See What You Eat !

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New detection platform for GMO screening

Eppendorf product overview

Instruments

Centrifuges

Pipettes

Thermomixers

Mastercycler

Workstations

Cell fusion

Cell manipulation

Consumables

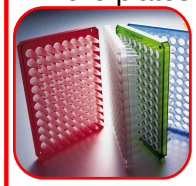
Micro test tubes

Pipette tips

Dispenser Combitips

PCR tubes

Micro plates



Cuvettes

Capillaries

BioChips/Arrays

Scanner

Hybridization system

Arrays

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DualChip® GMO

	Transformation event	Maize	Soybean	Rapeseed	Cotton
1	MON 15985				✓
2	MON 531				✓
3	MS8, RF3, MS8 X RF3			✓	
4	Bt176 maize	✓			
5	GA 21	✓			
6	MON 863	✓			
7	MON 1445				✓
8	MON 531 X MON 1445				✓
9	MON15985 X MON1445				✓
10	RRS // GTS 40/3/2 // MON 40-3-2		✓		
11	GT 73			✓	
12	MON603 // NK603	✓			
13	MON863 X MON603	✓			
14	MS1, RF1, MS1 X RF1			✓	
15	MS1, RF2, MS1 X RF2			✓	
16	GA21 X MON 810	✓			
17	MON 810	✓			
18	MON863 X MON810	✓			
19	NK 603 X MON 810	✓			
20	T45			✓	
21	Bt11	✓			
22	T25	✓			
23	TC 1507 // DAS1507	✓			
24	Topas 19/2 // HCN92			✓	

DualChip® GMO

- **Detection of all EU approved events**
- **One analysis with 24 results**
- **effective screening method**
- **System solution**

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New Detection of GMOs

One System – One Solution

DualChip – Silverquant-Scanner

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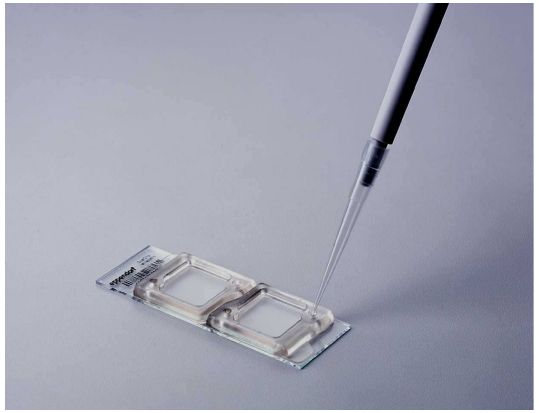
Eppendorf DualChip® Microarray Systems

- **Easy Handling:**
  - Safe experimental workflow due to the reliable EP frames
  - Barcode label on each chip for identification
- **High Reproducibility and Sensitivity:**
  - Chamber ensures consistent hybridization volume
  - Unique probe design for higher signal-to-noise ratios
- **Minimized false positive/ negatives:**
  - DualChip: probes tested in hybridizations to ensure real data
  - Integrated control system
- **Do-it-yourself software platform:**
  - No extensive need of bioinformatics
  - Software generates final result



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Dualchip®GMO Principle

Genetic elements detected with the current DualChip GMO design:

screening elements

- CaMV 35S Promoter (P35S)
- Nopaline Synthase Terminator (Tnos)
- Phosphinothricin N-acetyltransferase (Pat)
- Cry1Ab delta-endotoxin (Cry1Ab)
- 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)
- The junction between the Nopaline synthase promoter and the neomycin p
- hosphotransferase II gene (Pnos-nptII)

Specific reference elements

- Invertase (maize)
- Cruciferin (rapsseed)
- Lectin (soybean)
- rBCL (plant universal)

Control element

- Cauliflower Mosaic Virus (CaMV)

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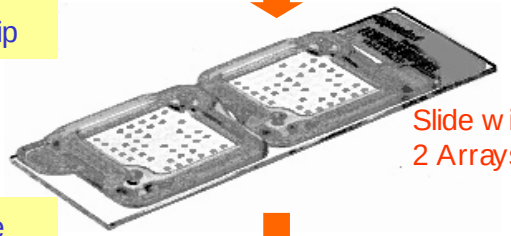
DualChip®GMO Procedure

DNA extraction from GMO sample

Genomic DNA

Amplification through multiplex PCR with biotinylated primers

Hybridization of the PCR products on 1 GMO Screening Chip



Slide with 2 Arrays

Detection of positive hybridization with Sliverquant detection

Data analysis and results

3 h

1h

1h 30 m

10 min

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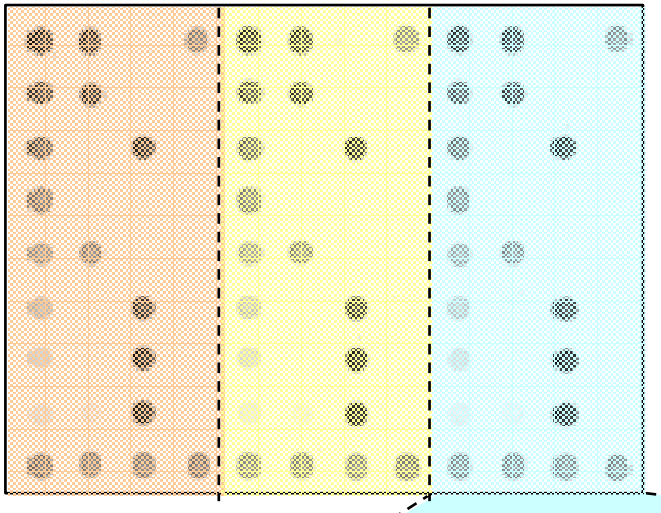
DualChip® GMO Design



Each spot is present in triplicate providing reliable results

- **sensitivity: 0.1%**
- **accuracy rate: 95%**

det ctl 1	positive hyb ctl	negative det ctl	det ctl 4
det ctl 2	P 35 S	T nos	negative det ctl
det ctl 3	negative det ctl	PCR ctl	Pnos-nptII
det ctl 4	CaMV	EPSPS-1	EPSPS-2
det ctl 5	Cry1Ab-1	Cry1Ab-2	Cry1Ab-3
det ctl 6	Pat	Maize	Rapeseed
det ctl 7	negative det ctl	Plant	Soybean
det ctl 8	det ctl 9	positive hyb ctl	negative hyb ctl
det ctl 4	det ctl 4	det ctl 4	det ctl 4

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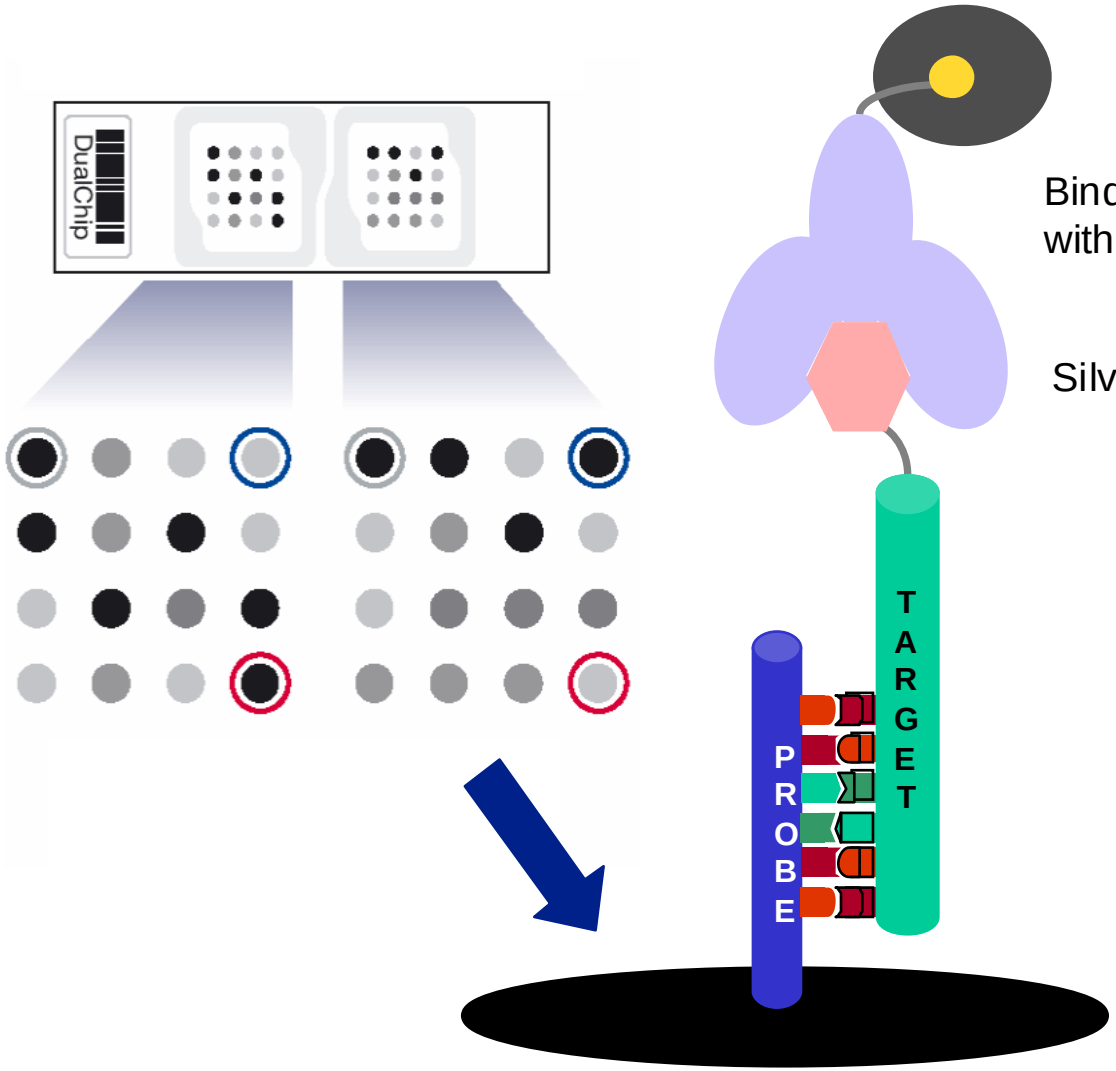
Silverquant® - Principle of Staining



Binding to hybridized biotinylated DNA with **Anti-Biotin Gold-conjugate**

Silver staining reaction

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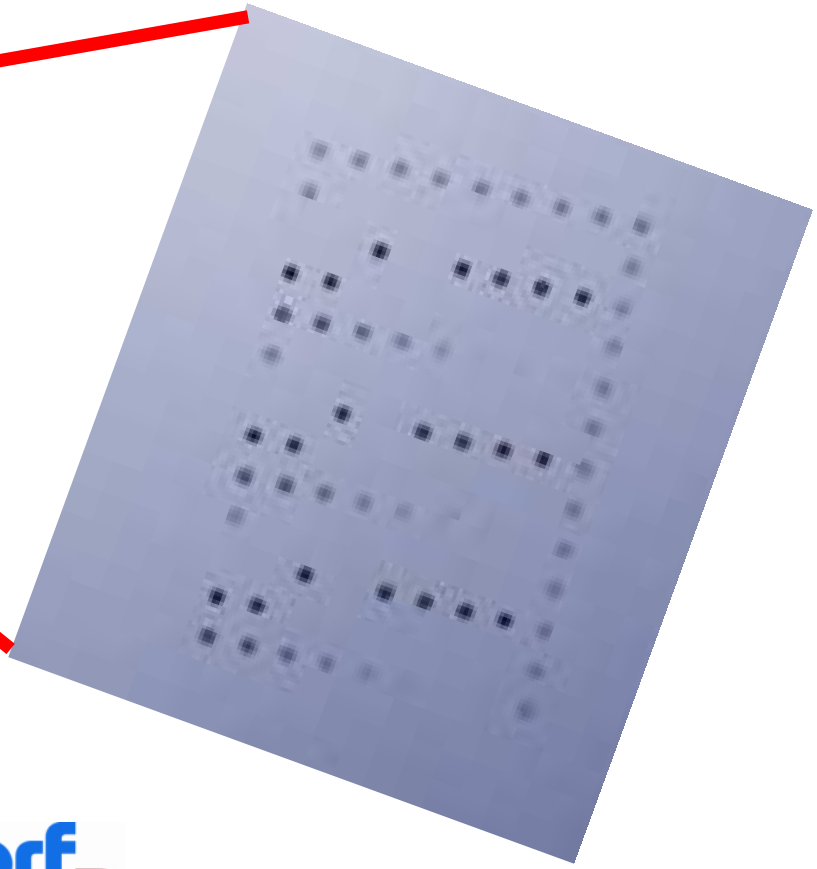
I. Alexandre et al.
Analytical Biochemistry 2001; 295, 1-8

Silverquant® - Staining



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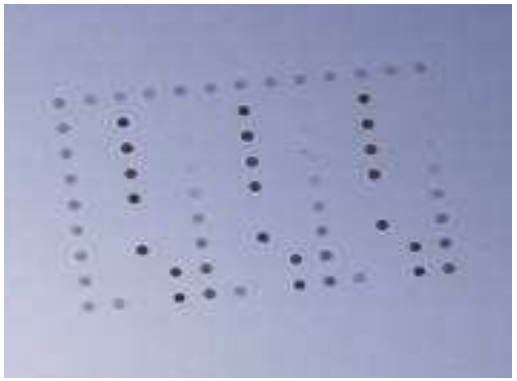
Silverquant® - Detection

- High sensitivity
- Very low background
- Robust and reproducible protocol
- Standardized workflow
- compatible with a wide range of substrates (e.g. glass, plastics)
- Open for other applications



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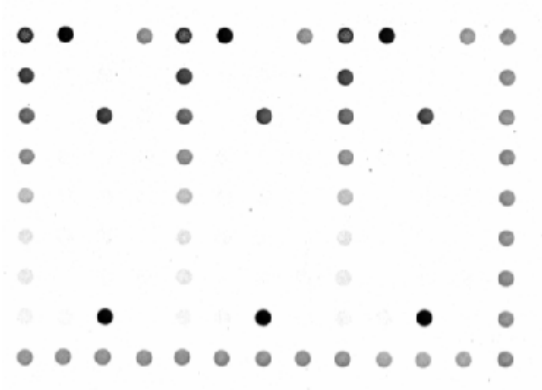
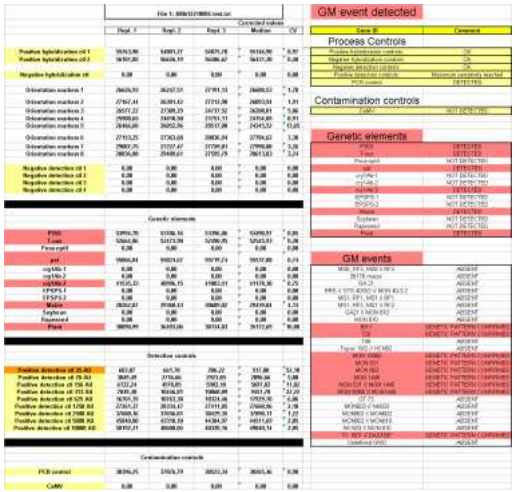
Silverquant® Software and Data Analysis

Array-Images



Array-Quantification



Data-Mining

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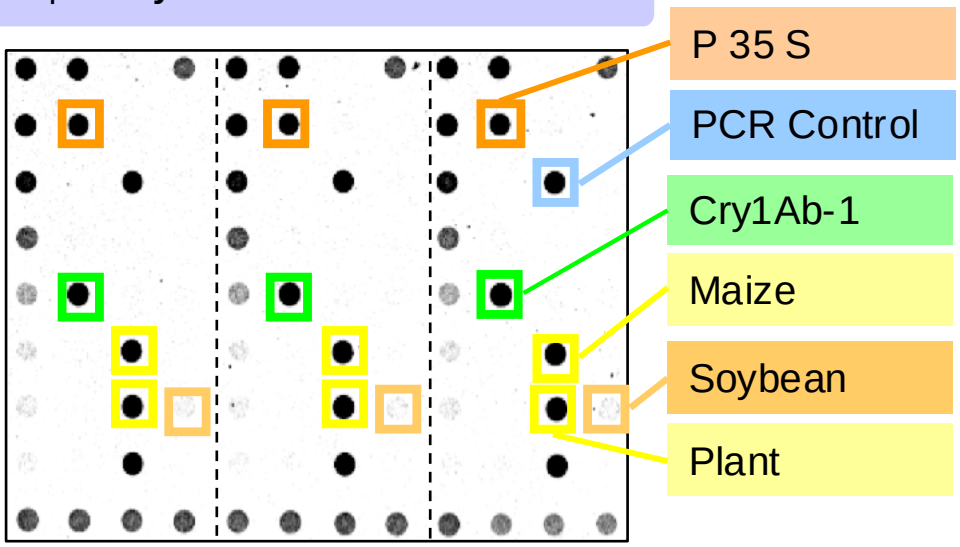
DualChip®GMO – An Example

Sample: **soy contamination in Bt 176**



			Corrected values		
	Repl. 1	Repl. 2	Repl. 3	Median	CV
	Genetic elements				
P35S	62548,09	62359,24	62657,45	62521,59	0,24
T-nos	0,00	0,00	39,27	13,09	173,21
Pnos-nptII	0,00	0,00	0,00	0,00	0,00
pat	0,00	0,00	0,00	0,00	0,00
cry1Ab-1	61053,99	61233,61	61599,54	61295,71	0,45
cry1Ab-2	0,00	0,00	0,00	0,00	0,00
cry1Ab-3	0,00	0,00	0,00	0,00	0,00
EPSPS-1	0,00	0,00	0,00	0,00	0,00
EPSPS-2	0,00	0,00	0,00	0,00	0,00
Maize	50826,14	50230,00	51217,90	50758,01	0,98
Soybean	1164,20	1017,90	1018,33	1074,49	6,53
Rapeseed	0,00	0,00	0,00	0,00	0,00
Plant	62732,34	62854,25	63614,31	63066,97	0,76

GM event detected	
Gene ID	**Comment**
Process Controls	
Positive hybridization controls:	OK
Negative hybridization controls:	OK
Negative detection controls:	OK
Positive detection controls:	Maximum sensitivity reached
PCR control	DETECTED
Contamination controls	
CaMV	NOT DETECTED
Genetic elements	
P35S	DETECTED
T-nos	NOT DETECTED
Pnos-nptII	NOT DETECTED
pat	NOT DETECTED
cry1Ab-1	DETECTED
cry1Ab-2	NOT DETECTED
cry1Ab-3	NOT DETECTED
EPSPS-1	NOT DETECTED
EPSPS-2	NOT DETECTED
Maize	DETECTED
Soybean	NOT DETECTED
Rapeseed	NOT DETECTED
Plant	DETECTED
GM events	
MS8, RF3, MS8 X RF3	ABSENT
Bt176 maize	GENETIC PATTERN CONFIRMED
GA 21	ABSENT
RRS // GTS 40/3/2 // MON 40-3-2	ABSENT
MS1, RF1, MS1 X RF1	ABSENT
MS1, RF2, MS1 X RF2	ABSENT
GA21 X MON 810	ABSENT
MON 810	ABSENT
Bt11	ABSENT
T25	ABSENT
T45	ABSENT
Topas 19/2 // HCN92	ABSENT
MON 15985	ABSENT
MON 531	ABSENT
MON 863	ABSENT
MON 1445	ABSENT
MON 531 X MON 1445	ABSENT
MON15985 X MON1445	ABSENT
GT 73	ABSENT
MON603 // NK603	ABSENT
MON863 X MON603	ABSENT
MON863 X MON810	ABSENT
NK 603 X MON 810	ABSENT
TC 1507 // DAS1507	ABSENT
Undefined GMO	ABSENT

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DualChip®GMO Advantages

- **14 real results** per array
- Clear indication of the **GM event**
- Integrated **Control reaction**
- Easy handling of **unknown compositions**
- **No time-consuming** individual GMO screening necessary
- More **transparency** despite of GMO complexity
- **Upgradable** technology

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Technology Validation

EUROPEAN COMMISSION
DIRECTORATE GENERAL JOINT RESEARCH CENTRE (JRC)
INSTITUTE FOR HEALTH AND CONSUMER PROTECTION
BIOTECHNOLOGY & GMOs Unit
COMMUNITY REFERENCE LABORATORY FOR GENETICALLY MODIFIED FOOD AND FEED

Community Reference Laboratory
CRL
GM Food and Feed

Description of the CRL-GMFF Validation Process

1. Introduction

The Community Reference Laboratory for GM Food and Feed (CRL-GMFF) was established by Regulation (EC) No 1829/2003 on genetically modified food and feed. The objectives and tasks of the CRL-GMFF are outlined in the Annex of the same Regulation. The operations of the CRL-GMFF are carried out in line with Regulation (EC) No 641/2004 on detailed rules for the implementation of Regulation (EC) No 1829/2003 (implementing measures).

The CRL conducts the scientific assessment of the documentation provided by the applicant for authorisation, for its completeness and compliance to the European legislation. If the information provided about the methods and samples fulfils the method acceptance criteria set by the European Network for GMO Laboratories (ENGL – "Definition of minimum performance requirements for analytical methods of GMO testing"), the CRL initiates the validation process of the detection method, making use of the control samples and the samples of food and feed provided by the applicant.

The validation process is carried out in collaboration with laboratories members of the European Network of GMO Laboratories (ENGL) and must be completed within a period of six months after the European Food and Safety Authority (EFSA) has declared an application as valid.

Description of the CRL-GMFF Validation Process – December 2006

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Technology Validation

2. Overview of steps in validation process

The validation process consists of the following steps (Figure 1):

- Step 1. Reception of documentation and material provided by the applicant;
- Step 2. Scientific assessment of documentation and data;
- Step 3. Experimental testing of the samples and methods;
- Step 4. Collaborative trial;
- Step 5. Reporting to the European Food Safety Authority (EFSA).

Step 4: Collaborative trial

The inter-laboratory study, called collaborative trial, for method validation is organised by the CRL-GMFF according to the requirements defined in the 'IUPAC Protocol for the Design, Conduct and Interpretation of Method-performance Studies' (Horwitz, W. 1995. Pure and Appl. Chem, 67, 331-343), and in the international standard (ISO) 5725 on "Accuracy -Trueness and Precision - of Measurements Methods and Results" (ISO, 1994). The experimental work is carried out by e.g. twelve or more European laboratories, members of the ENGL.

Technology Validation

Step 5: Reporting to the European Food Safety Authority (EFSA)

Upon proper statistical treatment, performed by the CRL-GFMFF, of data collected during the collaborative trial, the results are reported to the European Food Safety Authority (EFSA) and published, together with the validated protocols, on the CRL-GMFF web site.

In particular, the CRL-GMFF prepares the following type of documents:

1) Validation report, reporting the results of the validation study;

2) Validated protocols, containing the detailed description of the validated method(s).

In addition, the CRL-GMFF compiles a technical report which includes all information recorded during the experimental testing of samples and methods.

The CRL-GMFF carries out the entirety of its operations according to ISO 9001:2000 (certificate number: CH-32232) and ISO 17025:2005 (certificate number: DAC-PL-0459-06-00)

DualChip®GMO Validation – General Specifications

- Validated as qualitative screening technology
- Elements validated individually
- Set of 12 laboratories

  min. of 8 laboratories required for data analysis
- method validation - sample DNA extraction not included
- 95% accuracy rate fixed as acceptance criteria (like qPCR)
- test samples provided as blind samples

DualChip®GMO Validation – Experimental Workload

- 168 days of lab work
  (14 days per laboratory)
- 3360 PCR reactions
  (280 PCR reactions per laboratory)
- 840 micorarrays to be used
  (70 micorarrays per laboratory)

DualChip®GMO Validation - Preliminary Results

- Results analyzed from 8 laboratories
- Good inter-lab reproducibility
- LOD of 0.1% GMO with an accuracy rate of 95% confirmed
- accuracy rate of 95% for 0.045% GMO >80% (>95% for P35S and Tnos)
- Non plant DNA samples: no detection of false positives

DualChip®GMO

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