Service Description

Content

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GMO Analysis

Real Time PCR (Polymerase Chain Reaction) is used to detect specific DNA segments used in genetically modified plants (GMO). The classical procedure in GMO analysis consists of screening → variety identifications → quantification of the identified variety/ies. Alternatively, relevant varieties can also be identified or quantified directly.

The decision to use a particular screening strategy is influenced by the factors of risk assessment, maximum possible information density, cost and time. No investigation strategy can guarantee a complete coverage of all possible GMOs. We will be happy to advise you on questions regarding the composition of parameters so that you can optimally tailor your monitoring program to your requirements.

GVO - Screening

Screening parameters are used to detect as many different GMOs as possible. The **limit of detection** (**LOD**) in raw material is **5 to 20 target DNA copies** depending on the system.

Screening	What it provides ...	... und what it doesn't
2-parameter Screening	Many globally relevant GM plants contain either the 35S promoter (p35S), the NOS terminator (tNOS), or both (e.g. Roundup Ready® soybean-1 MON Ø4Ø32-6)	A 2-parameter screening is not enough for detecting relevant GMOs.
3-parameter Screening	For soya, canola, mustard, rice, grain and mixed products we recommend a 3-parameter-screening. This way the GMO-canola GT73/RT73 (MON ØØØ73-7) and the Roundup Ready®-Soya-2 (MON89788) amongst others will be detected. Depending on origin and kind of product, different 3-parameter-screenings are reasonable.	For some GMOs, a 3-parameter-screening does not suffice. Therefore, an additional identification test is necessary for such GMOs.
4-parameter Screening	These screening combos are particularly suitable for mixed and processed products (e.g. convenience food, spices, compound feed). Here, significantly more GMOs are detected; thus, you also have a higher detection reliability regarding GMOs that do not have EU market approval. Based on these screenings, it is often possible to narrow down the circle of possible candidates even before GM variety identification.	If a GMO does not contain any of the screening-parameters, it cannot be found by the screening (only by a direct event-specific analysis).
6-parameter Screening	You want a very high density of information? Then you are well equipped with a 6-parameter-screening. Depending on your type of question different 6-parameter-screenings can be reasonable. Compared to the stepwise procedure you have a significant time advantage.	We gladly advise you with any specific questions you might have!
7-parameter Screening	In addition to the 6-parameter-screening the Cauliflower Mosaic Virus (CaMV) will be tested, which provides a significant time advantage in comparison to the stepwise procedure.	

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Screening	GMO-Screening-packages
2-parameter Screening	p35S + tNOS pNOS-*npt*II-construct + FP967-construct (Flax)
3-parameter Screening	p35S + tNOS + epsps-Gene[1] p35S + tNOS + pFMV tNOS + pat[3]- + epsps-Gene[1] p35S + tNOS + cry1Ab/cry1Ac (Rice) epsps[1]- + pat[3]- + bar-Gene p35S + tNOS + pat-Gene[3] p35S + tNOS + pNOS-nptII-construct (e.g. apple, papaya)
4-parameter Screening	p35S + tNOS + epsps-Gene[1] + pNOS-nptII-construct p35S + tNOS + Border-M[2] + pat-Gen[3] (Soya)
6-parameter Screening	p35S + tNOS + epsps[1]- + pat[3]- + bar-Gene + CaMV-ID p35S + tNOS + epsps[1]- + pat[3]- + bar-Gene + pNOS-nptII-construct p35S + tNOS + Border-M[2] + pat-Gene[3]+ 305423 + CV127 (esp. for soya)
7-parameter Screening	p35S + tNOS + epsps[1]- + pat[3]- + bar-Gene + pNOS-nptII-construct + CaMV-ID
Further parameter combinations for your specific problem / situation are possible on request!	

[1] CTP2-CP4epsps-construct

[2] Border-M: Captures at least the GM-soya-varieties MON89788, MON87701, MON87705, MON87708, MON87769 and MON87751. Additionally, GM-varieties of other genus are captured: GM-canola GT73 and MON88302, GM-Corn MON89034, MON88017, MON87460 and MON 87427 as well as the GM-sugar beet H7-1.

[3] pat: Captures e.g. the GM-soya-varieties A2704-12, A5547-127, DAS-68416-4, DAS-44406-6, SYHTOH2, DAS-81419-2

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GMO – identification and quantification

Variety-ID	Method and what it provides
For identifications, specific gene sections are detected, using Real Time-PCR. The statement shows whether a certain GM variety (or a kind of plant or virus) was detected.	Depending on the positive screening parameters of the initial analysis, possible GMOs can be identified, assigned and excluded. To find the right decision for follow-up tests, amongst others we consult the current market situation, global amounts of cultivation and our vast experience. Our analyses are done according to the protocols of the Joint Research Centre belonging to the EU Commission (JRC) and DIN standards. For several testing systems no JRC-protocol exists, in these cases we work according to peer-reviewed publications.

Limit of detection	The limit of detection (LOD) in raw materials is < 5 to 40 target DNA copies (~ 0.01 % – 0.05 %) depending on the system.

Quantification	Method and what it provides
The relative quantification using Real Time-PCR gives a statement about the genomic relation.	Once the GMO-variety is identified, quantification offers you information about the compliance with the threshold value (relevant for EU approved varieties). Should the variety identification show an unauthorized variety you can skip the quantification (there is an exception to the "zero-tolerance" policy for feed: according to EU Regulation (EU) 619/2011 some GMOs will be tolerated up to 0.1 % under certain circumstances). For the quantitative determination of the GMO content, the calibration curve method is applied (relative quantification). Thereby separate calibration curves are established: one for the specific GMO gen and a second for the species-specific reference gen. Based on the calibration curves the GMO content in relation to the reference gene is determined. This procedure does not give any statement about masses. All varieties of which reference genes and adequate detection methods are available can be quantified.

Limit of quantification	The limit of quantification (LOQ) is < 0.1 % and (amongst others) depends on the kind of matrix.

Our analyses are done according to the protocols of the Joint Research Centre belonging to the EU Commission (JRC) and DIN standard. For several testing systems no JRC-protocol exists, in these cases we work according to peer-reviewed publications.

As experienced specialists we are pleased to support you with your specific questions like the analytic of cotton, papaya and honey, also concerning marketability.

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Identification / Quantification	ID (qual. PCR)	Quant (% PCR)	GMO Variety
Virus	●		CaMV (Cauliflower Mosaic Virus; no GMO)
Soya	●	●	DAS 68416-4 (DAS-68416-4)
	●	●	A2704-12 (LibertyLink; ACS-GMØØ5-3)
	●	●	A5547-127 (LibertyLink; ACS-GMØØ6-4)
	●	●	BPS-CV127-9 (BPS-CV127-9)
	●	●	DP-305423 (DP-3Ø5423-1)
	●	●	DP-356043 (DP-356Ø43-5)
	●	●	FG72 (MST-FGØ72-2)
	●	●	MON87701-Soya ((MON877Ø5-6)
	●	●	MON87705 (MON877Ø5-6)
	●	●	MON87708 (MON-877Ø8-9)
	●	●	MON87769 (MON-87769-7)
	●	●	Roundup Ready-Soya-1 (GTS 40-3-2)
	●	●	Roundup Ready-Soya-2 (MON89788)
	●	●	DAS 44406-6 (DAS-444Ø6-6)
	●	●	DAS 81419-2 (DAS-81419-2)
Maize	●	●	3272 (SYN-E3272-5)
	●	●	5307 (SYN-Ø53Ø7-1)
	●	●	98140 (DP-Ø9814Ø-6)
	●	●	Bt11 (SYN-BTØ11-1)
	●	●	Bt176 (Maximizer; SYN-EV176-9)
	●	●	DAS 59122 (Herculex; DAS-59122-7)
	●	●	DAS 40278-9 (DAS-4Ø278-9)
	●	●	GA-21 (Roundup Ready; MON-ØØØ21-9)
	●		LY038 (REN-ØØØ38-3)
	●	●	MIR162 ((SYN-IR162-4)
	●	●	MIR604 (SYN-IR6Ø4-5)
	●	●	MON810 (YieldGard; MON-ØØ81Ø-6)
	●	●	MON863 (YieldGard; MON-ØØ863-5)
	●	●	MON87460 (MON-8746Ø-4)
	●	●	MON88017 ((MON88Ø17-3)
	●	●	MON89034 (MON89Ø34-3)
	●	●	MON87427 (MON-87427-7)
	●	●	NK603 (Roundup Ready: MON-ØØ6Ø3)
	●	●	T25 (LibertyLink; ACS-ZMØØ3-2)
	●	●	TC1507 (Herculex; DAS-Ø15Ø7-1)

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Identification / Quantification	ID (qual. PCR)	Quant (% PCR)	GMO Variety
Canola	●	●	73496 (DP-Ø73496-4)
	●	●	HCN 92 (Topas 19/2; ACS-BNØØ7-1)
	●	●	MS8 (ACS-BNØØ5-8)
	●		Oxy-235
	●	●	RF3 (ACS-BNØØ3-6)
	●	●	Roundup Ready-Canola (GT73/RT73; MON-ØØØ73-7)
	●	●	T45 (HCN 28, LibertyLink; ACS-BNØØ8-2)
	●	●	MON88302 (MON-883Ø2-9)
Flax	●		FP967 CDC „Triffid“
Rice	●		Bt63
	●		LL601 (LibertyLink)
	●	●	LL62 (LibertyLink; ACS-OSØØ2-5)
Sugar Beet	●	●	H7-1 (KM-ØØØ71-4)
Potato	●	●	Amflora (EH92-527-1)
Alfalfa	●	●	J101 (MON-ØØ1Ø1-8)
Cotton	●	●	MON531 (MON-ØØ531-6)
	●	●	15985 (MON-15985-7)
	●	●	MON1445 (MON-Ø1445-2)

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GMO - analytical spectra for VLOG / according to VLOG-specifications

As VLOG-acknowledged laboratory we offer analytical spectra that meet the recommendations of the "Ohne Gentechnik" (non GMO) production- and testing standard (Laboratory Guideline in its respective current version). Of course, we guarantee compliance with the requirements for laboratories.

Matrix	Analysis	
Feed		
Compound Feed without soy	Soy is no ingredient:	Estimation of the soy-mass (ELISA)
	+ Maize as ingredient:	NK603 ID+ TC1507 ID + MON810 ID + MON89034 ID
	+ Canola as ingredient:	GT73 ID + bar-Gene
Compound Feed with soy	Soy as ingredient:	RRS-1 % + RRS-2 % + A2704-12 ID + A5547-127 ID
	+ Maize as ingredient:	NK603 ID+ TC1507 ID + MON810 ID + MON89034 ID
	+ Canola as ingredient:	GT73 ID
Raw product	Soy:	RRS-1 % + RRS-2 % + A2704-12 ID + A5547-127 ID
	Maize:	p35S + tNOS
	Canola: Canola alternative:	tNOS + CTP2-CP4epsps + pat (LL-construct) Estimation of the soy-mass (ELISA) + GT73 ID + bar-Gene
Food		
Rice/ Rice products	p35S + tNOS + cry1Ab/cry1Ac-sequence	
Honey	p35S + tNOS + Border-M or p35S tNOS + bar + CTP2-CP4epsps + pat	
Others	We are happy to coordinate the strategies for GMO analysis of other individual feeds, raw materials, (food) ingredients, intermediate products or foodstuffs with you, taking into account composition and origin.	

Abbreviation
RRS-1 ("Roundup Ready-Soya-1") = GTS 40-3-2
RRS-2 ("Roundup Ready-Soya-2") = MON89788
% = Quantification
ID = Identification

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Allergen analytics

There are several ways you can have your samples tested for allergens: **ELISA** (protein level), **PCR** (genome level DNA), and chemical-enzymatic.

Compared to **ELISA, PCR** is often more specific and has fewer cross-reactions as well as matrix effects. Due to our semi-quantitative method, quantity estimations in the form of a range are also possible on request. LOD / LOQ per allergen and method are available on request.

Group	Allergen	PCR	ELISA	chem. enzym
Others	Soya	●	●	
	Lupine	●	●	
	Sesame	●	●	
	Mustard (S. alba, B. juncea, B. nigra)	●		
	Gluten (Gliadin) (PCR: Wheat)	●	●	
	Celery	●		
	Oat	●		
	Barley	●		
	Rye	●		
Nuts At the moment it is not possible to detect the aller-gen group „nuts“ with only one test.	Peanut	●	●	
	Hazelnut	●	●	
	Almond	●	●	
	Walnut	●		
	Macadamia	●		
	Cashew	●		
	Pistachio	●		
	Brazil nut	●		
	Pecan nut	●		
	Coconut	●		
Milk	Milk (ß-Lactoglobulin+Casein)		●	
	β-Lactoglobulin		●	
	Casein		●	
	Lactose and Galactose (2 Tests)			●
Egg	Egg		●	
	Lysozyme		●	
Fish & others	Fish (multi species method for bony fishes)	●		
	Crustaceae	●	●	
	Mollusca	●		
Insects	Insects	●		

Further parameters on request!

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Authenticity testing

Identification of species using Real Time PCR

Plant and animal species analysis	Method and what it provides
The analysis states whether a certain gene sequence of a plant or animal species was detected.	Using Real Time-PCR (Polymerase Chain Reaction) specific plant and animal DNA segments are detected. The analytic statement is whether certain DNA sequences were detected (yes/no). Our analyses are done according to the protocols of the Joint Research Centre belonging to the EU Commission (JRC) and DIN standards. For several testing systems no JRC-protocol exists, in these cases we work according to peer-reviewed publications or self-developed testing systems.
Limit of detection	The limit of detection (LOD) in raw material is 10 to 20 target DNA copies depending on the system.

Plant species				
Apricot	Cotton	Cashew	Peanut	Barley
Hazelnut	Potato	Coconut	Flax	Lupine
Alfalfa	Macadamia	Maize	Almond	Brazil nut
Papaya	Pecan nut	Pistachio	Canola (*Brassica napus* specific)	Canola (Brassicaceen)
Rice	Rye	Celery	Mustard (yellow + brown / black)	Sesame
Soya	Walnut	Wheat	Sugar Beet	Further parameters on request!

Animal species				
Barbary / Muscovy duck	Crustaceans (multi species method for most Crustaceans)	Bonito (Katsuwonus pelamis)	Duck (incl. Muscovy Duck)	Fish (multi species method for bony fishes)
Goose	House cricket (Achaeta domesticus)	Chicken	Insects	Meal worm beetle (Tenebrio molitor)
Molluscs (multi species method for most Molluscs)	Horse (incl. Donkeyl)	Turkey	Cow	Sheep
Pig	Ostrich	Water buffalo	Goat	animal DNA (mammals and poultry)

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Identification of species using sequencing (Barcoding)

Sequencing	Methods: Vertebrates / land plants / crustacean / molluscs
Normally, at least two different mitochondrial gene sequences (vertebrates as well as crustaceans and molluscs) or at least two different plastid/nucleus-encoded gene sequences (terrestrial plants) are sequenced. Subsequently, the species at hand is determined by alignment of the DNA sequence with different databases. This method can only be used for monoproducts. Larger admixtures of another species can lead to the fact that the sequence data are not evaluable or only the second species is identified (if it contains the most DNA).	
Informative value	
Sequencing can be used not only to identify a specific known species, but also to determine whether and which unknown species are present that deviate from the declaration. In individual cases, only the genus may be identifiable. In contrast to PCR, it is not necessary to know in advance which animal or plant species is being searched for.	

Identification of species using Next-Generation-Sequencing (NGS)

NGS	Methods: Vertebrates / land plants
This technique is also known as metabarcoding. After extraction of the DNA, two different mitochondrial gene sequences (vertebrates) or one plastid and one nuclear-encoded gene sequence (terrestrial plants) are amplified by PCR. All amplicons of the different species are then sequenced simultaneously and in both directions. Subsequently, bioinformatic processing of the obtained sequences including quality control is performed. Afterwards, the determined different sequences are used for the identification of the respective species contained in a sample by comparison with a database.	
Informative value	
The result of the procedure is an identification of those species which are contained in the sample (mixed sample or monoproduct) with sufficiently high DNA proportions. The sample can thus be analyzed for the expected ingredients as well as for the presence of unknown and unexpected admixtures. The limit of detection is approximately between 0.5% and 5% depending on DNA content, species and tissue type. The method is primarily qualitative, but the number of different sequences obtained can be included for evaluation. The detected species are reported in the order of the number of sequences found. You will get an estimation if unexpected species in a sample could be due to accidental contamination.	

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Vegan tests (partly usable for vegetarian products)

Packaget	PCR test	ELISA test
Vegan test light	Mammal and poultry + fish (bone fishes)	-
Vegan test	Mammal and poultry + fish (bone fishes)	Egg
Vegan test sensitive	Mammal and poultry + fish (bone fishes)	Egg + milk
Vegan test complete	Mammal and poultry + Cow mitochondrial + fish + molluscs + crustacean + insects	Egg
Vegan test complete sensitive	Mammal and poultry + fish (bone fishes) + molluscs + crustacean + insects	Egg + milk
Optional: Lactose + Galactose (enzymatic test)		

Ethics test Halal / Haram

Detection of Pig-DNA, Horse/Donkey-DNA and ethanol
PCR: Pig / pork (incl. wild boar) Enzymatic: Ethanol (analysis performed by Tentamus Lab bilacon GmbH)
Optional: PCR: Horse (incl. donkey)

Gender determination (using Real Time PCR)

Gender determination	Method and typical use cases
Bovine	A DNA region of the Y-chromosome of male bovine animals is detected by real-time PCR; in addition, a detection of bovine animals in general is used as a reference system. A typical use case is the authenticity testing of heifer meat.
Pig	A DNA region of the Y-chromosome of male pigs is detected by Real Time PCR; in addition, a detection of pigs in general is used as a reference system. A typical use case is boar odor in meat.
Chicken	A DNA region of the sex chromosome W of female chickens is detected by real time PCR; in addition, a detection of chicken and rooster is used as reference system. A typical use case is the detection of brother cock meat.

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Detection of soft wheat contaminations

Soft wheat portion	Method (Real Time PCR)
in durum wheat	Using Real Time Polymerase chain reaction (PCR) we detect two sequence sections and quantify them using standard curves, one of which is specific for soft wheat (Triticum aestivum incl. subspecies like spelt) and one of which is specific for total wheat. The quantification limit (LOQ/determination limit) of the method is 3%.
In spelt products	Using Real Time Polymerase chain reaction (PCR) we detect three sequence sections, two of which are specific for different soft wheat varieties (Triticum aestivum) and one of which is specific for total wheat. The analysis is semi quantitative using single point calibration (specification as < 5% soft wheat or as range, e.g. 5-10%).

Estimation of the amount of Bonito in tuna conserves

Bonito portion	Method (Real Time PCR)
in tuna conserves	Testing for Bonito (Katsuwonus pelamis) and Fish. Estimation using ΔΔct-method. The Limit of quantification (LOQ) is < 1 %.

Species identification of Barbary duck

Species-ID	Method (Real Time PCR)
Barbary Duck	DNA sequences specific for Barbary / Muscovy / Mulard duck are detected. The statement depends on detection of certain DNA sequences (yes or no). Beijing and Mallard ducks cannot be identified using the detection system. The Limit of detection (LOD) is 5 copies.

Estimation of the amount of persipan in marzipan

Apricot	Method (Real Time PCR)
in almond / marzipan	Species specific DNA-sequences for almond and apricot are detected, e.g., in marzipan or almond paste. Estimation using ΔΔct-method. The limit of detection is <10 target DNA copies (almond) or <50 target DNA (apricot).

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Estimation of the amount of cow milk in milk products

Cow milk	Method (Real Time PCR)
in sheep-/ goat cheese, -milk, -whey or -yoghurt	Using Real Time-PCR, mitochondrial DNA sequences specific for cow, sheep and goat are detected. Estimation using ΔΔct-Method. The Limit of detection is < 1 target DNA copies.
in buffalo products (e.g. buffalo mozzarella)	Using Real Time-PCR, mitochondrial DNA sequences specific for cow and water buffalo are detected. Estimation using ΔΔct-Method. The Limit of detection is < 1 target DNA copies.

Estimation of the amount of BRW in goat- and sheep-milk products

BRW	Method (ELISA)
in dairy products of other vertebrates (e.g. goat or sheep whey or milk powder)	The concentration of an antibody or antigen is measured in a test solution. One of the reactants is enzyme labelled that lead to colorimetric detection. The test is calibrated for BRW (bovine rennet whey) with a protein content of 11%. The limit of detection (LOD) is 0.1%; The limit of quantification (LOQ) is 0.25%

Analysis of CMS species

Ogura sequence	Method (Real Time PCR)
Occurrence: cabbage varieties	The cytoplasmatic male sterility (CMS) is brought in hybrid plants by cell fusion technology. A certain DNA section which is found in CMS species is detected by using the Real-Time Polymerase Chain Reaction (PCR). The Limit of detection (LOD) in raw materials is 50 DNA-copies.

Verification of varieties using FLA (apples or potatoes)

Verification of varieties	Method (Real Time PCR, fragment length analysis)
Potatoes	After the DNA-extraction specific SNPs are amplified using PCR. Their length is then determined using fragment length analysis. This is performed parallel for 6 apples / potato tubes per sample. The occurring fragment length patterns are matched with an internal database. As this database contains only a limited number of apple/potato varieties, it cannot be ruled out that varieties not included in this database may have identical patterns. For the selection of suitable primers, an indication of the expected apple/potato variety is necessary.
Apples	

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