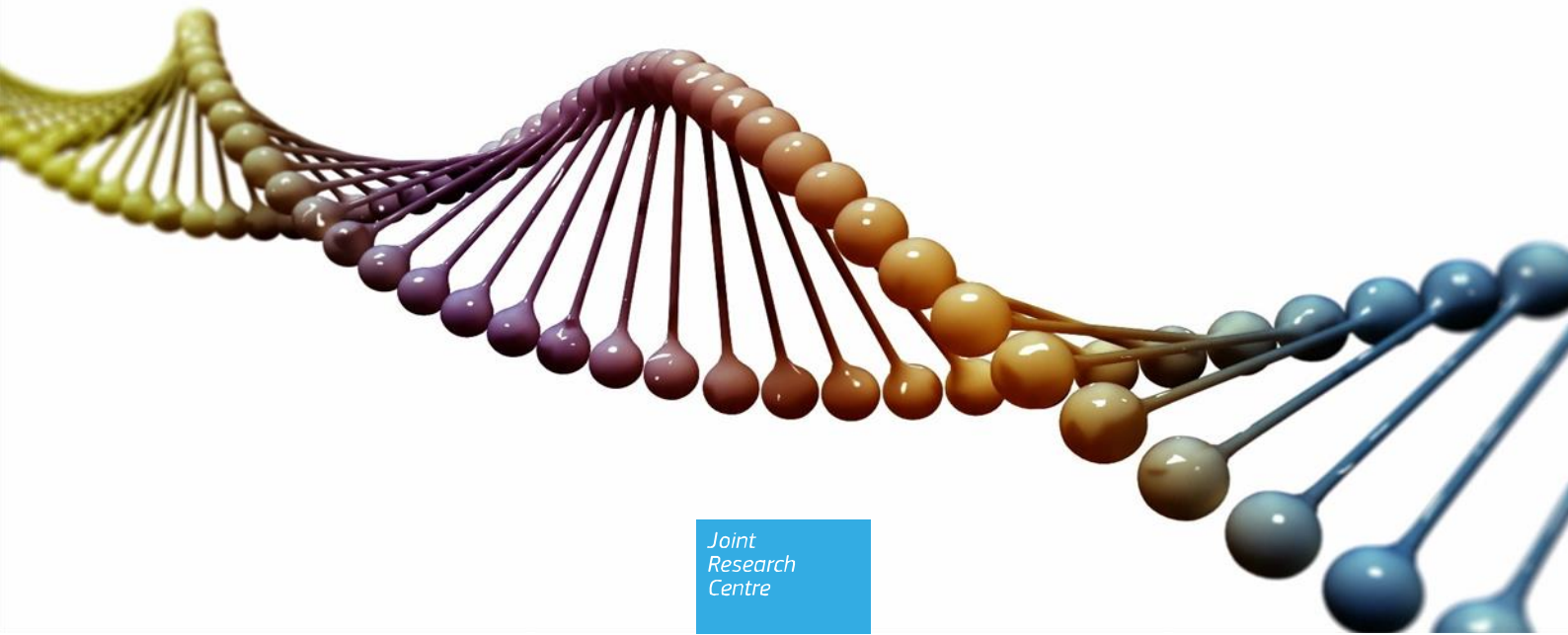
European Commission

Report on the Verification of the Performance of GMB151 and DAS-44406-6 event-specific PCR-based Methods applied to DNA extracted from GM Stack GMB151 x DAS-44406-6 soybean

Validation Report

Savini, C., Maretti, M., Mazzara, M., Vincent, U.

2025



Joint Research Centre

Contact information
European Commission
Directorate General Joint Research Centre
Directorate F – Health and Food
European Union Reference Laboratory for GM Food and Feed
Food & Feed Compliance (F.5)
Via E. Fermi, 2749.
I-21027 Ispra (VA), Italy

Functional mailbox: JRC-EURL-GMFF@ec.europa.eu

EU Science Hub
https://joint-research-centre.ec.europa.eu

JRC141238

Ispra: European Commission, 2025

How to cite this report: European Commission: Joint Research Centre, Savini, C., Maretti, M., Mazzara, M. and Vincent, U., *Report on the Verification of the Performance of GMB151 and DAS-44406-6 event-specific PCR-based Methods applied to DNA extracted from GM Stack GMB151 x DAS-44406-6 soybean,* European Commission, Ispra, 2025, JRC141238.

Contents

Abstract

An application was submitted by BASF Agricultural Solutions, to request the authorisation of the genetically modified stack (GM stack) GMB151 x DAS-44406-6 soybean (nematode resistant and herbicide tolerant), for food and feed uses, import and processing, in accordance with articles 5 and 17 of Regulation (EC) N° 1829/2003 GM Food and GM Feed. The unique identifier assigned to GM stack GMB151 x DAS-44406-6 soybean is BCS-GM151-6 x DAS-444Ø6-6.

The GM stack GMB151 x DAS-44406-6 soybean has been obtained by conventional crossing between the genetically modified soybean events: GMB151 and DAS-44406-6, without any new genetic modification.

The EURL GMFF has previously validated individually, and declared fit for purpose, the detection methods for the single events GMB151 and DAS-44406-6 (see https://gmo-crl.jrc.ec.europa.eu/method-validations). In line with the approach defined by the ENGL (see ENGL document "Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing" https://gmo-crl.jrc.ec.europa.eu/guidance-documents) the EURL GMFF has carried out only an *in-house* verification of the performance of each validated method when applied to genomic DNA extracted from GM stack GMB151 x DAS-44406-6 soybean.

The results of the *in-house* verification led to the conclusion that the individual methods meet the ENGL performance criteria also when applied to genomic DNA extracted from the GM stack GMB151 x DAS-44406-6 soybean.

This report is published at https://gmo-crl.jrc.ec.europa.eu/method-validations.

Quality assurance

The EURL GMFF is ISO/IEC 17025:2017 accredited [certificate number: BELAC 268 TEST (Flexible Scope for determination of Genetically Modified content in % (m/m) and % (cp/cp) in food and feed by DNA extraction, DNA identification and Real-time PCR and for determination of Genetically Modified content in % (cp/cp) in food and feed by DNA extraction and digital PCR)].

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

Report on the Verification of the Performance of GMB151 and DAS-44406-6 event-specific PCR-based Methods applied to DNA extracted from GM Stack GMB151 x DAS-44406-6 soybean

Validation Report

30/01/2025

European Union Reference Laboratory for GM Food and Feed

1 Introduction

The EU legislative system (1, 2) for genetically modified food and feed foresees 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 GM stack 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 EURL 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 EURL GMFF report in the overall opinion concerning the risk assessment and potential authorisation of the assessed stack. In line with the approach defined by the ENGL (see ENGL document "Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing" https://gmo-crl.jrc.ec.europa.eu/guidance-documents) the EURL GMFF carries out an *in-house* verification of the performance of each event-specific methods if this method has previously been validated by the EURL GMFF for the parental single-line event and these events have been stacked by conventional crossing. These criteria are met for the GM stack GMB151 x DAS-44406-6 soybean.

Upon reception of methods, samples and related data (step 1), the EURL 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 (EU) No 503/2013 (Annex III).

The results of the *in-house* verification study were evaluated with reference to ENGL method performance requirements (3) and to the validation results on the individual events.

2 Dossier reception and acceptance (step 1)

BASF Agricultural Solutions submitted the detection methods, data demonstrating their adequate performance when applied to genomic DNA extracted from the stack, and the corresponding control samples of DNA extracted from the GM stack soybean GMB151 x DAS-44406-6 and from non GM soybean. The dossier was found to be complete and was thus moved to step 2.

3 Scientific assessment (step 2)

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.

3.1 DNA extraction

A method for DNA extraction from soybean was previously evaluated by the EURL GMFF with regard to its performance characteristics and was considered valid, i.e. fit the purpose of providing soybean DNA of appropriate quality and amount for being used in subsequent PCR experiments.

Annex III to Reg. (EU) No 503/2013 (2) requires the applicant to discuss the validity and limitations of the detection methods in the various types of foods and feeds (matrices) that are expected to be placed on the market. To this regard the applicant stated that "the suitability of isolated DNA as an analyte for PCR based detection of GMOs is dependent on the quality, purity, and quantity of the DNA. Although the DNA extraction method can be applied to different food and feed matrices, the application of the method to certain complex and difficult processed matrices may require adaptation. In fact, food processes can influence the quality and intactness of the DNA contained in the final processed products. Other challenges of working with processed food and feed matrices is the presence of PCR inhibitors, which can reduce the efficiency and/or reproducibility of PCR and thus may contribute to inaccurate PCR results. Therefore, DNA extraction from certain of these processed matrices may require additional rounds of purification in order to achieve the quality standards needed for quantitative real-time PCR[1,2,3,4].

In conclusion, the DNA extraction module has been successfully validated on soybean seeds and grains, which is the most representative matrix of food and feed soybean samples in the context of the current application for authorization".

On a general note the EURL GMFF recommends that laboratories using this validated method for testing complex or difficult matrices always verify that the extracted genomic DNA is of sufficient quality.

The protocol for the DNA extraction method is available at https://gmo-crl.jrc.ec.europa.eu/summaries/A2704-12_soybean_DNAExtr_report.pdf.

Consequently, the EURL GMFF did not verify the DNA extraction method proposed by the applicant.

[1] Bauer, T. *et al.* – 2003. The effect of processing parameters on DNA degradation in food. Eur. Food Res. Technol., 217: 338-343
[2] Chen, Y. *et al.* -2005. Degradation of endogenous and exogenous genes of Roundup Ready soybean during food processing. J. Agric. Food Chem., 53: 10239-10243
[3] Lipp, M. *et al.* -2005. Polymerase chain reaction technology as analytical tool in agricultural biotechnology. J AOAC Int 88 (1):136–155
[4] Murray S.R. *et al.*, 2007. Use of quantitative real-time PCR to estimate maize endogenous DNA degradation after cooking and extrusion or in food products. J. Agric. Food Chem., 55: 2231-2239

3.2 PCR methods

Table 1 shows values of trueness (expressed as bias %) and precision (expressed as RSD_r %) calculated by the applicant for the two methods applied to GMB151 x DAS-44406-6 soybean genomic DNA. Means are the average of eighteen replicates obtained through six runs performed with a BIO-RAD CFX real-time PCR equipment. Percentages are expressed as GM DNA copy number / total DNA copy number x 100.

Table 1. Trueness (expressed as bias %) and precision (expressed as relative repeatability standard deviation, RSD_r %) provided by the applicant for the GMB151 and DAS-44406-6 methods applied to GM stack GMB151 x DAS-44406-6 soybean.

GMB151 *				
Sample GM %	**Expected value (GMO %)**			
	4.5	**2.0**	**0.9**	**0.08**
Mean	4.72	2.16	0.95	0.10
RSD_r (%)	4.04	5.67	9.57	10.91
Bias (%)	4.96	7.91	6.08	19.56
DAS-44406-6 *				
Sample GM %	**Expected value (GMO %)**			
	5.0	**2.0**	**0.9**	**0.085**
Mean	5.35	2.27	0.93	0.09
RSD_r (%)	7.01	9.80	8.91	15.74
Bias (%)	6.96	13.62	3.39	5.20

* Numbers are not rounded but are presented as reported by the applicant

Source: EURL GMFF data

3.2.1 Deviations from the validated methods introduced by the applicant

The applicant did not declare deviations from the validated method

The EURL GMFF verified the data and concluded that they were reliable and seemed to confirm that the methods meet the ENGL performance criteria (3). The dossier was therefore moved to step 3.

4 EURL GMFF experimental testing (step 3)

In step 3 the EURL GMFF implemented the two methods in its own laboratory and performed a verification of their performance when applied to genomic DNA extracted from GM stack GMB151 x DAS-44406-6 soybean.

Note: Numerical values presented in the following tables were rounded keeping two significant digits for values ≤ 1, two significant digits for values between 1 and 10 and three significant digits for values ≥ 10, unless otherwise reported. The calculations in the MS Excel files however were done over not rounded data. This approach might create small inconsistencies in the numerical values reported in the tables but it allows a higher precision in the final results.

4.1 Materials

The following control samples were provided by the applicant:

- genomic DNA extracted from soybean leaf homozygous for GMB151 x DAS-44406-6, as positive control sample.
- genomic DNA extracted from leaf of the conventional (non-GM) soybean, as negative control sample.

The EURL GMFF prepared test samples of different GMO concentrations by mixing genomic DNA extracted from GM stack GMB151 x DAS-44406-6 soybean with the non-GM soybean genomic DNA, in a constant amount of total soybean genomic DNA. The same GM concentrations as in the validation of the methods for the single lines were achieved. Table 2 shows the five GM concentrations used in the verification of the GMB151 and DAS-44406-6 methods when applying them to genomic DNA extracted from the GM stack GMB151 x DAS-44406-6 soybean.

Table 2. Percentage (GM %) of GMB151 and DAS-44406-6 in GMB151 x DAS-44406-6 stack genomic DNA contained in the verification samples.

GMB151 GM %* [(GM DNA / total soybean DNA x 100)]	**DAS-44406-6 GM %*** [(GM DNA / total soybean DNA x 100)]
0.10	0.10
0.50	0.40
0.90	0.90
2.0	2.0
4.5	5.0

* percentage expressed in copy number ratio

Source: EURL GMFF data

The protocols described by the applicant were implemented precisely in the EURL GMFF laboratory and were in accordance with the protocols already published for the individual GMB151 and DAS-44406-6 GM events (available at https://gmo-crl.jrc.ec.europa.eu/method-validations).

4.2 Experimental design

Eight PCR runs were carried out for each method. In each run, samples were analysed in parallel with both the GM-specific and the reference method lectin *(le1)*. Five GM levels were examined per run, each GM level in duplicate. PCR analysis was performed in triplicate for all samples. In total, for each method GMB151 and DAS-44406-6, the quantification of the five GM levels was performed 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 the GM %.

4.3 PCR methods

During the verification study, the EURL GMFF carried out parallel tests on DNA extracted from GM stack GMB151 x DAS-44406-6 soybean using the single detection methods previously validated for the respective single GM events GMB151 and DAS-44406-6.

For detection of GM soybean events GMB151 and DAS-44406-6, DNA fragments of 84-bp and 99-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 the fluorescent dye FAM (6-carboxyfluorescein) at the 5'-end of the probes and quenched at the 3'-end respectively by BHQ1 (Black Hole Quencher-1), in the probe specific to GMB151 amplicons, and by TAMRA, in the probe specific to DAS-44406-6 amplicons.

For quantification of GM soybean event GMB151, the taxon-specific reference method amplifies a 102-bp fragment of lectin (*Le1*), a soybean endogenous gene (GenBank accession number: K00821), using two lectin gene-specific primers and a gene-specific probe labelled with JOE and BHQ1.

For quantification of GM soybean event DAS-44406-6, the taxon-specific reference method amplifies a 74-bp fragment of lectin (*Le1*), a soybean endogenous gene (GenBank accession number: K00821), using two lectin gene-specific primers and a gene-specific probe labelled with FAM and TAMRA.

For the relative quantification of GM soybean events GMB151 and DAS-44406-6 standard curves are generated both for the GMB151 and DAS-44406-6 and for the lectin (*Le1*) method by plotting Cq values of the calibration standards against the logarithm of the DNA amount and by fitting a linear regression into these data. Thereafter, the Cq values of the unknown samples are measured and, by means of the regression formula, the relative amount of GMB151 and DAS-44406-6 DNA is estimated.

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

4.3.1 Deviations from the validated methods introduced by the EURL

No deviations from the original validated methods were introduced.

4.4 Results

Tables 3 and 4 present the values of the slopes of the different standard curves generated by the EURL GMFF when using DNA extracted from the GM stack, from which the PCR efficiency is calculated using the formula $[10^{(-1/slope)} - 1] \times 100$, and of the coefficient of determination (R^2) reported for all PCR systems in the eight runs, for GM soybean events GMB151 and DAS-44406-6. Slope values were rounded to two digits.

Table 3. Values of standard curve slope, PCR efficiency and R^2 coefficient for the GMB151 method on GM stack GMB151 x DAS-44406-6 soybean.

Run	GMB151			*Le1*		
	Slope	**PCR Efficiency (%)**	**R^2 coefficient**	**Slope**	**PCR Efficiency (%)**	**R^2 coefficient**
1	-3.51	93	1.00	-3.42	96	1.00
2	-3.39	97	1.00	-3.40	97	1.00
3	-3.48	94	1.00	-3.40	97	1.00
4	-3.41	96	1.00	-3.37	98	1.00
5	-3.39	97	1.00	-3.40	97	1.00
6	-3.48	94	1.00	-3.41	97	1.00
7	-3.41	96	1.00	-3.41	96	1.00
8	-3.45	95	1.00	-3.39	97	1.00
Mean	**-3.44**	**95**	**1.00**	**-3.40**	**97**	**1.00**

Source: EURL GMFF data

Table 4. Values of standard curve slope, PCR efficiency and R^2 coefficient for the DAS-44406-6 method on GM stack GMB151 x DAS-44406-6 soybean.

Run	DAS-44406-6			*Le1*		
	Slope	**PCR Efficiency (%)**	**R^2 coefficient**	**Slope**	**PCR Efficiency (%)**	**R^2 coefficient**
1	-3.41	97	1.00	-3.36	98	1.00
2	-3.37	98	1.00	-3.38	98	1.00
3	-3.26	103	1.00	-3.34	99	1.00
4	-3.34	99	1.00	-3.36	98	1.00
5	-3.34	99	1.00	-3.34	99	1.00
6	-3.39	97	1.00	-3.37	98	1.00
7	-3.32	100	1.00	-3.36	99	1.00
8	-3.29	102	1.00	-3.36	98	1.00
Mean	**-3.34**	**99**	**1.00**	**-3.36**	**98**	**1.00**

Source: EURL GMFF data

The mean PCR efficiencies of the GM and species-specific methods were 95% and 97% for GMB151 and *Le1*, and 99% and 98% for DAS-44406-6, and Le1 respectively. The mean R^2 coefficient of the methods was 1.00 in all cases. The data presented in Tables 3 and 4 confirm the appropriate performance characteristics of the two methods when tested on GM stack GMB151 x DAS-44406-6 soybean in terms of PCR efficiency and R^2 coefficient.

The EURL GMFF also assessed the values of trueness (expressed as bias%) and precision (expressed as relative repeatability standard deviation, RSD_r %) of the two methods applied to samples of DNA extracted from GM stack GMB151 x DAS-44406-6 soybean see Tables 5 and 6.

Table 5. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSDr %) of the GMB151 method applied to genomic DNA extracted from GM stack GMB151 x DAS-44406-6 soybean.

GMB151					
Unknown sample GM %	**Expected value (GMO %)**				
	4.5	**2.0**	**0.90**	**0.50**	**0.10**
Mean	4.6	2.1	0.93	0.53	0.11
SD	0.03	0.07	0.01	0.03	0.005
RSD_r (%)	0.65	3.3	1.3	5.0	4.4
Bias (%)	1.6	6.1	3.2	6.2	7.9

Source: EURL GMFF data

Table 6. Estimates of trueness (expressed as bias %) and relative repeatability standard deviation (RSDr %) of the DAS-44406-6 method applied to genomic DNA extracted from GM stack GMB151 x DAS-44406-6 soybean.

DAS-44406-6					
Unknown sample GM %	**Expected value (GMO %)**				
	5.0	**2.0**	**0.90**	**0.40**	**0.10**
Mean	4.8	1.9	0.85	0.38	0.09
SD	0.10	0.05	0.02	0.03	0.01
RSD_r (%)	2.0	2.7	2.9	6.9	8.8
Bias (%)	-3.8	-2.7	-5.5	-5.8	-11

Source: EURL GMFF data

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 should be less or equal to ± 25% across the entire dynamic range. As shown in Tables 5 and 6, the values range from 1.6% to 7.9% for GMB151 and from -11% to -2.7% for DAS-44406-6. Therefore, the two methods satisfy the above mentioned requirement throughout their respective dynamic ranges, also when applied to DNA extracted from GM stack GMB151 x DAS-44406-6 soybean.

Tables 5 and 6 also show the relative repeatability standard deviation (RSD_r) estimated for each GM level. According to the ENGL acceptance criteria and method performance requirements, the RSD_r values should be equal to or below 25%. As the values range between 0.65% and 5.0% for GMB151 and between 2.0% and

8.8% for DAS-44406-6, the two methods satisfy this requirement throughout their respective dynamic ranges when applied to DNA extracted from GM stack GMB151 x DAS-44406-6 soybean.

5 Conclusions

The performance of the two event-specific methods for the detection and quantification of soybean single line events GMB151 and DAS-44406-6, when applied to genomic DNA extracted from GM stack GMB151 x DAS-44406-6 soybean, meets the ENGL performance requirements, as assessed on the control samples provided by the applicant.

Therefore these methods, developed and validated to detect and quantify the single soybean events GMB151 and DAS-44406-6, can be equally applied for the detection and quantification of the respective events in DNA extracted from the GM stack GMB151 x DAS-44406-6 soybean, supposed that sufficient genomic DNA of appropriate quality is available.

6 References

1. Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed.

2. 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.

3. European Network of GMO Laboratories. Definition of minimum performance requirements for analytical methods of GMO testing - Part 1, Publications Office of the European Union, Luxembourg 2015, JRC 95544. https://gmo-crl.jrc.ec.europa.eu/guidance-documents

List of abbreviations and definitions

EURL GMFF	European Union Reference Laboratory for GM Food and Feed
PCR	Polymerase chain reaction
ENGL	European Network of GMO Laboratories

List of tables

Getting in touch with the EU

In person

All over the European Union there are hundreds of Europe Direct centres. You can find the address of the centre nearest you online (european-union.europa.eu/contact-eu/meet-us_en).

On the phone or in writing

Europe Direct is a service that answers your questions about the European Union. You can contact this service:

— by freephone: 00 800 6 7 8 9 10 11 (certain operators may charge for these calls),

— at the following standard number: +32 22999696,

— via the following form: european-union.europa.eu/contact-eu/write-us_en.

Finding information about the EU

Online

Information about the European Union in all the official languages of the EU is available on the Europa website (european-union.europa.eu).

EU publications

You can view or order EU publications at op.europa.eu/en/publications. Multiple copies of free publications can be obtained by contacting Europe Direct or your local documentation centre (european-union.europa.eu/contact-eu/meet-us_en).

EU law and related documents

For access to legal information from the EU, including all EU law since 1951 in all the official language versions, go to EUR-Lex (eur-lex.europa.eu).

EU open data

The portal data.europa.eu provides access to open datasets from the EU institutions, bodies and agencies. These can be downloaded and reused for free, for both commercial and non-commercial purposes. The portal also provides access to a wealth of datasets from European countries.

Science for policy

The Joint Research Centre (JRC) provides independent, evidence-based knowledge and science, supporting EU policies to positively impact society

EU Science Hub
Joint-research-centre.ec.europa.eu