

Generically Modified Organisms

Real-Time PCR detection and quantification





Commercially available genetically modified organisms (GMO) are plants whose genome was artificially inserted with DNA fragments from foreign species. Europe developed a strict regulation to give maximum protection of public health and of environment, while at the same time providing a science based regulatory structure where biotechnology can flourish.

European public opinion has expressed the desire of having an informed choice in what they are eating. Consequently, the European Union has introduced legislation on the traceability and detection of GMOs, including labelling of food and feed containing GMOs, or derived products thereof, above a defined threshold of fortuitous presence.

The development and application of reliable detection and quantitative analytical methods was essential for the implementation of the labelling rules. Real-time PCR is the gold standard in GMO analysis according to ISO norms. The molecular analysis required to comply with the current European Union GMO legislation consist of three distinct steps:

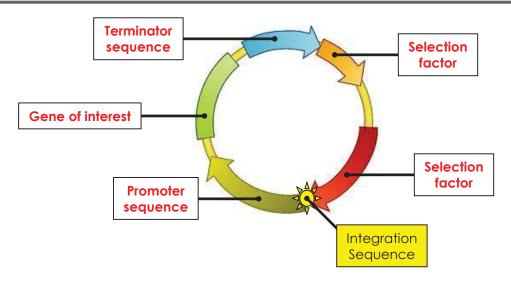
Detection Identification Quantification

**MODIfinder** is the product line developed by **Generon** with premium quality molecular biology reagents to provide the customer a "turn-key" solution for the detection and quantification of a wide selection of specific and generic GMO events according to EU norms.

Using MODIfinder products along with an appropriate DNA extraction method enables to test the presence of GMOs in the most diverse matrices: Bulk/Raw Ingredients, Semifinished products, Finished products.

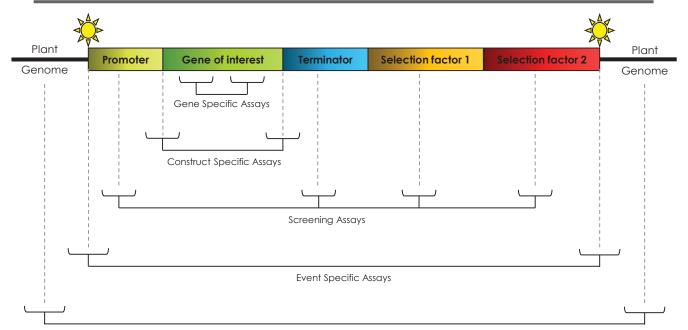
The gene construct is inserted in the recipient plant using a circular cloning vector composed of several elements, usually at least: the gene of interest, a promoter functioning as a start signal, a terminator functioning as a stop signal for regulation of gene expression and selection factors necessary for the segregation of the plasmid and of the transgenic cells (Figure 1).

Figure 1 – Circular structure of a plasmid to transfer genes into a plant genome



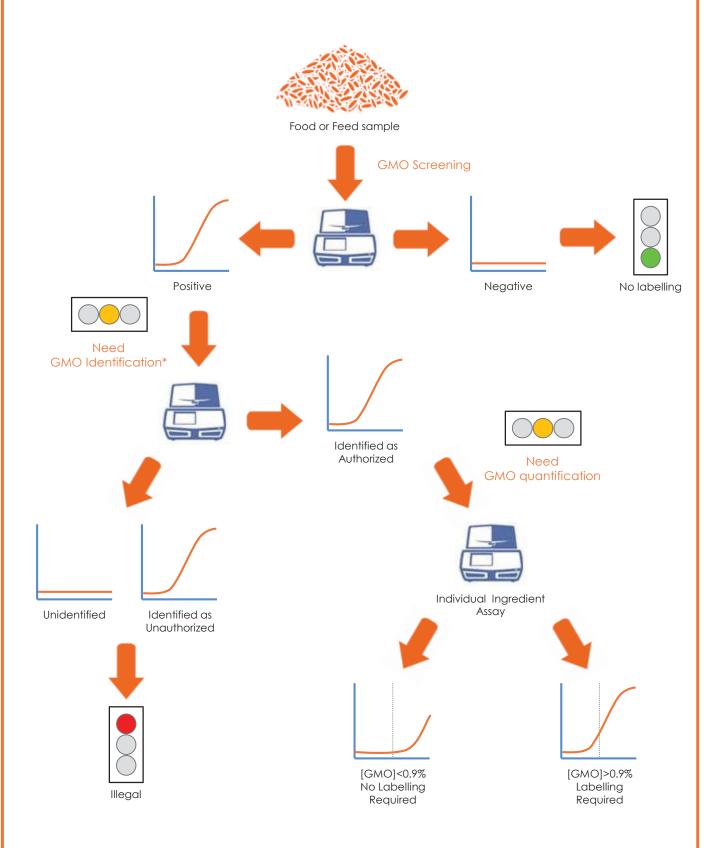
When the plasmid integrates into the plant genome it takes a linear structure that accounts for the different PCR-based GMO tests to detect it. These can be grouped into at least four categories corresponding to their level of specificity (Figure 2).

Figure 2 – Structure of a «transgene cassette» transferred into a plant genome and PCR tests



The current EU legislation covers all GMOs that have received Community authorization for the placing on the market, including food and feed, containing or consisting of GMOs. Examples are GM seeds, and bulk quantities or shipments of whole GM grain e.g. soybean and maize. The regulations also cover food and feed products which are derived from GMOs. This includes starch, highly refined oil or flour produced from a GM maize or lecithin from soybean or rapeseed. Regulation (EC) 1829/2003 calls for the labelling of food and feed products that intentionally contain authorized GMO, or have an adventitious (accidental and technically unavoidable) presence of authorized GMOs, above a threshold of 0.9% in otherwise non-GMO food and feed. Every time that a non-labelled product contains authorized GMOs, but at a level below 0.9%, evidence must be provided that such adventitious presence could not have been avoided by routine food and feed chain procedures. Above the 0.9% threshold the product must be labelled. Noteworthy when the sample contains a mixture of GMOs, each ingredient (analytically translated as content per taxon) may not exceed 0.9%, irrespective of its proportion in the final product. In contrast, unapproved GMOs are not permitted at any level.

Figure 3 – A decision tree for labelling GMO food and feed according to EU legislation



<sup>(\*)</sup> When testing complex matrices composed by more then one ingredient is fundamental to have knowledge of the different plant varieties (taxa) present in the sample in order to restrict the number of GM specific test to be performed.

As the number of genetically modified plants is increasing, it is necessary to use appropriate qualitative methods for GMO screening in food products. The basis for a qualitative GMO screening procedure is the use of control elements such as promoters, transcription terminators, and markers, such as resistance genes.

Negative responses from such a panel of screening methods eliminate the possibility of GMO presence in a test sample, but only if the selected screening methods cover all the GMOs to be detected.

#### MODIfinder™ Screening Assays collection includes:

- Real-Time assays for all the relevant screening target (including gene and construct specific
  assays) and allows their detection in single-plex or multiplex. Assays were developed following
  indications published in the JRC "Compendium of reference methods for GMO analysis" or in
  other peer reviewed scientific literature.
- Plant/Eukaryotic generic marker to determine the overall amplificability of the extracted DNA when ingredient are not known.

## **MODIfinder™ Screening Assays**

Description (target acronym)	50 Reactions kit			
MODIfinder 35S Promoter Assay (p35S)	PGE01A-50			
MODIfinder 35S Terminator Assay (†35S)	PGE03A-50			
MODIfinder NOS Terminator Assay (tNOS)	PGE04A-50			
MODIfinder 28S Assay	PGE06A-50			
MODIfinder NptII Assay	PGE07A-50			
MODIfinder PAT Assay	PGE14A-50			
MODIfinder BAR Assay	PGE15A-50			
MODIfinder EPSPS Assay	PGE16A-50			
MODIfinder FMV Promoter Assay (pFMV)	PGE17A-50			
MODIfinder Cry1Ab/Ac Assay	PGE25A-50			
MODIfinder NOS Promoter Assay (pNOS)	PGE27A-50			
B. Multiplex Real-Time PCR kits for GM markers detection				
Description	50 Reactions kit			
MODIfinder Assay 2-plex p35S(FAM) + tNOS(HEX)	PGE05A-50			
MODIfinder Assay 2-plex [NptII(FAM) + pNOS(Texas Red)]	PGE37A -50			
MODIfinder Assay 2-plex [NptII(FAM) + Pfmv(HEX)]	PGE40A -50			
MODIfinder MultiSCREEN Assay 3-plex [p35S(FAM)/tNOS (HEX)/pFMV(Cy5)]	PGE26A-50			
MODIfinder MultiSCREEN Assay 3-plex [p35\$(FAM)/tNO\$ (HEX)/pFMV(Cy5) + IAC(Texas Red)]	PGE26A-C-50			
MODIfinder MultiSCREEN Assay 3-plex NPTII(FAM)/PAT (HEX)/EPSPS Cy5)	PGE32A-50			
MODIfinder MultiSCREEN Assay 3-plex [ctp2cp4EPSPS(FAM)/PAT(HEX)/BAR(Texas Red)]	PGE38A-50			
MODIfinder MultiSCREEN Assay 4-plex p35S (FAM)/tNOS (HEX)/pFMV(Cy5)/BAR(Texas Red)	PGE34A-50			
MODIfinder MultiSCREEN Assay 4-plex NPTII (FAM)/PAT(HEX)/EPSPS (Cy5)/pNOS(Texas Red)	PGE35A-50			
MODIfinder MultiSCREEN Assay 4-plex [ctp2cp4EPSPS(FAM)/PAT(HEX)/BAR(Texas Red)/NPTII(c	y5)] PGE42A-50			
MODIfinder CP4EPSPS/BAR Duplex Assay [ctp2cp4EPSPS(FAM)+ BAR(HEX)]	PGE44A-50			
MODIfinder 35S Terminator/PAT Duplex Assay [35S terminator (FAM)+ PAT (HEX)]	PGE45A-50			
MODIfinder MultiSCREEN Assay 4-plex p35S(FAM)/tNOS(HEX)/pFMV(Cy5)/t35S(Texas Red)	PGE47A-50			
C. Special Screening kits				
Description	50 Reactions kit			
MODIfinder CaMV Assay	PGE02A-50			
MODIfinder CaMV (FAM)/FMV (HEX) Duplex Assay	PGE39A-50			
A positive screening for P35s or pFMV does not necessarily imply the presence of GMO-derived DNA. The detected genetic to the presence of the host organisms namely the cauliflower mosaic virus (CaMV) or the figwort mosaic virus (FMV). These environmental Mosaic Virus causing "false GM positivity" when present in infected cells (e.g. rapeseed) contaminating the s	se kits allow for the detection of the DN.			
MODIfinder MultiSCREEN <b>Soy</b> 2-plex CV127/DP305423+DP356043(HEX)/MON87708+MON87769(FAM)	PGE41A-50			
MODIfinder MultiSCREEN <b>Soy</b> 3-plex CV127/DP305423/MON87701	PG\$16A-50			
This kit allows for the detection of these GM events all together in a single reaction: CV127 (FAM); DP305423 (HEX); MON8770	1 (Cy5).			
MODIfinder MultiSCREEN <b>Soy</b> 3-plex A2704/DP356043/A5547	PG\$18A-50			
This kit allows for the detection of these GM events all together in a single reaction: A2704 (FAM); DP356043 (HEX); A5547 (Cy	75).			
MODIfinder MultiSCRFEN <b>Sov</b> 3-plex cv127+mon87701/dp305423+dp356043/mon87708+mon87769	PG\$21A-50			

MODIfinder MultiSCREEN **Soy** 3-plex cv127+mon87701/DP305423+DP356043/mon87708+mon87769 This kit allows for the detection of these GM events all together in a single reaction: CV127+MON87701 (FAM); DP305423+DP356043 (HEX); MON87708+MON87769 (Cy5).

This kit allows for the detection of these GM events all together in a single reaction: LY038 (FAM); 98140(HEX); DAS40278-9 (Cy5).

PGS21A-50

MODIfinder MultiSCREEN **Canola** 3-plex Ms8/T45/Rf3 PGZ31A-50

This kit allows for the detection of these GM events all together in a single reaction: Ms8 (FAM); T45 (HEX); Rf3 (Cy5).

MODIfinder MultiSCREEN Corn LY038/DAS40278-9 Assay 2-PLEX PGC28A-50

#### D. Kits for DNA Amplifiability verification

Description	50 Reactions kit
MODIfinder 28S (eukaryotic DNA) Assay Assay	PGE06A-50
MODIfinder Chloroplast ENDO Assay	PGE08A-50

For quantitation, the copy number of the GM PCR target relative to genome copies of the corresponding species is important because the principle of quantitation of GM content is to compare the relative ratio of these two. Usually, a PCR targeting a species-specific single copy gene is used to determine the amount of the species derived DNA, for example the soybean lectin gene for soya-derived DNA. Thus, we also need PCR methods for the reference targets (taxon specific endogenous reference gene).

These methods also help in the upstream identification of the ingredients present in an unknown and complex matrix, thus facilitating the identification of the contaminating, when the sample is found positive at a first screening.

Taxon specific assays can also be used to determine the overall amplificability of the extracted DNA when the ingredient is known.

# MODIfinder™ Taxon Specific Assays

#### A. Singleplex Real-Time PCR kits for corn

Description	50 Reactions kit
MODIfinder Zeine Assay	PGE10A-50
MODIfinder Invertase Assay	PGE11A-50
MODIfinder ADH1 Assay	PGE23A-50
MODIfinder HMG Assay	PGE24A-50

### B. Singleplex Real-Time PCR kits for other taxon

Description	50 Reactions kit
MODIfinder Soy (Lectin) Assay	PGE09A-50
MODIfinder Flax Assay	PGE12A-50
MODIfinder Rice Assay	PGE13A-50
MODIfinder Canola (Rapeseed) Assay	PGE19A-50
MODIfinder Potato Assay	PGE20A-50
MODIfinder Cotton Assay	PGE21A-50
MODIfinder Sugar Beet Assay	PGE22A-50
MODIfinder Tomato Assay	PGE28A-50
MODIfinder Wheat Assay	PGE29A-50

#### C. Multiplex Real-Time PCR kits for taxa screening

Description	50 Reactions kit
MODIfinder MultiENDO Corn/Soy Assay 2-PLEX	PGE43A-50
This kit allows for a convenient detection of these taxa all together in a single reaction: Corn (FAM); Soy (HEX).	
MODIfinder MultiENDO Corn/Soy/Rapeseed/Cotton Assay 4-PLEX	PGE33A-50
This kit allows for a convenient detection of these taxa all together in a single reaction: Corn (FAM); Soy (HEX); Ro	apeseed (Texas Red); Cotton (Cy5).

MODIfinder™ Identification Assays were developed to detect the only unique signature of a transformation event that is the junction at the integration locus between the recipient genome and the inserted DNA. The identification of the GMO contaminating the matrix under investigation is a fundamental step when considering the labelling rules enforced in Europe.

Quantitation of GMO contamination must be in fact relative to something. In European legislation it refers to the presence w/w relative to the ingredient and not to the whole product. Therefore knowing exactly which is the event to quantify is crucial.

Noteworthy, even the event-specific methods have their limitations. When two GMOs are crossed (e.g. two different GM maize such as T25 and Mon810), the resulting hybrid offspring may contain the genetic modifications including the signatures of both events and will be indistinguishable from its two parents in a PCR test (unless the test is performed on DNA from a single plant, tissue or kernel/seed/grain). This phenomenon is referred to as **gene stacking**.

All the assays allows for qualitative DNA detection with a detection limit of 0.01% w/w depending on matrix and DNA preparation. Reaction volume 30  $\mu$ l (sample 12  $\mu$ l). Each assay box contains a positive and negative control.

Next tables list all the events (excluding the stacked events for clarity) registered in the Euginius project database, one of the most complete public database of GM available, their approval status [ Approved Not Approved Approved with restrictions ] and the screening markers associated [Reported and detected by MODIfinder screening assays Reported as a modified variant and not detected by MODIfinder screening assays] modified. Available MODIfinder event specific kits part number are reported on the right.

	Event	EU Approval	P-35S	T-35S	son-d	SON-+	FMV	NPT II	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
"T													
	32138												
	4114												
	5307												PGC24A
	676												
	678												
	680												
	B16 (DLL25)												PGC06A
	Bt10 maize												
	B†11												PGC04A
	B†176												PGC01A
	BVLA430101												
	CBH351												
	TC1507												PGC08A
	DAS40278												PGC22A
	DAS59122												PGC11A
	DAS59132												
	DBT418												PGC12A
	Event 3272												PGC17A
	Event 98140												PGC13A
	GA21												PGC07A
	LY038												PGC16A
	MIR162												PGC14A
	MIR604												PGC10A
	MON80100												
	MON802												
	MON809												
	MON810												PGC02A
	MON832												
	MON863												PGC09A
	MON87403												
	MON87411												PGC27A
	MON87419												
	MON87427												PGC25A
	MON87460												PGC23A
	MON88017												PGC19A
	MON89034												PGC20A
	Ms3												
	Ms6												
	MZHG0JG												
	MZIR098												
	NK603												PGC05A
	T14												
	T25												PGC03A
	TC6275												
	VCO-01981-5												PGC26A

## MODIfinder™ GM Soy Identification Assays

<b>,</b> T	Event	EU Approval	P-35S	T-35S	SON-d	SON-4	FMV	II LAN	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
	260-05												
	A2704-12												PGS01A
	A2704-21												
	A5547-127												PGS03A
	A5547-35												
	CV127												PGS08A
	DAS44406												PG\$12A
	DAS68416												PG\$13A
	DAS81419												PG\$14A
	DP305423												PGS05A
	DP356043												PGS06A
	FG72												PG\$11A
	GTS 40-3-2												PGS02A
	GU262												
	MON87701												PG\$07A
	MON87705												PGS09A
	MON87708												PG\$15A
	MON87712												
	MON87751												PG\$19A
	MON87754												
	MON87769												PG\$10A
	MON89788												PGS04A
	SYHT0H2												PGS20A
	W62												
	W98												

# MODIfinder™ GM Rice Identification Assays

Event	EU Approval	P-35S	1-358	SON-d	\$ON-4	FMV	NPT II	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
B†63												PGR03A
Bt-ZJ22												
Golden Rice 2												
Kefeng6												
KMD1												
LLRICE06												
LLRICE601												PGR01A
LLRICE604												
LLRICE62												PGR02A
T1c-19												

# MODIfinder™ GM Cotton Identification Assays

Event	EU Approval	P-35S	1-358	SON-d	\$ON-	FMV	NPT	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
10215												
10222												
10224												
19-51A												
281-24-236												PGT03A
3006-210-23												PGT04A
31807												
31808												
BXN10211												
COT102												
COT67B												
DAS81910												
Event 1												
GHB119												PGT09A
GHB614												PGT07A
LLCotton25												PGT05A
MON1076												
MON1445												PGT02A
MON 15985												PGT06A
MON1698												
MON531												PGT01A
MON757												
MON88701												
MON88913												PGT08A
T303-3												
T304-40												PGT10A

# $\mathbf{MODIfinder}^{\mathsf{TM}} \ \mathbf{GM} \ \mathbf{Sugarbeet} \ \mathbf{Identification} \ \mathbf{Assays}$

Event	EU Approval	P-35S	T-35S	son-d	SON-+	FMV	NPT II	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
GTSB77												
H7-1												PGB01A
SBVR111												
T120-7												

# $\mathbf{MODIfinder}^{\mathsf{TM}} \ \mathbf{GM} \ \mathbf{Rapeseed} \ (\mathbf{Canola}) \ \mathbf{Identification} \ \mathbf{Assays}$

Event	EU Approval	P-35S	T-35S	SON-d	SON-4	FMV	II LAN	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
23-18-17												
23-198												
73496												PGZ10A
Falcon GS 40/90												
GT200												
GT73												PGZ03A
HCN10												
HCR1												
Liberator												
MON88302												PGZ11A
MPS961												
MPS962												
MPS963												
MPS964												
MPS965												
Ms1												PGZ09A
Ms8												PGZ01A
OXY-235												PGZ08A
Rf1												PGZ06A
Rf2												PGZ07A
Rf3												PGZ02A
T45												PGZ04A
Topas 19/2												PGZ05A

# $\mathbf{MODIfinder}^{\mathsf{TM}} \ \mathbf{GM} \ \mathbf{Flax} \ \mathbf{Identification} \ \mathbf{Assays}$

Event	EU Approval	P-35S	1-355	SON-d	t-NOS	FMV	NPT II	EPSPS	PAT	BAR	Cry1Ab/Ac	MODIfinder Assay
FP967												PGF01A

Event	EU Approval	P-35S	1-358	SON-d	SON-+	FMV	II TAN	EPSPS	PAT	BAR	Cry1Ab/Ac
AM04-1020											
ATBT04-06											
ATBT04-27											
ATBT04-30											
ATBT04-31											
ATBT04-36											
AV43-6-G7											
Bt10 potato											
BT12 potato											
BT16 potato											
BT17 potato											
BT18 potato											
BT23 potato											
BT6 potato											
E12											
E24											
EH92-527-1											
F10											
F37											
G11											
H37											
H50											
HLMT15-15											
HLMT15-3											
HLMT15-46											
J3											
J55											
J78											
PH05-026-0048											
RBMT15-101											
RBMT21-129											
RBMT21-152											
RBMT21-350											
RBMT22-082											
RBMT22-186											
RBMT22-238											
RBMT22-262											
SEMT15-02											
SEMT15-15											
SPBT02-5											
SPBT02-7											
V11											
W8											
X17											
Y9											

MODIfinder Assay

PGP01A

MODIfinder™ Quantitative kits were developed to quantify the presence of a GMO in a sample according to EU indications.

For quantitation, the copy number of the GM PCR target relative to genome copies of the corresponding species is important because the principle of quantitation of GM content is to compare the relative ratio of these two. Usually, a PCR targeting a species-specific single copy gene is used to determine the amount of the species derived DNA, for example the soybean lectin gene for soya-derived DNA.

Using Real-Time PCR Quantitation can be done by comparison with a standard curve obtained by using DNA extracts from reference materials. In standard curve quantitation, the final quantitative estimate is based on comparing estimated quantity of GM to estimated quantity of reference TAXA.

All MODIfinder™ Quantitative kits allows for qualitative DNA detection with a detection limit down to 0.01% w/w depending on matrix and DNA preparation. Reaction volume 30 µl (sample 12 µl). Each kit contains: event specific primers and probe mix (50 test, 30 µl reaction end volume); taxon specific primers and probe mix (50 test, 30 µl reaction end volume); standard solutions at scalar target GM concentration; taxon DNA standard solutions at scalar concentration; negative control. Standard solutions are obtained extracting lot traceable reference materials using lon-Force DNA Extraction kit.

# MODIfinder™ Quantitative Assays

### A. Quantitative kits for CORN GM events

Description	50 Reactions kit
MODIfinder Corn Bt176 Quantitative	PGC01Q-50
MODIfinder Corn MON810 Quantitative	PGC02Q-50
MODIfinder Corn T25 Quantitative	PGC03Q-50
MODIfinder Corn BT11 Quantitative	PGC04Q-50
MODIfinder Corn NK603 Quantitative	PGC05Q-50
MODIfinder Corn GA21 Quantitative	PGC07Q-50
MODIfinder Corn TC1507 Quantitative	PGC08Q-50
MODIfinder Corn MON863 Quantitative	PGC09Q-50
MODIfinder Corn MIR604 Quantitative	PGC10Q-50
MODIfinder Corn DAS59122 Quantitative	PGC11Q-50
MODIfinder Corn 98140 Quantitative	PGC13Q-50
MODIfinder Corn MIR162 Quantitative	PGC14Q-50
MODIfinder Corn Event 3272 Quantitative	PGC17Q-50
MODIfinder Corn MON88017 Quantitative	PGC19Q-50
MODIfinder Corn MON89034 Quantitative	PGC20Q-50
MODIfinder Corn DAS40278 Quantitative	PGC22Q-50
MODIfinder Corn MON87460 Quantitative	PGC23Q-50
MODIfinder Corn Event 5307 Quantitative	PGC24Q-50
MODIfinder Corn MON87427 Quantitative	PGC25Q-50
MODIfinder Corn VCO-01981-5 Quantitative	PGC26Q-50
MODIfinder Corn MON87411 Quantitative	PGC27Q-50

#### B. Quantitative kits for SOY GM events

Description	50 Reactions kit
MODIfinder Soybean A2704-12 Quantitative	PGS01Q-50
MODIfinder Soybean GTS 40-3-2 (RoundUp Ready) Quantitative	PGS02Q-50
MODIfinder Soybean A5547-127 Quantitative	PG\$03Q-50
MODIfinder Soybean MON89788 Quantitative	PGS04Q-50
MODIfinder Soybean DP305423 Quantitative	PG\$05Q-50
MODIfinder Soybean DP356043 Quantitative	PG\$06Q-50
MODIfinder Soybean MON87701 Quantitative	PG\$07Q-50
MODIfinder Soybean CV127 Quantitative	PG\$08Q-50
MODIfinder Soybean MON87705 Quantitative	PGS09Q-50
MODIfinder Soybean MON87769 Quantitative	PG\$10Q-50
MODIfinder Soybean FG72 Quantitative	PG\$11Q-50
MODIfinder Soybean DAS44406 Quantitative	PG\$12Q-50
MODIfinder Soybean DAS68416 Quantitative	PG\$13Q-50
MODIfinder Soybean DAS81419 Quantitative	PG\$14Q-50
MODIfinder Soybean MON87708 Quantitative	PG\$15Q-50
MODIfinder Soybean MON87751 Quantitative	PG\$19Q-50
MODIfinder Soybean SYHT0H2 Quantitative	PGS20Q-50

#### MODIfinder™ Quantitative Assays

#### C. Quantitative kits for COTTON GM events

Description	50 Reactions kit
MODifinder Cotton MON531 Quantitative	PGT01Q-50
MODifinder Cotton MON1445 Quantitative	PGT02Q-50
MODifinder Cotton 281-24-236 Quantitative	PGT03Q-50
MODifinder Cotton 3006-210-23 Quantitative	PGT04Q-50
MODIfinder Cotton LL 25 Quantitative	PGT05Q-50
MODIfinder Cotton MON15985 Quantitative	PGT06Q-50
MODIfinder Cotton GHB 614 Quantitative	PGT07Q-50
MODIfinder Cotton MON88913 Quantitative	PGT08Q-50
MODIfinder Cotton GHB119 Quantitative	PGT09Q-50
MODIfinder Cotton T304-40 Quantitative	PGT10Q-50

#### D. Quantitative kits for RAPESEED (Canola) GM events

Description	50 Reactions kit
MODIfinder Canola MS8 Quantitative	PGZ01Q-50
MODIfinder Canola RF3 Quantitative	PGZ02Q-50
MODIfinder Canola RT73 Quantitative	PGZ03Q-50
MODIfinder Canola T45 Quantitative	PGZ04Q-50
MODIfinder Canola HCN92 Quantitative	PGZ05Q-50
MODIfinder Canola RF1 Quantitative	PGZ06Q-50
MODIfinder Canola RF2 Quantitative	PGZ07Q-50
MODIfinder Canola OXY-235 Quantitative	PGZ08Q-50
MODIfinder Canola M\$1 Quantitative	PGZ09Q-50
MODIfinder Canola DP073496 Quantitative	PGZ10Q-50
MODIfinder Canola MON88302 Quantitative	PGZ11Q-50
E. Quantitative kits for other taxa GM events	

Description	50 Reactions kit
MODIfinder Sugar beet RURH7-1 Quantitative	PGB01Q-50
MODIfinder Potato AMFLORA Quantitative	PGP01Q-50
MODIfinder Rice LL62 Quantitative	PGR02Q-50

#### F. Quantitative kits for screening markers

Description	50 Reactions kit
MODIfinder 35S Promoter Quantitative	PGE01Q-50
MODIfinder tNOS Quantitative	PGE04Q-50

Gene stacking creates a problem not only with respect to identification, but it also creates a potential problem for quantitation. As an example, if a gene-stacked GM maize is authorized, then 0.6% gene-stacked hybrid shall be treated as a 0.6% GM maize according to EU legislation but PCR tests would report 0.6% GM maize A + 0.6% GM maize B, that is 1.2% GM maize altogether. Euginius Database lists: 39 Stacked GMO approved or approved with restrictions; 135 Stacked GMO not approved for any application.

### Taxa with no event approved in EU

The Euginius Project Database reports also GM of taxa that have no actual or expired approval in EU thus there is no MODIfinder identification assay developed for them. Nevertheless it's important to trace them as they can lead to positivity at screening difficult to trace the cause.

Plant	Event	EU Approval	P-35S	1-358	son-d	SON-4	FMV	NPT II	EPSPS	PAT	BAR	Cry1Ab/Ac
Alfalfa	J101											
Alfalfa	J163											
Alfalfa	KK179											
Apple	GD743											
Apple	G\$784											
Apple	NF872											
Bean	Embrapa 5.1											
Bentgrass	ASR368											
Cicory	RM3-3											
Cicory	RM3-4											
Cicory	RM3-6											
Eggplant	EE-1											
Melon	Melon A											
Melon	Melon B											
Papaya	16-0-1											
Papaya	18-2-4											
Papaya	55-1											
Papaya	63-1											
Papaya	Huanong No. 1											
Papaya	X17-2											
Pineapple	EF2-114											
Plum	C5											
Squash	CZW-3											
Squash	ZW20											
Sugarcane	CTB141175/01-A											
Tomato	1345-4											
Tomato	35 1 N											
Tomato	5345											
Tomato	8338											
Tomato	B tomato											
Tomato	Da tomato											
Tomato	Ftomato											
Tomato	FLAVR SAVR											
Tomato	Nema282F											
Turnip	ZSR500											
Turnip	ZSR502											
Turnip	ZSR503											
Wheat	MON71700											
Wheat	MON71800											

**MODIfinder™ SemiQ Controls** are purified DNA extracted from traceable reference materials at a defined concentration.

When used as cut-off positive controls in GM detection experiments performed on matrices derived from single ingredients (e.g. soy grains, lecithin, corn, corn starch...) when unknown samples are normalized at the same DNA concentration, they allow for a convenient estimation of contamination level without the need of more expensive classical quantification experiments.

MODIfinder semiQ controls are available in 120 µl vials at a standard concentration of 3 ng DNA/µl and a 0.2% GM contamination, custom concentrations and cut-off are available on demand.

Measure the Normalize Compare Cq Extract **DNA** concentration concentration Run PCR of SemiQ Control the unknown of the DNA at that of experiments and sample extracted SemiQ Control unknown sample

## **MODIfinder™ Semi-Q Controls**

#### A. Screening markers Semi-Q Controls

Cat.#	Marker	Cat.#	Marker	Cat.#	Marker
PGE01R	35S Promoter (p35S)	PGE07R	NptII	PGE17R	FMV promoter (pFMV)
	(1 /			PGE25R	Cry1Ab/Ac
PGE03R	35S Terminator (†35S)	PGE14R	PAT	PGE27R	NOS promoter (pNOS)
PGE04R	NOS terminator (tNOS)	PGE15R	BAR	PGE36R	MultiSCREEN New Soy
PGE05R	p35S + tNOS Duplex	PGE16R	EPSPS	r GE36K	MOIIISCREEN NEW 309

#### B. Events Specific Semi-Q Controls

Cat.#	Marker	Cat.#	Marker
PGC01R	MODIfinder Corn Bt176 SemiQ Control	PGS11R	MODIfinder Soybean FG72 SemiQ Control
PGC02R	MODIfinder Corn MON 810 SemiQ Control	PGS12R	MODIfinder Soybean DAS44406 SemiQ Control
PGC03R	MODIfinder Corn T25 SemiQ Control	PGS13R	MODIfinder Soybean DAS68416 SemiQ Control
PGC04R	MODIfinder Corn BT11 SemiQ Control	PGS14R	MODIfinder Soybean DAS81419 SemiQ Control
PGC05R	MODIfinder Corn NK603 SemiQ Control	PGS15R	MODIfinder Soybean DAS87708 SemiQ Control
PGC07R	MODIfinder Corn GA21 SemiQ Control	PGS19R	MODIfinder Soybean MON87751 SemiQ Control
PGC08R	MODIfinder Corn TC1507 SemiQ Control	PGS20R	MODIfinder Soybean SYHT0H2 SemiQ Control
PGC09R	MODIfinder Corn MON 863 SemiQ Control	PGT01R	MODifinder Cotton MON 531 SemiQ Control
PGC10R	MODIfinder Corn MIR 604 SemiQ Control	PGT02R	MODifinder Cotton MON 1445 SemiQ Control
PGC11R	MODIfinder Corn DAS 59122 SemiQ Control	PGT03R	MODifinder Cotton 281-24-236 SemiQ Control
PGC13R	MODIfinder Corn 98140 SemiQ Control	PGT04R	MODifinder Cotton 3006-210-23 SemiQ Control
PGC14R	MODIfinder Corn MIR 162 SemiQ Control	PGT05R	MODIfinder Cotton LL 25 SemiQ Control
PGC17R	MODIfinder Corn Event 3272 SemiQ Control	PGT06R	MODIfinder Cotton MON 15985 SemiQ Control
PGC19R	MODIfinder Corn MON88017 SemiQ Control	PGT07R	MODIfinder Cotton GHB 614 SemiQ Control
PGC20R	MODIfinder Corn MON89034 SemiQ Control	PGT08R	MODIfinder Cotton MON 88913 SemiQ Control
PGC22R	MODIfinder Corn DAS 40278 SemiQ Control	PGT09R	MODIfinder Cotton GHB119 SemiQ Control
PGC23R	MODIfinder Corn MON87460 SemiQ Control	PGT10R	MODIfinder Cotton T304-40 SemiQ Control
PGC24R	MODIfinder Corn Event 5307 SemiQ Control	PGZ01R	MODIfinder Canola MS8 SemiQ Control
PGC25R	MODIfinder Corn MON87427 SemiQ Control	PGZ02R	MODIfinder Canola RF3 SemiQ Control
PGC26R	MODIfinder Corn VCO-01981-5 SemiQ Control	PGZ03R	MODIfinder Canola RT73 SemiQ Control
PGC27R	MODIfinder Corn MON87411 SemiQ Control	PGZ04R	MODIfinder Canola T45 SemiQ Control
PGS01R	MODIfinder Soybean A2704-12 SemiQ Control	PGZ06R	MODIfinder Canola RF1 SemiQ Control
PGS02R	MODIfinder Soybean GTS 40-3-2 SemiQ Control	PGZ07R	MODIfinder Canola RF2 SemiQ Control
PGS03R	MODIfinder Soybean A5547-127 SemiQ Control	PGZ08R	MODIfinder Canola OXY-235 SemiQ Control
PGS04R	MODIfinder Soybean MON89788 SemiQ Control	PGZ09R	MODIfinder Canola MS1 SemiQ Control
PGS05R	MODIfinder Soybean DP305423 SemiQ Control	PGZ10R	MODIfinder Canola DP073496 SemiQ Control
PGS06R	MODIfinder Soybean DP356043 SemiQ Control	PGZ11R	MODIfinder Canola MON88302 SemiQ Control
PGS07R	MODIfinder Soybean MON87701 SemiQ Control	PGP01R	MODIfinder Potato AMFLORA SemiQ Control
PGS08R	MODIfinder Soybean CV127 SemiQ Control	PGR02R	MODIfinder Rice LL62 SemiQ Control
PGS09R	MODIfinder Soybean MON87705 SemiQ Control	PGB01R	MODIfinder Sugar beet RURH7-1 SemiQ Control
PGS10R	MODIfinder Soybean MON87769 SemiQ Control		

The basis of digital PCR (dPCR) is to quantify the absolute number of targets present in a sample, using limiting dilutions, PCR and Poisson statistics.

At Generon we developed a **MODIfinder DigIT** a portfolio of kits based on Bio-Rad ddPCR systems for quantitative analysis of GMO in food and feed samples. Digital PCR is an alternative technique for quantifying gene copy number which may provide more accurate measurements than other approaches currently available as it is not dependent on amplification efficiency. Of particular importance, ddPCR measurements are made independent of any calibrator and, therefore, this technique has the potential to be considered as a primary method for use in certification of nucleic acid reference materials.

The measurement principle has a high metrological quality and can overcome several of Real-Time GMO analysis difficulties by transforming exponential data from conventional PCR to digital signals that simply indicate whether or not amplification has occurred after a defined number of cycles.

Thanks to the collaboration with major food testing entities and ddPCR technology implemented at the R&D department, Generon is able to provide **MODIfinder ddPCR quantified controls** copy number counted positive controls for any target including some GMO that have no standard material available.

## **MODIfinder™ DigIT Kits**

Cat.#	Description	Cat.#	Description
DGE01K	MODIfinder DigIT 35S kit	DGE24K	MODIfinder DigIT HMG kit
DGE04K	MODIfinder DigIT tNOS kit	DGE34K	MODIfinder DigIT pFMV/DP356043 +
DGE05K	MODIfinder DigIT p35S/tNOS duplex kit		DP305423 kit
DGE07K	MODIfinder DigIT NPTII kit	DGC02K	MODIfinder DigIT Corn MON 810 kit
DGE09K	MODIfinder DigIT Lectin kit	DGC20K	MODIfinder DigIT Corn MON89034 Assay
DGE14K	MODIfinder DigIT PAT kit	DG\$01K	MODIfinder DigIT Soybean A2704 kit
DGE15K	MODIfinder DigIT BAR kit	DG\$02K	MODIfinder DigIT Soybean GTS 40-3-2
DGE17K	MODIfinder DigIT pFMV kit		(RoundUp Ready) kit
DGE19K	MODIfinder DigIT Canola ENDO kit	DG\$03K	MODIfinder DigIT Soybean A5547 kit
DGE21K	MODIfinder DigIT Cotton ENDO kit	DGS06K	MODIfinder DigIT Soybean DP356043 kit

Each kit includes all the reagents necessary to perform 96 reactions buffer ddPCR, target specific oligos (primers and probe) at optimized concentration. Positive and negative controls. Droplet generator oil and disposable plastics are not included.

# MODIfinder™ ddPCR Quantified Amplicons/Plasmids/RM DNA Extracts

Cat.#	Description
PGC02D	MODIfinder Corn MON 810 ddPCR Quantified Reference Material
PGC05D	MODIfinder Corn NK603 ddPCR Quantified Reference Material
PGC06D	MODIfinder Corn CBH-351 ddPCR Quantified Amplicon
PGC12D	MODIfinder Corn DBT 418 ddPCR Quantified Amplicon
PGC20D	MODIfinder Corn MON89034 ddPCR Quantified Reference Material DNA extract
PGF01D	MODIfinder Flax FP967 ddPCR Quantified Amplicon
PGR01D	MODIfinder Rice LL601 ddPCR Quantified Amplicon
PGR03D	MODIfinder Rice BT63 ddPCR Quantified Amplicon
PGS01D	MODIfinder Soybean A2704-12 ddPCR Quantified Reference Material
PGS02D	MODIfinder Soybean GTS 40-3-2 (RoundUp Ready) ddPCR Quantified Reference Material
PGS03D	MODIfinder Soybean A5547-127 ddPCR Quantified Reference Material
PGS04D	MODIfinder Soybean MON89788 ddPCR Quantified Reference Material
PGS06D	MODIfinder Soybean DP356043 ddPCR Quantified Reference Material
PGS07D	MODIfinder Soybean MON87701 ddPCR Quantified Reference Material
PGZ05D	MODIfinder Canola HCN92 ddPCR Quantified Amplicon



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