



Federal Office of
Consumer Protection
and Food Safety

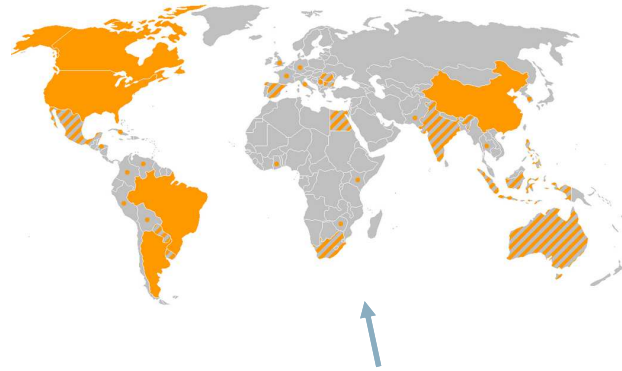
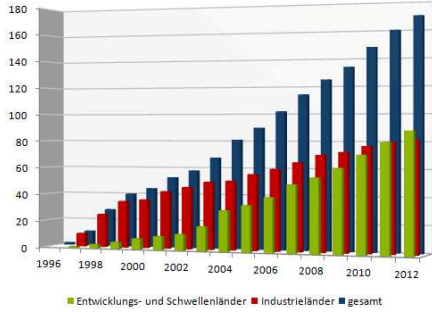


European Network of GMO Laboratories



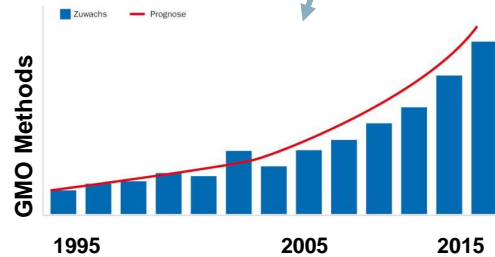
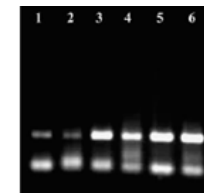
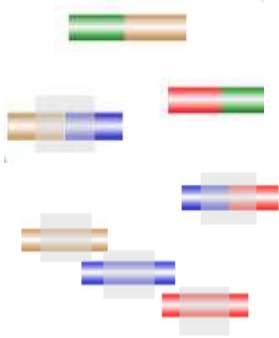
Challenges and what the future holds for GMO analysis: ENGL perspective

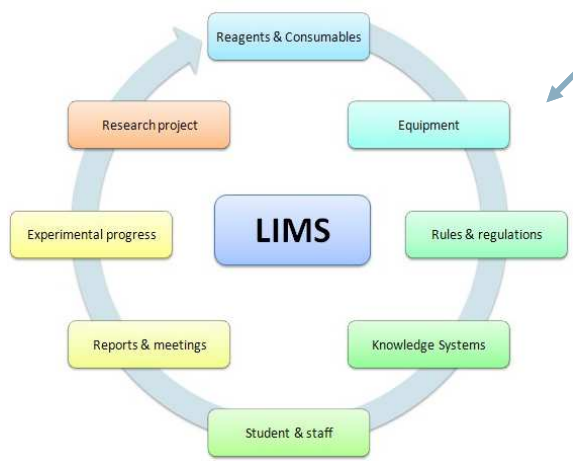
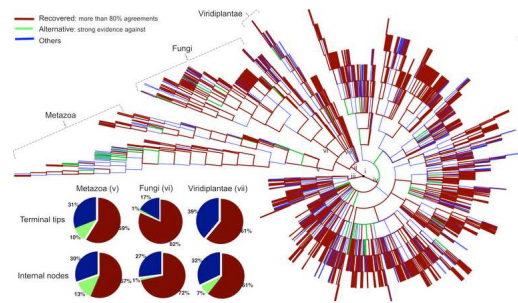
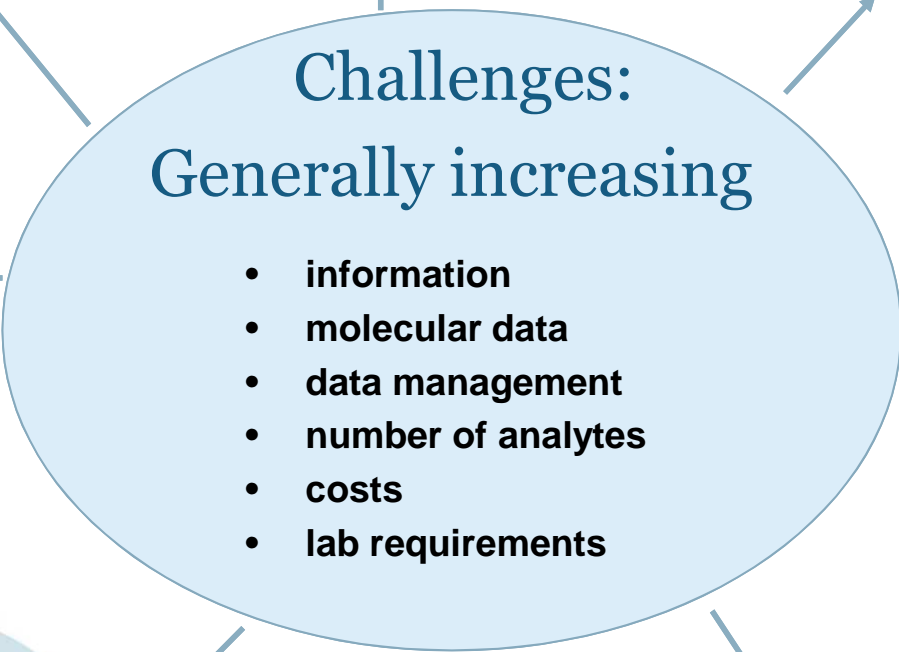
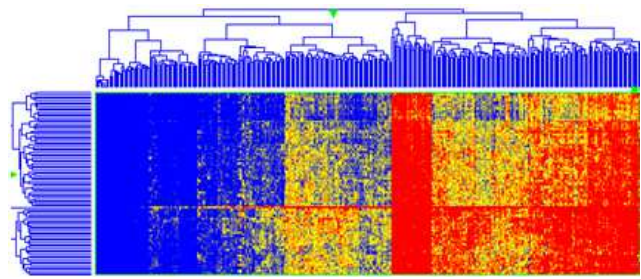
**Lutz Grohmann
Federal Office of Consumer Protection and Food Safety
Berlin, Germany**



**Challenges:
Constantly increasing**

- number of GMOs
- cultivation area
- crop diversity
- genetic elements
- number of methods
- technologies





Finding the needle in the haystack, but cheap and without delay!





GMO Screening Strategies - Challenges

- **Cover the increasing number of GMOs**
- **Select the „best“ methods (fit-for-purpose)**
- **Constantly update your GMO matrix**
- **Sustain efficiency**

- ✓ Harmonised guidelines for strategies
- ✓ Databases and Web-tools



Guidelines for Screening Strategy



TECHNICAL SPECIFICATION

CEN/TS 16707

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

October 2014

ICS 67.050

English Version

Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Polymerase chain reaction (PCR) based screening strategies

Produits alimentaires - Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés - Stratégies de criblage basées sur l'utilisation de la réaction de polymérisation en chaîne (PCR)

Lebensmittel - Verfahren zum Nachweis von gentechnisch veränderten Organismen und ihren Produkten - Strategien für das Screening mit Polymerase-Kettenreaktion (PCR)

General principles of GMO screening strategies
by using the [matrix approach](#) / and [requirements](#) for its use

Content:

- Combination of targets for screening
- Analysis of the output of the first screening
- Additional screening tests
- GM event identification
- Interpretation of PCR results
- PCR method performance criteria and validation
 - Absolute LOD
 - Specificity and reference materials
 - Robustness
 - False-positive / false-negative rates
 - Probability of detection (POD)



Pre-spotted plates (PSP) project

Screening PSP

(6 Screening Targets / 7 Species / 3 Events)

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG	p35s	HMG	p35s	HMG	p35s	HMG	p35s	HMG	p35s	HMG	p35s
B	Lec	tNOS	Lec	tNOS	Lec	tNOS	Lec	tNOS	Lec	tNOS	Lec	tNOS
C	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS	CruA	CTP2-EPSPS
D	Sah7	PAT	Sah7	PAT	Sah7	PAT	Sah7	PAT	Sah7	PAT	Sah7	PAT
E	UGP	BAR	UGP	BAR	UGP	BAR	UGP	BAR	UGP	BAR	UGP	BAR
F	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab	PLD	Cry1Ab
G	GS	CV127	GS	CV127	GS	CV127	GS	CV127	GS	CV127	GS	CV127
H	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423	DAS-40278	DP-305423
	30		20		10		5		1		NTC	

soy & maize PSP

(13 soy events / 19 maize events)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Lec	GTS 40-3-2	Lec	GTS 40-3-2	Lec	GTS 40-3-2	Lec	GTS 40-3-2	Lec	GTS 40-3-2	Lec	GTS 40-3-2
B	AZ704	MON	AZ704	MON	AZ704	MON	AZ704	MON	AZ704	MON	AZ704	MON
C	A5547	MON	A5547	MON	A5547	MON	A5547	MON	A5547	MON	A5547	MON
D	CV127	MON	CV127	MON	CV127	MON	CV127	MON	CV127	MON	CV127	MON
E	DAS 68416	MON	DAS 68416	MON	DAS 68416	MON	DAS 68416	MON	DAS 68416	MON	DAS 68416	MON
F	305423	MON	305423	MON	305423	MON	305423	MON	305423	MON	305423	MON
G	DP-356043	MON	DP-356043	MON	DP-356043	MON	DP-356043	MON	DP-356043	MON	DP-356043	MON
H	FG72	MON	FG72	MON	FG72	MON	FG72	MON	FG72	MON	FG72	MON

	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG	E3272	E5307	E98140	BT11	Bt176	DAS-40278	DAS-59122	GA21	LY038	MIR162	MIR604
B	MON	MON	MON	MON	MON	MON	NK603	T25	TC1507			
C	HMG	E3272	E5307	E98140	BT11	Bt176	DAS-40278	DAS-59122	GA21	LY038	MIR162	MIR604
D	MON	MON	MON	MON	MON	MON	NK603	T25	TC1507			
E	HMG	E3272	E5307	E98140	BT11	Bt176	DAS-40278	DAS-59122	GA21	LY038	MIR162	MIR604
F	MON	MON	MON	MON	MON	MON	NK603	T25	TC1507			
G	HMG	E3272	E5307	E98140	BT11	Bt176	DAS-40278	DAS-59122	GA21	LY038	MIR162	MIR604
H	MON	MON	MON	MON	MON	MON	NK603	T25	TC1507			



www.euginius.eu

Euginius GMO - Database Detection Tools



EUGINIUS Home **GMO** Detection Analysis Authorisation

European GMO Initiative for a Unified Database System

- [Euginius news](#)
- [Going online](#)
- [New Release](#)
- ~~[Where do I find](#)~~
- [GMO Details](#)
- [Methods](#)
- [Analysis module](#)
- [Authorisation](#)
- [Safety literature](#)
- [GMO-related web](#)
- [Suggest new GM](#)
- [Give feedback](#)

GMO show	Authorisation	Species	RM available	ctb2/cnf4-epsps	P-35S/par (GL-C)	Inos-RHD (C)	CS-bar-STRHY	E-FMV, P-FMV (C)	CS-cnf4/Ab-BAC	P-35S GalMV (C)
ASR368		Agrostis stolonifera	no	-1	-1	1	-1	-1	-1	1
GTSB77		Beta vulgaris	no	3	-3	-3	-3	1	-1	3
H7-1		Beta vulgaris	yes	3	-3	-3	-3	1	-1	-3
H7-1 x SBVR111		Beta vulgaris	no	1	-1	1	-1	1	-1	-1
SBVR111		Beta vulgaris	no	-1	-1	1	-1	-1	-1	-1
T120-7		Beta vulgaris	no	-3	3	-3	-3	-1	-1	3
Falcon GS 40/90		Brassica napus	yes	-3	3	-3	-3	-1	-1	3
GT200		Brassica napus	no	2	-2	-2	-2	1	-1	-2
GT73		Brassica napus	yes	3	-3	-3	-3	1	-1	-3
HCN10		Brassica napus	no	-1	1	-1	-1	-1	-1	-1
Liberator		Brassica napus	no	-3	3	-3	-3	-1	-1	3
MON88302		Brassica napus	yes	2	-2	-2	-2	3	-1	-2
MON88302 x Ms8		Brassica napus	no	-1	-1	1	1	1	-1	-1
MPS965		Brassica napus	no	-1	-1	1	-1	-1	-1	-1
Ms1		Brassica napus	yes	-3	-3	3	3	-1	-1	-3
Ms1 x Rf1		Brassica napus	no	-3	-3	3	3	-1	-1	-3
Ms1 x Rf2		Brassica napus	no	-2	-2	2	2	-1	-1	-2
Ms8		Brassica napus	yes	-3	-3	3	3	-1	-1	-3
Ms8 x Rf3		Brassica napus	no	-3	-3	3	3	-1	-1	-3



How to detect GMOs not covered by the screening tests?

Soybean (8)

	<i>ctp2 epsps</i>	<i>P-35S</i>	<i>bar</i>	<i>P-FMV</i>	<i>T-nos</i>	<i>cryIAb/AC</i>
	QL-CON-00-008	QT-ELE-00-004	QL-CON-00-011	QL-ELE-00-015	QL-ELE-00-014	QL-ELE-00-016
305423 Soybean (DP-305423-1)	0	0	0	0	0	0
CV127 Soybean (BPS-CV127-9)	0	0	0	0	0	0
MON87701 Soybean (MON-87701-2)	0	0	0	0	0	2
MON87769 Soybean (MON-87769-7)	0	0	0	0	0	0
MON87708 Soybean (MON-87708-9)	0	0	0	0	0	0
DAS-68416-4 Soybean (DAS-68416-4)	0	0	0	0	0	0
GMO event DAS-44406-6 Soybean (DAS-44406-6)	0	0	0	0	0	0
DAS-81419-2 Soybean	0	0	0	0	0	0

Legend:

- 0 No amplification predicted
- 1 Amplification predicted, imperfect annealing*
- 2 Amplification predicted, perfect annealing

How to detect GMOs not covered by the screening tests?

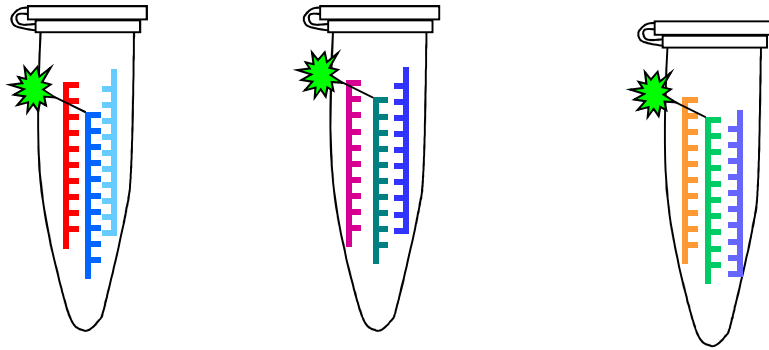
Maize (4)

	<i>ctp2 epsps</i>	<i>P-35S</i>	<i>35S-pat</i>	<i>P-FMV</i>	<i>bar</i>	<i>T-nos</i>	<i>cry1Ab/Ac</i>	<i>pat</i>
	QL-CON-00-008	QT-ELE-00-004	QL-CON-00-011	QL-ELE-00-015	QL-ELE-00-011	QL-ELE-00-014	QL-ELE-00-016	QL-ELE-00-021
LY038 Maize (REN-00038-3)	0	0	0	0	0	0	0	0
DAS-40278-9 Maize (DAS-40278-9)	0	0	0	0	0	0	0	0
Maize (VCO-01981-5)	0	0	0	0	0	0	0	0
BVLA430101 Maize	0	0	0	0	0	0	0	0

Legend:

- 0 No amplification predicted
- 1 Amplification predicted, imperfect annealing*
- 2 Amplification predicted, perfect annealing

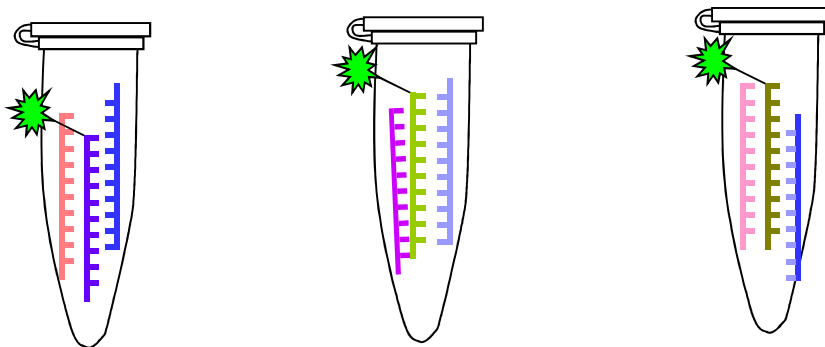
* Up to a maximum of 2 gaps and 2 mismatches for each primer



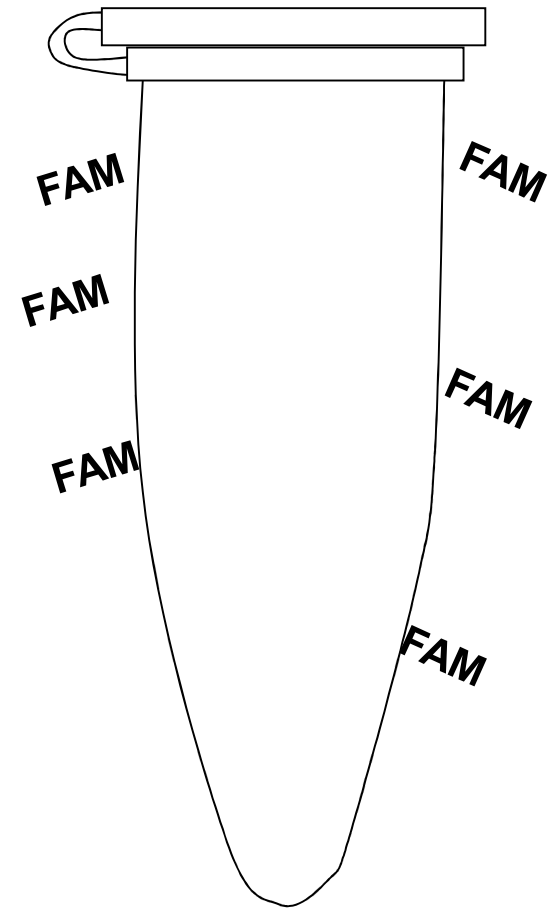
MON 87701 MON87708 MON 87769

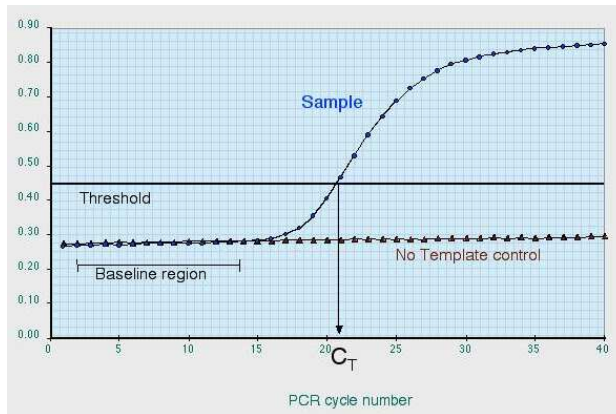
**6 validated event-specific
real-time PCR methods of the EURL GMFF**

DP-305423 CV-127 DAS-68416-4

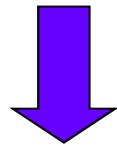


Pilot Study: Multiplex event-specific real-time PCR Screening

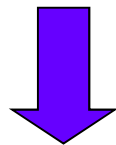




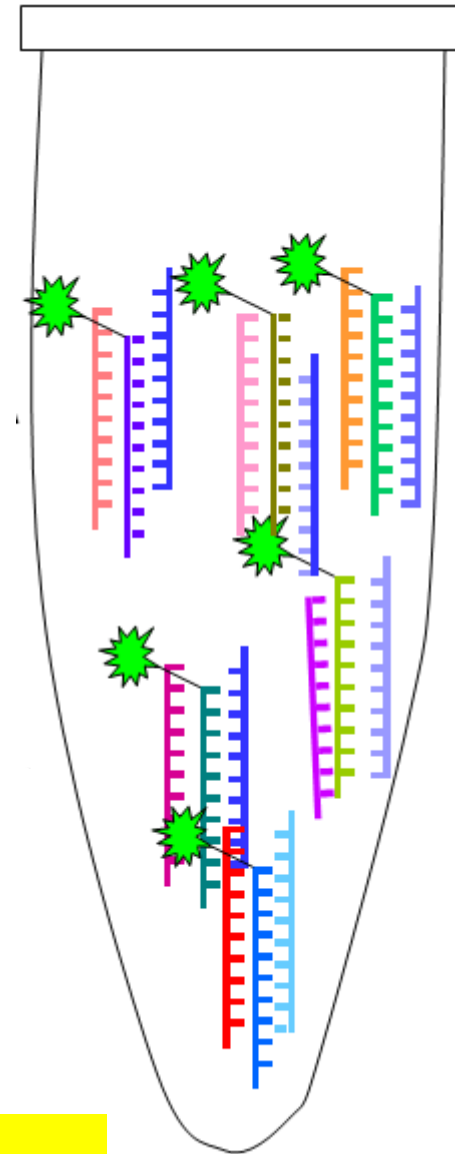
Positive FAM signal



**one or more soybean event(s)
are present**



**further analysis in single event-
specific real-time PCR tests**



✓ Verified in bioinformatic tests performed by the
JRC/EURL-GMFF Bioinformatics Unit



Results of the Collaborative Trial (2015)

- Participation of 16 labs testing 12 blinded soya flour samples (mixtures with 0.1% and 0.03% GM content)
- only 1 false-positive results (out of 360 results)
- 10 false-negative results for 0.03% samples (2.8%)
- 4 false-negative results for 0.1% samples (1,1%)
- All labs were able to detect at least 20 target copies

ENGL Activities






ENGL Working Groups (concluded)



Title	Status	Downloads
Working group on Method Performance Requirements (WG-MPR)	Concluded	Mandate Final report
Working group on Sample Preparation Procedure (WG-SPP)	Concluded	Mandate Final report
Working group on identification of stacked GM events (WG-IGSE)	Concluded	Mandate Final report
Working group on "Method Verification" (WG-MV)	Concluded	Mandate Final report
Working group on "unauthorised GMOs" (WG-UGM)	Concluded	Mandate Final report

ENGL Working Groups (active)



Title	Status	Downloads
Working group on digital PCR (WG-dPCR)	Active 	Mandate
Working group on update of methods (WG-UpMeth)	Active 	Mandate
Working group on unit of measurement (WG-UoM)	Active 	Mandate
Working group on Seed Testing (WG-ST)	Active 	Mandate
Working group on Detection, Interpretation and Reporting on the presence of authorised and unauthorised genetically modified materials (WG-DIR)	Active 	Mandate



1st Workshop on Species Identification

MBG Unit – JRC – Ispra
14 April 2015 (9:30 – 17:00)

STATEMENTS

The participants to the 1st Workshop on Species Identification organised by the Molecular Biology and Genomics Unit (MGB) at the JRC, Ispra on 14 April 2015, agree on the following general statements.

≤There is an emerging request for reliable and harmonised species identification and quantification, inter alia for allergen detection. This can be achieved by collecting and evaluating existing detection methods. To assess and compare these methods, criteria based on existing experience should be identified and agreed upon.

The establishment of a European Network for Species Identification (ENSI) was proposed by the meeting. It should serve as an expert platform for discussing and addressing technical problems common to the different fields in which Species Identification is applied/needed for regulatory purpose. The meeting agreed that such a network would be a concrete step towards a necessary better harmonisation of species identification within the EU.

Due to its recognised role and experience in the area of GMOs (ENGL, EURL GMFF) the participants suggested that the MBG Unit would lead and drive the establishment of the network, benefitting from the experience made with the ENGL.

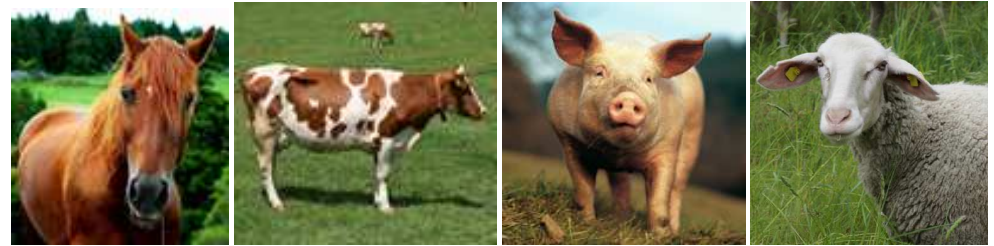


DNA-based/ Molecular Biology Methods

- **Animal Species**
- **Allergens**
- **Food Microbes (Pathogens)**

1st Workshop on Species Identification

MBG Unit – JRC – Ispra
14 April 2015 (9:30 – 17:00)



STATEMENTS

The starting point could be identification of fish species and of the five most relevant meat species: beef, pork, mutton, turkey and chicken.

There is also a need to harmonise the strategies for the methods application. This should include non-food applications, e.g. for custom control or environmental monitoring.

The Network could play a pivotal role in the development, implementation and standardisation of new emerging techniques for the accurate and robust identification and quantification of species in complex products.

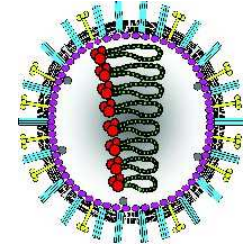


Fatstock prices (rounded)

- Pig 1,45 €/kg
- Cattle (Cow) 3,30 €/kg
- Turkey 1,40 €/kg
- Lambs 5,00 €/kg
- Horses 0,50 €/kg

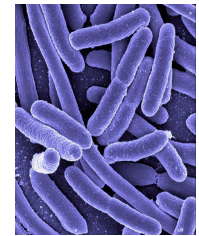


What is next?



What falls under the **G**enetically **M**odified **O**rganism legislation ?

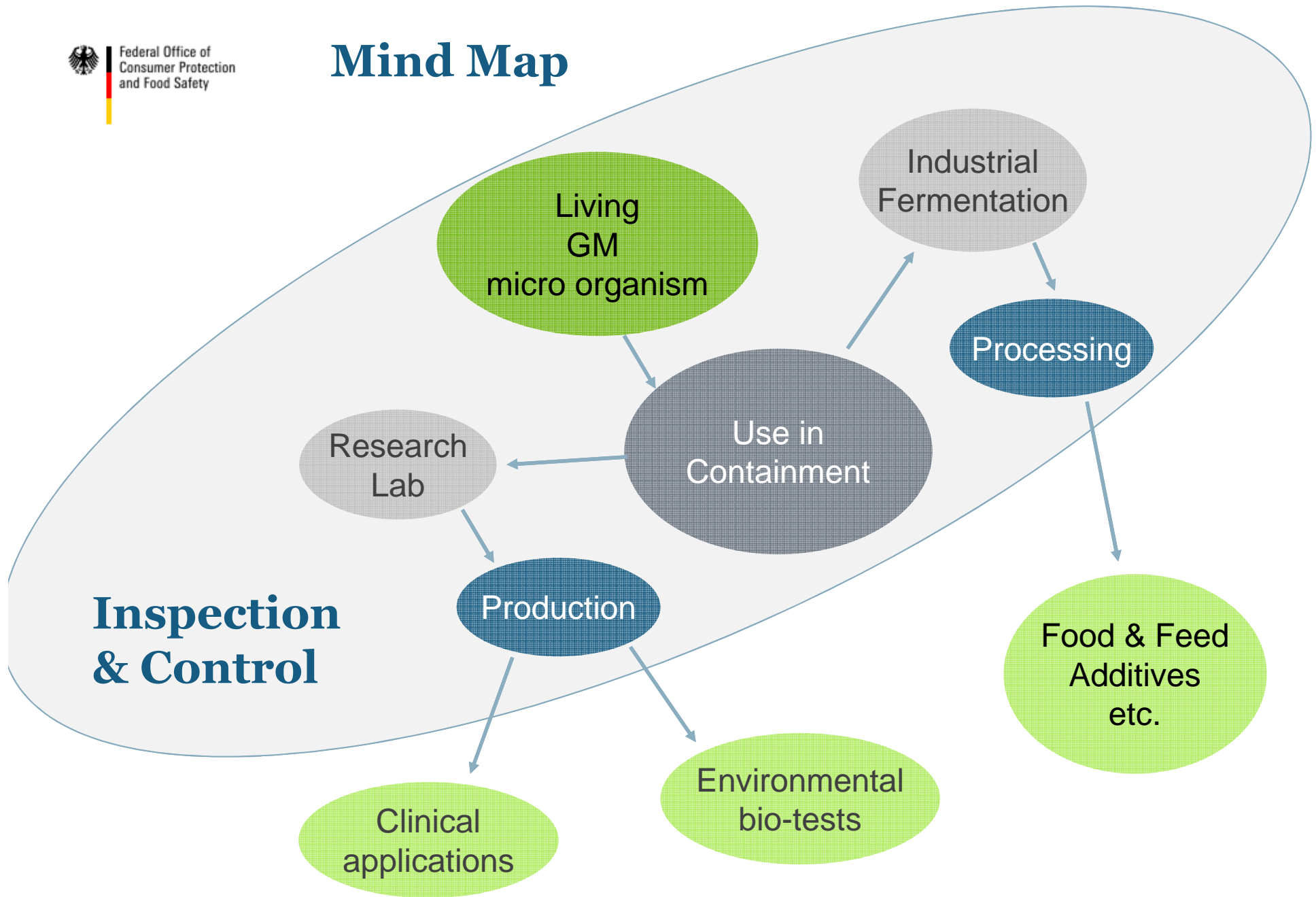
- an organism whose genetic material has been altered using **genetic engineering techniques**
- microorganisms (viruses, bacteria), plants, animals
 - living organisms
 - processed material (food/feed products)



Excluded: Vitamins, enzymes, amino acids etc.
produced with the help of GM microorganisms
Free of recombinant DNA, production strain

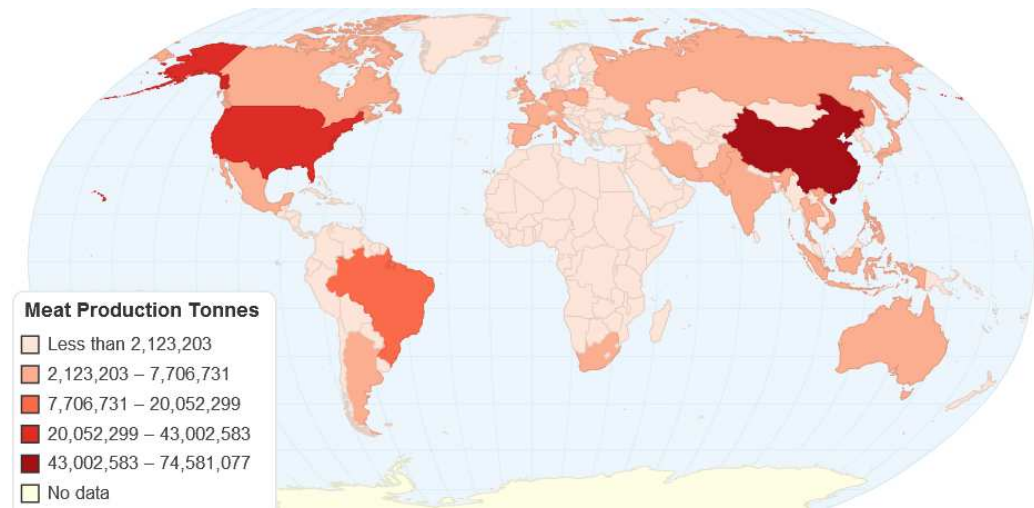


Mind Map

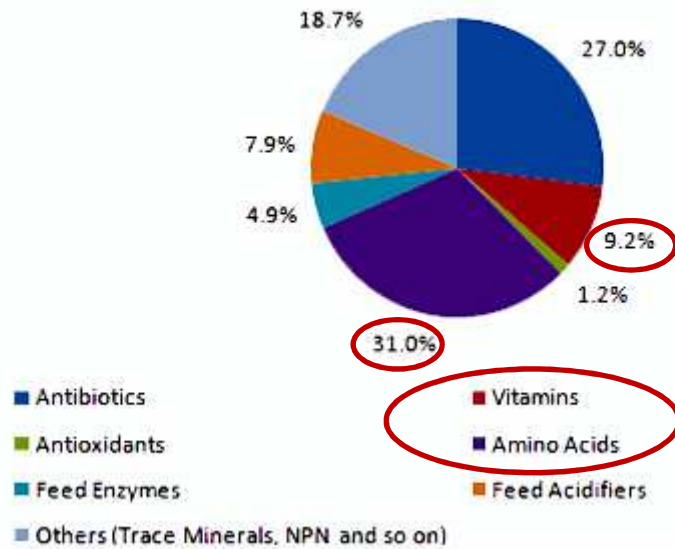


Global market for animal feed additives

was estimated to be 14,4 billion \$
 in 2012 and is expected
 to reach 19,5 billion in 2020



Animal feed additives market revenue share, by product, 2012



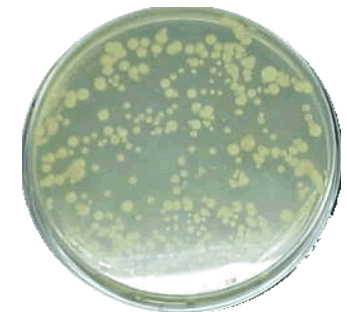
Vitamin B2 (riboflavin) used as feed additive

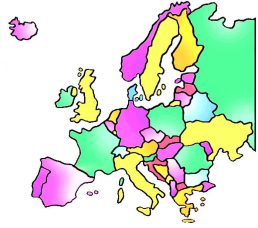
Sept. 2014
**Notification in the Rapid Alert System Food
and Feed (RASFF)**

***Importer notified for a lot of 3 tons of Vitamin B2
from China:***

Presence of living (spore-forming) ***Bacillus subtilis***
production strain

- recombinant DNA (rib operon on chromosome, 3 plasmids)





Possibilities for detecting and identifying crops produced with **New Plant Breeding Techniques**



- **Genome Editing – single nucleotide changes**
- **Removal of few base pairs**



- **How to Detect ?**
- **How to distinguish from conventionally bred crops ?**



GMOs authorised in EU



Food & Feed

60-70 GMOs approved for
import and marketing

19 GMO applications
pending for approval

Cultivation

Only 1 GMO
authorised: MON810

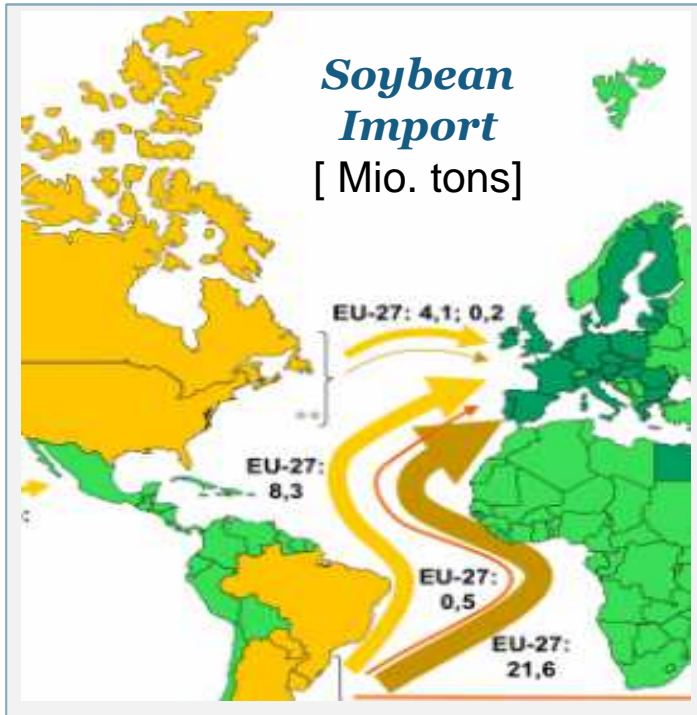
Only 1.5% of total maize
cultivation area in EU



8 GMO applications
pending for
cultivation approval

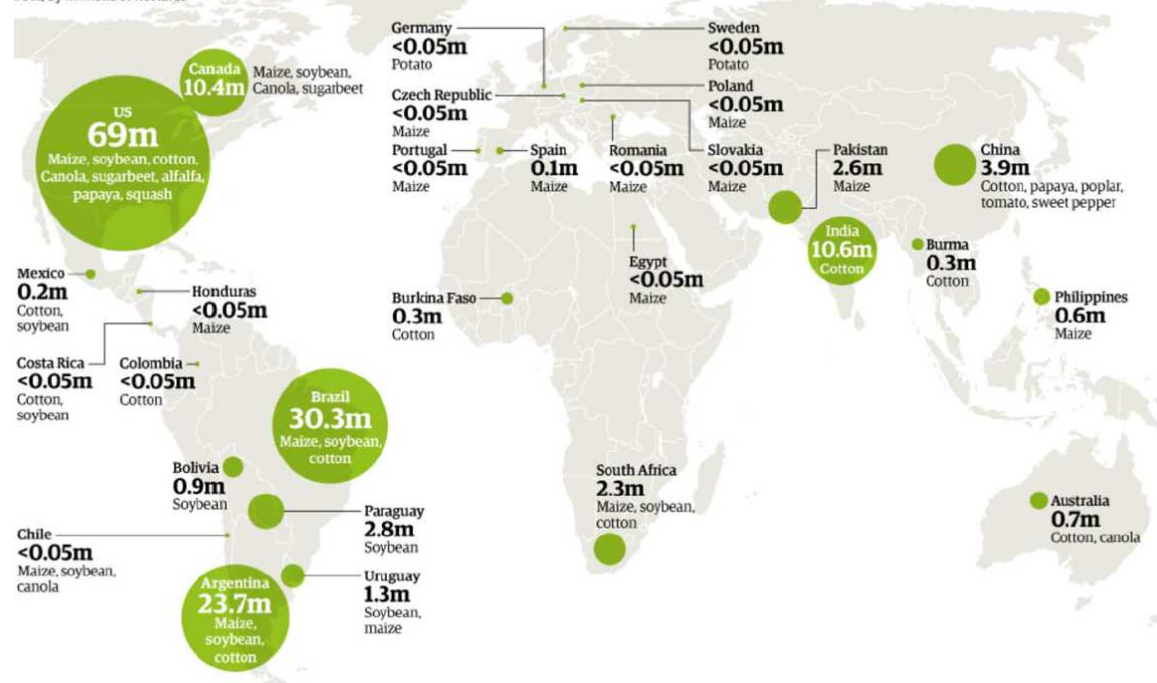


Authorised or not ?

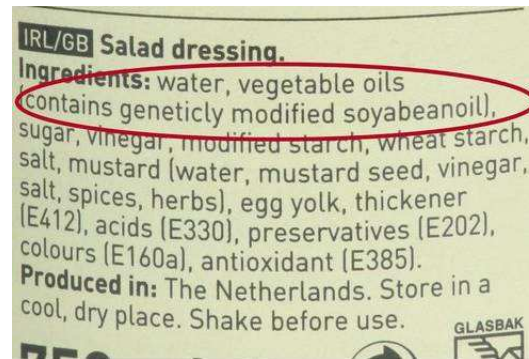


Global status of commercial GM crops

2011, by millions of hectares



Correct labelling ?





GVO Findings 2014



RASFF Portal

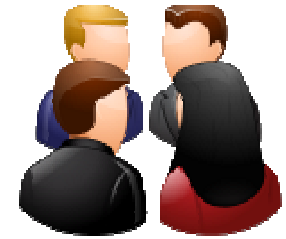
Search results

Search criteria | Notified from 01/01/2014 till 31/12/2014

	Classification	Date of case	Country	Type	Product Category	Subject
1	information for follow-up	01.09.2014	Belgium	food	cereals and bakery products	unauthorised genetically modified (Bt176 maize) pop corn from Argentina
2	border rejection	27.08.2014	Belgium	food	cereals and bakery products	unauthorised genetically modified (Cry1Ab) rice vermicelli from China
3	information for attention	14.08.2014	Norway	food	fruits and vegetables	unauthorised genetically modified (presence of p35S and tNOS) papaya in syrup from Thailand
4	information for attention	08.08.2014	United Kingdom	food	fruits and vegetables	unauthorised genetically modified green papayas from Thailand
5	border rejection	22.07.2014	Germany	food	cereals and bakery products	unauthorised genetically modified rice flour from China
6	border rejection	03.07.2014	Germany	food	fruits and vegetables	unauthorised genetically modified papaya from Thailand
7	border rejection	25.06.2014	Slovenia	food	cereals and bakery products	unauthorised genetically modified rice cakes from China
8	information for attention	10.04.2014	Netherlands	food	dietetic foods, food supplements, fortified foods	unauthorised genetically modified (p35S and tNos present) papaya powder from the United States



Challenges for Global Networking



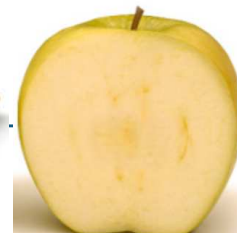
Exchange of information on GMOs

- in-the-pipeline
- approved for any use



What is basically required ?

- molecular data for screening
- (event-specific) PCR detection methods
- (certified) reference material



Thank you for your attention!

Contact:

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Consumer Protection
and Food Safety

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