Tris EDTA
Tris	Tris(hydroxymethyl)aminomethane
CTAB	cetyltrimethylammonium bromide

3. Description of the method

3.1 Scope and applicability

The method for DNA extraction described below is suitable for the isolation of high quality genomic DNA from maize seeds, grains and flour. Application of the method to other matrices may require adaptation and possible further specific validation. In all cases a thorough verification of the quality of the extracted genomic DNA is recommended.

3.2 Practicability

The DNA extraction method described below requires only standard molecular biology equipment, e.g. a centrifuge, an incubator and pipettes.

3.3 Principle

The basic principle of the DNA extraction consists of first releasing the DNA present in the matrix into aqueous solution and further purifying the DNA from PCR inhibitors. The present method starts with a lysis step (terminal lyses in the presence of Tris HCl, EDTA, CTAB and 2-mercaptoethanol) followed by removal of contaminants such as polysaccharides, polyphenols and proteins by extraction with phenol and chloroform.

DNA precipitates are then generated by using isopropanol and ethanol precipitation. The pellet is dissolved in low salt buffer. Remaining inhibitors are removed with a Wizard[®] mini column DNA clean up system. The cleaned DNA is re-suspended in H₂O to be used for further PCR applications.

3.4 Extraction of genomic DNA from maize seeds or grains

1. Weight out 5 grams of ground maize seeds into a 50 mL conical tube.
2. Add to each sample a 25 mL solution containing 24.25 mL pre-warmed CTAB extraction buffer, 0.5 mL 2-mercaptoethanol, and 0.25 mL of 10 mg/mL proteinase K (final concentration 2% 2-mercaptoethanol and 100 µg/mL proteinase K).
3. Incubate for 60 min at 65°C. After 30 minutes of incubation, vigorously mix the tube on the vortex at maximum speed for 20 seconds.
Make sure that the exhaust is open and placed over the incubator exhaust.
4. Cool the tube on the bench for 5 minutes.
5. Add 20 mL of phenol:chloroform:isoamylalcohol (PCI:25:24:1, pH 6.7). Cap the tube and mix vigorously on a vortex at maximum speed for 20 seconds.
6. Centrifuge for 40 minutes at 3400 rpm at room temperature (to separate the aqueous and organic phases).
Make sure that the exhaust is open and placed over the centrifuge.
7. Transfer 19.0-19.5 mL upper aqueous phase to a new 50 mL conical tube.
8. Add 14 mL of -20°C isopropanol and mix gently by inversion.
Set the centrifuge at 4°C and let it rotate empty for 10 minutes. This step is done to cool down the centrifuge.
9. To precipitate the DNA place the tube at -20°C for 20 minutes.
10. To pellet the DNA, centrifuge the tubes at 3399 rcf for 40 minutes at 4°C in the swinging rotor bucket.
11. Remove isopropanol by pouring in a waste bottle.
12. Perform a quick spin (1 minute, 3399 rcf) to bring down the residue isopropanol and pipette with a P1000 to leave the pellet dry.
13. Perform a second quick spin (1 minute, 3399 rcf) to bring down the final residue isopropanol and pipette with a P200 to leave pellet dry.

14. Dry the pellet at room temperature for 10 minutes at room temperature (no longer)
Because the pellet still contains a lot of polysaccharides it is difficult to dissolve the pellet in TE. Therefore the rigorous dissolving steps below need to be strictly followed. Some small precipitates may still be visible by the end of the dissolving procedure. Even with small precipitates, the DNA solution can be used for further chloroform extraction clean-up.
15. Loosen the pellet from the tube by pipetting 1 mL pre-warmed (65°C) TE pH 8.0 on the pellet.
16. Vortex for 1 minute to partially dissolve the pellet.
17. Incubate the pellet for 15 minutes at 65°C.
18. Add 1 mL pre-warmed (65°C) TE pH 8.0.
19. Vortex again for 30 seconds to 1 minute to further dissolve the pellet
20. Incubate the pellet a second time for 15 minutes at 65°C
21. Add 2 mL of pre-warmed (65°C) TE pH 8.0
22. Vortex again for 30 seconds to 1 minute to further dissolve the pellet.
23. Incubate the pellet a final time for 10 minutes at 65°C
24. Vortex briefly and add 9 mL of pre-warmed (65°C) TE pH 8.0 and mix again
25. Cool down the tubes for 5 minutes at room temperature.
26. Add 40 µl of 10 mg/mL RNase A and incubate for 30 minutes in a 37°C water bath.
Prepare the high speed rotor in the centrifuge. Set the rotor to "rotor 12169", the speed to 13000 rcf and the time to 10 minutes.
27. Perform chloroform extraction by adding 12 mL of chloroform:isoamylalcohol (24:1) to the 13 mL DNA solution.
Make sure to tape the tube caps with Parafilm to avoid spilling of the chloroform when extracting.
28. Mix vigorously by inversion for 60 seconds and centrifuge for 10 minutes 13000 rcf at room temperature
29. Transfer the upper aqueous phase (12 mL) to a clean 50 mL tube
30. Repeat chloroform extraction step 26
31. Transfer the upper aqueous phase (11-11.5 mL) to a clean 50 mL tube.
32. Add half a volume (5.5 mL) of 7.5M ammonium acetate
33. Gently mix by inversion
34. Add two volumes (22 mL) of absolute ethanol
35. Mix by inversion (10 times) and place tubes at -20°C for 30 minutes.
Set the centrifuge at 4°C and let it rotate empty for 10 minutes. This step is only to cool down the centrifuge.
36. Centrifuge at 13000 rcf to 10 minutes at 4°C to pellet the DNA.
37. Rinse the pellet twice with 20 mL of 70% ethanol, loosening the pellet from the side of the tube.
38. After the second wash step remove the residual ethanol by using a short spin (10 seconds) and using a P1000 pipette to eliminate the residual ethanol.
39. Dry the pellet at 65°C for maximum of 10 minutes.
40. Loosen the pellet from the tube by pipetting 1 mL pre-warmed (65°C) TE pH 8.0 on the pellet.

41. Vortex the pellet for 30 seconds to 1 minute to partially dissolve the pellet.
42. Incubate the pellet for 15 min at 65°C
43. Add 1 mL pre-warmed (65°C) TE.
44. Vortex briefly to further dissolve the pellet
45. Place the DNA overnight at 4°C to further dissolve
46. Transfer the 2 mL DNA solution into a clean 15 mL tube
47. Centrifuge at 15 000 rcf for 10 minutes at room temperature to pellet the precipitate
48. Transfer the DNA solution to a clean 2.0 mL microcentrifuge tube without disturbing the pellet and store the DNA at 4°C

3.5 Wizard® clean-up kit for the purification of DNA

- The binding capacity of 1 mL of resin is approximately 20 µg of DNA
 - The sample volume must be between 50 and 500 µl, the sample volume of this experiment was 100µl
 - The resin must be thoroughly mixed before use. If crystals or aggregates are present, dissolve them by warming the resin to 37°C for 10 minutes. Cool to 25-30°C before use
 - Use one Wizard mini column for each sample
1. Attach the provided syringe to the Luer-Lock extension of each mini column and insert the column into the vacuum manifold
 2. Add 1 mL of resin to 1.5 mL microcentrifuge tube, add 100 µl sample to the resin and mix by inverting several times
 3. Transfer the resin/DNA mix into the syringe barrel. Apply a vacuum to draw the solution through the mini column. Break the vacuum to the mini column.
 4. Add 2 mL of 80% isopropanol to the syringe barrel, and re-apply a vacuum to draw the solution through the mini column
 5. Dry the resin by continuing to draw the vacuum for 30 seconds after the solution had been pulled through the mini column (no more than 30 seconds)
 6. Transfer the mini column to a 1.5 mL micro centrifuge tube and centrifuge for 2 minutes at maximum speed (10 000x *g* or rcf) to remove residual isopropanol
 7. Transfer the mini column to a new 1.5 mL micro centrifuge tube
 8. Apply 50 µl of pre-warmed (65°C) MilliQ
 9. Incubate for 1 minute
 10. Centrifuge the mini column for 20 seconds at 10 000x *g* or rcf to elute the bound DNA
 11. Remove and discard the mini column. The purified DNA may be stored at 4°C or -20°C.

4. Testing of the DNA extraction method by the EU-RL GMFF

The EU-RL GMFF tested the method proposed by the notifier and described above, on samples consisting of flour provided by the notifier. DNA extraction procedures should result in repeatable recovery, fragmentation profile, concentration and PCR quality of DNA extracts.

The extracted DNA should be of suitable quantity and quality for the intended purpose^d.

4.1 DNA extraction

Five kg of conventional flour were received by the applicant. DNA was extracted from six test portions (replicates), each one composed of 5 g, following the method described in paragraph 3.4 "Extraction of genomic DNA from maize seeds or grains". The procedure was repeated over three days, for a total of 18 DNA extractions.

4.2 DNA concentration, yield and repeatability

The concentration of the extracted DNA solutions was determined by fluorescence detection using the PicoGreen dsDNA Quantitation Kit (Molecular Probes).

Each DNA extract was measured in triplicate, and the three values were averaged. DNA concentration was determined on the basis of a five point standard curve ranging from 1 to 500 ng/ μ L using a Biorad VersaFluor fluorometer.

The average DNA concentration and the yield are reported in Table 1 below.

Table 1. Overall DNA concentration (ng/ μ L) and yield of extracted samples

	Concentration (ng/μL)	Yield (μg)
Overall average	171.83	1.72
Standard deviation of all samples	14.27	0.14
Coefficient of variation (%)	8.31	8.31

Table 2 reports the data of DNA concentration and yield for the 18 individual extracted samples.

^d EURL/ENGL guidance document "Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing" (<http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>)

Table 2. DNA concentration (ng/ μ L) and yield of extracted samples

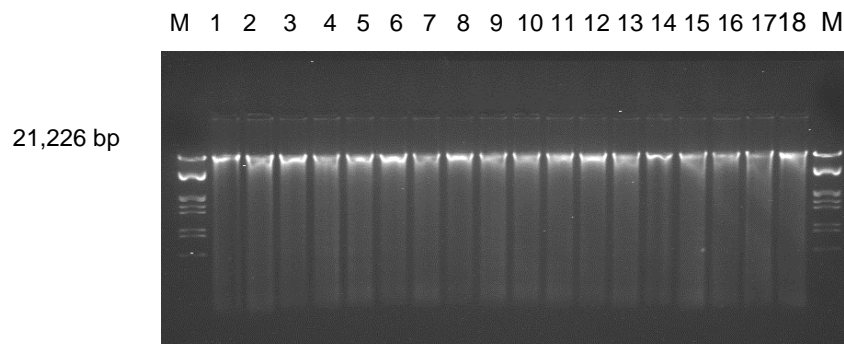
Sample	Concentration (ng/ μ L)	Yield (μ g)
1	166.0	1.66
2	162.0	1.62
3	185.0	1.85
4	178.0	1.78
5	170.0	1.70
6	165.0	1.65
7	154.0	1.54
8	156.0	1.56
9	185.0	1.85
10	165.0	1.65
11	167.0	1.67
12	158.0	1.58
13	168.0	1.68
14	165.0	1.65
15	213.0	2.13
16	184.0	1.84
17	184.0	1.84
18	168.0	1.68

Note: In yellow boxes samples extracted on day 1; in green boxes samples extracted on day 2; in blue boxes samples extracted on day 3.

4.3 DNA Fragmentation

The size of the extracted DNA was evaluated by analysing it on a 1.0% agarose gel electrophoresis, to check that the DNA is not excessively fragmented for subsequent analyses. On the agarose gel, 4 μ L of the DNA solutions are loaded (Figure 1).

Figure 1. Agarose gel electrophoresis of eighteen genomic DNA samples extracted from maize flour



Lanes 1-6: samples extracted on day 1, lanes 7-12: samples extracted on day 2, lanes 13-18: samples extracted on day 3; M: Lambda DNA/*EcoRI*+*HindIII* molecular weight markers.

The extracted genomic DNA samples appeared as distinct high molecular weight DNA fluorescent banding patterns migrating through the gel. None of the DNA samples showed indication of significant degradation.

4.4 Purity / Absence of PCR inhibitors

In order to assess the purity and to conduct a test for the presence of PCR inhibitors, the extracted DNA solutions were adjusted to a concentration of 40 ng/ μ L (hereafter referred as "undiluted" samples).

Subsequently, fourfold serial dilutions (1:4, 1:16, 1:64, 1:256) of each extract were prepared with TE low buffer (1 mM Tris, 10 μ M EDTA, pH 8.0) and analysed in triplicate using a real-time PCR system detecting the target sequence of the endogenous gene aldolase. The C_q values obtained for "undiluted" and diluted DNA samples are reported in Table 3.

Table 3. Cq values of undiluted and fourfold serially diluted DNA extracts after amplification of maize gene *aldolase*

Cq values					
DNA extract	Undiluted (30 ng/ μ L)	Diluted extracts			
		1:4	1:16	1:64	1:256
1	23.83	25.46	27.04	29.25	31.25
2	24.18	25.67	27.45	29.39	31.44
3	23.98	25.63	27.35	29.46	31.46
4	23.77	25.33	27.24	29.17	31.40
5	23.85	25.53	27.25	29.53	31.48
6	23.76	25.59	27.56	29.47	31.63
7	23.73	25.47	27.24	29.45	31.39
8	23.79	25.69	27.41	29.63	31.35
9	23.75	25.58	27.25	29.44	31.42
10	23.95	25.52	27.57	29.54	31.52
11	23.81	25.40	27.31	29.31	31.38
12	23.71	25.32	27.19	29.25	31.44
13	23.42	25.26	27.14	29.24	31.50
14	23.96	25.67	27.50	29.61	31.76
15	24.21	26.08	27.78	29.74	32.09
16	23.99	25.74	27.54	29.38	31.49
17	23.83	25.42	27.30	29.33	31.28
18	24.13	25.84	27.67	29.66	31.62

Note: In yellow boxes samples extracted on day 1; in green boxes samples extracted on day 2; in blue boxes samples extracted on day 3.

To measure inhibition, the Cq values of the four diluted samples were plotted against the logarithm of the dilution and the Cq values for the "undiluted" samples (40 ng/ μ L) were extrapolated from the equation calculated by linear regression.

Subsequently, the extrapolated Cq values for the "undiluted" samples were compared with the measured Cq data. It is assumed that PCR inhibitors are present if the measured Cq value for the "undiluted" sample is > 0.5 cycles from the calculated Cq value. Table 4 below reports the comparison of extrapolated Cq values versus measured Cq values for all samples and the values of linearity (R^2) and slope of all measurements.

Table 4. Comparison of extrapolated Cq values versus measured Cq values (amplification of maize aldolase gene)

DNA extraction	R ²	Slope	Cq extrapolated	mean Cq measured	ΔCq*
1	0.9951	-3.254	23.35	23.83	0.48
2	0.9972	-3.196	23.68	24.18	0.50
3	0.9980	-3.255	23.57	23.98	0.41
4	0.9970	-3.309	23.29	23.77	0.48
5	0.9959	-3.338	23.42	23.85	0.43
6	0.9982	-3.329	23.55	23.76	0.21
7	0.9973	-3.319	23.39	23.73	0.34
8	0.9962	-3.186	23.72	23.79	0.07
9	0.9967	-3.275	23.49	23.75	0.26
10	0.9991	-3.319	23.54	23.95	0.41
11	0.9994	-3.313	23.36	23.81	0.45
12	0.9986	-3.396	23.19	23.71	0.52
13	0.9973	-3.459	23.08	23.42	0.34
14	0.9975	-3.381	23.55	23.96	0.42
15	0.9943	-3.321	23.92	24.21	0.29
16	0.9978	-3.171	23.77	23.99	0.23
17	0.9985	-3.261	23.42	23.83	0.41
18	0.9989	-3.210	23.87	24.13	0.26

Note: In yellow boxes samples extracted on day 1; in green boxes samples extracted on day 2; in blue boxes samples extracted on day 3.

*delta Cq = abs (Cq extrapolated - Cq measured)

According to ENGL definition of minimum performance requirements for analytical methods of GMO testing^e the expected slope for a PCR with 100% efficiency is -3.3; the accepted average value should be in the range of -3.6 and -3.1. In addition the average value of R^{2f} shall be ≥0.98.

The table indicates that all but one ΔCq values of extrapolated versus measured Cq are ≤ 0.5. DNA extraction of sample number 12 was slightly inhibited (ΔCq value=0.52) but still not compromising the inhibition run which for the rest of the points was within the ENGL minimum performance requirements. The R² coefficient of linear regression is > 0.98 for all DNA samples and the slopes of the curves are between -3.1 and -3.6 for all samples.

^e EURL/ENGL guidance document "Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing" (<http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>)

^f R² is the correlation coefficient of a standard curve obtained by linear regression analysis.

5. Conclusions

The results confirm that the extraction method from maize flour provided by the applicant produces DNA of suitable quantity and quality for subsequent PCR-based analysis.

Although the method was submitted under Regulation (EC) No 641/2004, the in-house validation was performed under Regulation (EU) No 503/2013 and it was concluded that the method is applicable to maize samples provided by the applicant in accordance with the requirements of the (EC) Regulations mentioned above.

If applied to complex food or feed products containing maize, because of the known difficulties in extracting high quality and quantity of DNA from such materials, particular care must be taken with regard to verifying the suitability of the extracted DNA for subsequent analyses.

6. References

1. Richards M., Reichardt M. and Rogers S., 1994. Preparation of genomic DNA from plant tissue. Unit 2.3 Supplement 27/45. In: Current Protocols in Molecular Biology. John Wiley&Sons. Inc.
2. Doyle J.J. and Doyle J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12(1):13-15
3. Hupfer C., Hotzel H., Sachse K., Engel K.H., 1997. Detection of genetically modified insect-resistant Bt maize by means of polymerase chain reaction. Z Lebensmittel Unters. Forsch A (1997) 205:442-445
4. COMMISSION REGULATION (EC) No 641/2004 of 6 April 2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 of the European Parliament and of the Council as regards the application for the authorisation of new genetically modified food and feed, the notification of existing products and adventitious or technically unavoidable presence of genetically modified material which has benefited from a favourable risk evaluation. Official Journal of the European Union L102/14
5. COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union L 157/1

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