The Chinese rice case

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The Chinese rice case

- Overview on rice products contaminated by unauthorized GM rice
- Food and Veterinary Office inspections in China
- European Commission decisions
- EURL technical guidance document

Unauthorized GM rice in rice products: a long lasting story

No GM rice are authorized in the EU

112 notifications in the RASFF since 2006 regarding
5 identified GM rice and Pubi-cry target

The Rapid Alert System for Food and Feed (RASFF) was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. This exchange of information helps Member States to act more rapidly and in a coordinated manner in response to a health threat caused by food or feed.

Unauthorized GM rice in rice products: a long lasting story

In September 2006, rice products from China contaminated with an unauthorized GM rice: **Bt63**

Total of 49 notifications in the RASFF (alert, information and border rejection) coming from 12 european countries (last one Dec. 2011)

RASFF Portal

Notifications list : 49 results

Search criteria | **Subject** *BT63*

<< First << << Previous 100 << Notifications **1 to 49** of 49 >> Next 100 >> >> Last >>

	Classification	Date of case	Last change	Reference	Country	Subject	Product Category	Type
1.	alert	07/09/2006	04/04/2008	2006.0575	FR	unauthorised genetically modified (BT63) rice used to manufacture rice sticks from China	cereals and bakery products	food
2.	information	30/03/2007		2007.AVH	GR	unauthorised genetically modified (rice BT63) rice protein concentrate from China (Hong Kong)	feed for food-producing animals - (obsolete)	Feed
3.	information	18/04/2007		2007.AYT	GR	unauthorised genetically modified (rice BT63) rice protein concentrate from China	feed for food-producing animals - (obsolete)	Feed
4.	alert	20/07/2007	07/09/2007	2007.0489	IT	unauthorised genetically modified (RICE BT63) rice noodles from China	cereals and bakery products	food
5.	alert	22/11/2007	19/12/2007	2007.0856	DE	unauthorised genetically modified (presence of BT63 strand) rice noodles vermicelli from China, via the Netherlands	cereals and bakery products	food
6.	information	19/03/2008	18/06/2010	2008.0320	FR	unauthorised genetically modified (rice BT63) rice vermicelli from China	cereals and bakery products	food
7.	border rejection	22/05/2008	06/06/2008	2008.AUB	ES	unauthorised genetically modified (rice BT63) rice protein concentrate from China	feed for food-producing animals - (obsolete)	feed
8.	information	18/06/2008	28/08/2008	2008.0724	SI	unauthorised genetically modified (rice BT63) rice powder from China	cereals and bakery products	food
9.	information	26/06/2008	16/03/2009	2008.0763	SE	unauthorised genetically modified (Bt63 rice) rice noodles from China	cereals and bakery products	food
10.	border rejection	26/06/2008	20/10/2008	2008.AZS	GB	unauthorised genetically modified (rice BT63) rice vermicelli from China, via China (Hong Kong)	cereals and bakery products	food
11.	border rejection	26/06/2008	10/07/2008	2008.AZT	GB	unauthorised genetically modified (rice BT63) rice noodles from China (Hong Kong)	cereals and bakery products	food
12.	information	11/07/2008	18/07/2008	2008.0829	GB	unauthorised genetically modified (BT63 rice) rice-flour noodles from China	cereals and bakery products	food
13.	border rejection	04/08/2008	10/03/2010	2008.BFJ	GB	unauthorised genetically modified (rice BT63) rice noodles from China, via China (Hong Kong)	cereals and bakery products	food
14.	border rejection	27/08/2008	02/02/2011	2008.BJA	NL	unauthorised genetically modified (BT63: ct39.9) rice noodles from China	cereals and bakery products	food
15.	border rejection	05/01/2009	05/01/2009	2009.AAU	DE	unauthorised genetically modified (BT63 Rice) instant rice noodles from China	cereals and bakery products	food
16.	alert	16/06/2009	13/07/2011	2009.0781	SE	unauthorised genetically modified (Bt63 rice) fine rice vermicelli from China (Hong Kong), via the Netherlands	cereals and bakery products	food
17.	border rejection	31/03/2010	31/03/2010	2010.APJ	GB	unauthorised genetically modified (Bt63) rice vermicelli from China	cereals and bakery products	food
18.	border rejection	31/03/2010	31/03/2010	2010.API	GB	unauthorised genetically modified (Bt63) rice macaroni from China	cereals and bakery products	food

Unauthorized GM rice in rice products: a long lasting story

In March 2010, rice products from China contaminated with others unauthorized GM rice:
presumably Kefeng 6 and **KMD1**

From this date, a total of 37 + 12 notifications in the RASFF (alert, information and border rejection).

Unauthorized GM rice in rice products: a long lasting story

Globalization also applies for GM contamination...

The USA

LL601 and **LL62** GM rices (10 alerts, first in 2006)

India and Pakistan

GM contamination in basmati rice (presence of **Pubi-cry**)

4 alerts (2011 and 2012)

Considering the alerts…and the lack of guarantee…

- Two audits were conducted in China by the Food and Veterinary Office (DGSANCO)

- Two Decisions were taken by the EC

Audits carried out by the EC in China

- November 2008 (DGSANCO 2008-7834)

- April 2011 (DGSANCO 2011-6208):

  3 technical recommandations out of 6

  - **Review the control strategy** for detecting GMO on the market, in particular to address the risk that **GM rice events other than Bt63** may be present in rice products intended for export to the EU by ensuring that representative samples are taken from seeds, feed and food on the market.
  - Review the analytical procedures to **increase the sensitivity of the test methods** used for detecting GMO in seed, feed and food on the market.
  - Consider requiring the provision of test methods and a **single source positive control material** for GM rice events entering field trials, in order to ensure that the surveillance and testing regime for GMO on the market and the pre-export controls can be adapted at an early stage addressing the potential risks.

Decision 2008-289-EC

on emergency measures regarding the unauthorised GMO Bt 63 in rice products

- Conditions for first placing on the market: analytical report based on a construct-specific method (D. Mäde et al.)
- Control measures: random sampling and analysis
- Recovery of costs: bear by the operators responsible for the first placing on the market (in case of a non-compliance)

Decision 2011-884-EU

on emergency measures regarding unauthorised GM rice in rice products originating from China

- Analytical report + health certificate for each lot
  - If absent: re-dispatching to the country or destroyed
  - If present: CA shall analyse a sample with a **frequency of 100 %**
- Sampling and analytical procedure are specified and detailed in an EURL-GMFF technical guidance document
- Recovery of costs: bear by the operators responsible for the first placing on the market

EURL-GMFF Technical Guidance

Technical guidance document linked to the Decision 2011-284-EU

- Sample's preparation
- Screening strategy
- Interpretation of the results

EURL-GMFF Technical Guidance

Preparation of the laboratory sample

4 sub samples of 240 g each

For grains: 2.5 kg

Grinding

4 sub samples of 125 g each

For processed food or feed: 0.5 kg

EURL-GMFF Technical Guidance

Preparation of the laboratory sample

- Two DNA extractions per sub-sample
- Total of 8 DNA extractions per sample
- 4 times more work than required (ISO standards)

PCR: on which targets ?

At this point the story gets complicated...

EURL-GMFF Technical Guidance

PCR strategy

For previous contamination with « known » GM rice such as Bt63, KeFeng6... we can manage...

...provided we have PCR methods and positive control (thanks to EURL and ENGL members)

But for unknown GM rice?

Testing of Real-time PCR methods on GM rice

- Few information on the GMO developed in China
- Limited number of control samples
- Focus on screening for GM presence (not identification)

Universe of "GM rice originating from China"

Key document (forwarded by DG SANCO)

Annu. Rev. Entomol. 2011. 56:81-101 doi: 10.1146/annurev-ento-120709-144810
Insect-Resistant Genetically Modified Rice in China: From Research to Commercialization
Chen, Shelton, and Ye
(and Supplemental Material)

Supporting documents

Eur Food Res Technol (2011) Vol 232:351–359
Integrated structure and event-specific real-time detection of transgenic cry1Ac/SCK rice Kefeng 6
Changqing Su ., et al.

Anal Bioanal Chem (2011) Vol 400:1433–1442
Development and validation of real-time PCR screening methods for detection of cry1A.105 and cry2Ab2 genes in genetically modified organisms
Dinon A.Z.., et al.

Eur Food Res Technol (2011) Vol 232:351–359
Integrated structure and event-specific real-time detection of transgenic cry1Ac/SCK rice Kefeng 6
Su C., et al.

J Sci Food Agric (2011) DOI 10.1002/jsfa.4421
Effects of genetically modified T2A-1 riceon faecal microflora of rats during 90 day supplementation
Yuan Y., et al.

Eur Food Res Technol (2011) 232:297–305
Event-specific qualitative and quantitative detection of transgenic rice Kefeng-6 by characterization of the transgene flanking sequence
Wang W-X., et al.

A Matrix representation of the insect/herbicide resistance GM rice Universe (date: July 2011)

Marker	*Cry1Ac*	*Cry1Ab/Ac*	*Cry1Ab*	*cry1C*	*Cry2a*	*hpt*	*nptII*	*GUS*	*bar*	*CpTi*	P-35S	T-35S	T-nos	P-actin	P-ubi
GMO event															
Kefeng-6	+	+	+			+				+	+	+	+	+	+
IIyou Kefeng-6 (hybrid line)	+					+				+	+	+	+		
Huahui 1		+											+	+	
Bt63	+	+											+	+	
KMD1 (Kemingdao)			+			+	+	+			+				+
LLRice 601									+		+				
LLRice 62									+		+	+			
Event T103-10 (Xa21-IR72)											+				
Event 11586 (Golden Rice)													+		
GM II-Youming 86									+		+		+		
Bt aizawai 7-29											+				
Bt Xiushui 11			+												+
Minghui 63a				+											+
GM Minghui 63b					+										+
IR72 ; Minghui 63c		+												+	
GM Minghui 86a										+				+	
Eyi 105; Ewan 5											+				
Xiushui 11; Chunjiang 11											+				
Jijing81; Jijing 88; Tong 887											+				
Minghui86b	+									+				+	
Minghui63d, Zhenshan97A, MaxieA		+													
Zhuxian B														+	
Eyi105, Ewan5															+
Zhongua91(a)			+						+					+	
Zhongua91(b)			+						+					+	
Xiushui110			+												+

Abbreviations: "x": corresponding target is present in the GMO;
Marker specifications: "CryIAb", "CryIAc", "CryIAb/Ac", 'CryIC", "Cry2a": coding regions of *Bacillus thuringiensis* insect resistance genes; hpt": hygromycin phosphotransferase of *Escherichia coli*.;
"nptII": coding region of the neomycine-phosphotransferase gene; "GUS": β-glucuronidase gene of *Escherichia coli*; "bar": coding region of the phosphinotricine acetyl-transferase herbicide resistance gene ; "CpTi": Cowpea Trypsin Inhibitor gene from *Vigna Unguiculata*; "P-35S" and T-35S: promotor (P) and terminator (T) of the 35S gene from Cauliflower Mosaic Virus; "T-nos": terminator (T) of the nopaline synthase gene of *Agrobacterium tumefasciens*; "P-actin": promoter (P) of the rice actin gene; 'P-ubi": promoter (P) of the rice ubiquitin gene

GM rice Screening approach

AIM : Broad-range screening approach

The most relevant targets are:

Coverage: P-35S & T-Nos

- 35S promotor from Cauliflower mosaic virus
- T-NOS: Nopaline Synthase terminator from *Agrobacterium tumefaciens*

Discrimination: CryIAb/Ac

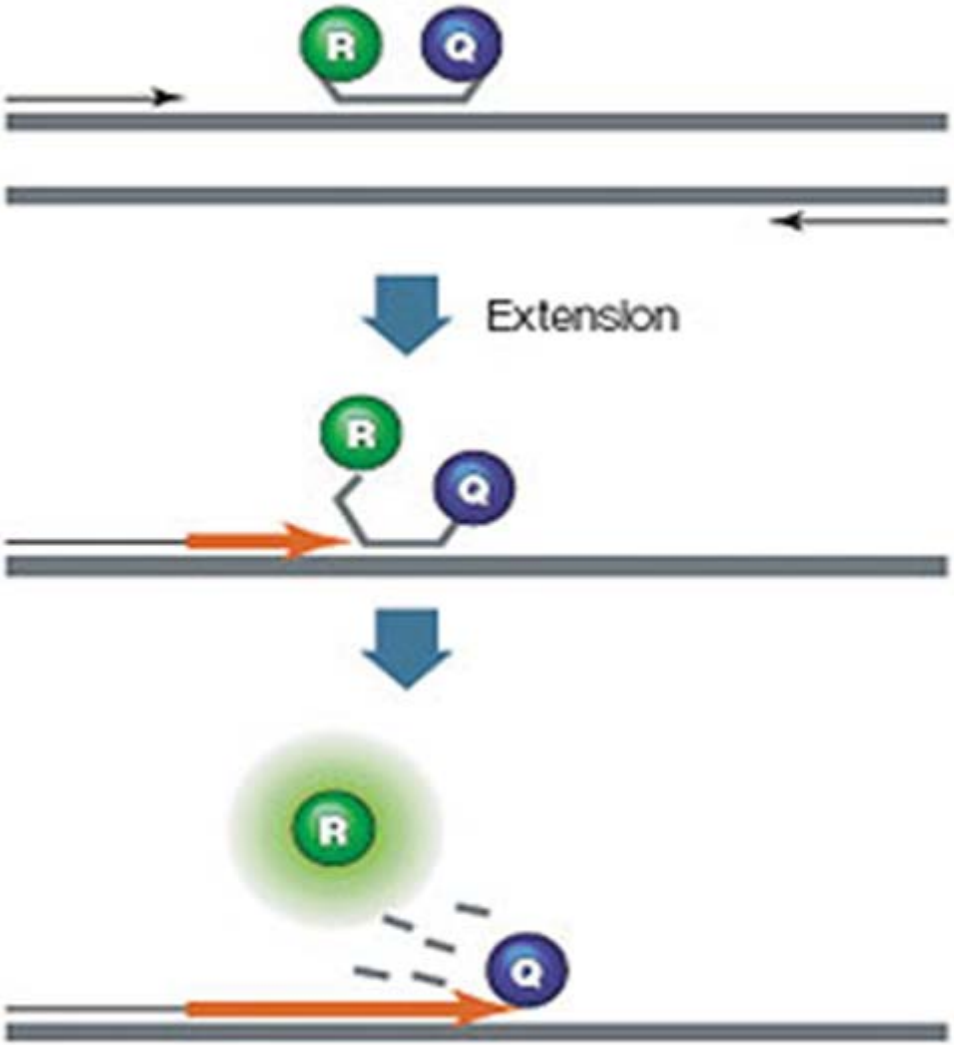
- Toxin CryIAb from *Bacillus thuringiensis* Insect resistance

GM rice Screening approach

Once the targets defined: which PCR chemistry?

- Probe/Reporter technology (Taqman®...)
- Fluorescent dyes (SYBR®Green...)

Fluorescently-labelled Probes



During annealing, the TaqMan probe binds to the target sequence

During extension, the probe is partially displaced and the reporter is cleaved. The free reporter fluoresces

R Reporter

Q Quencher

Validated Taqman® screening methods (Compendium)

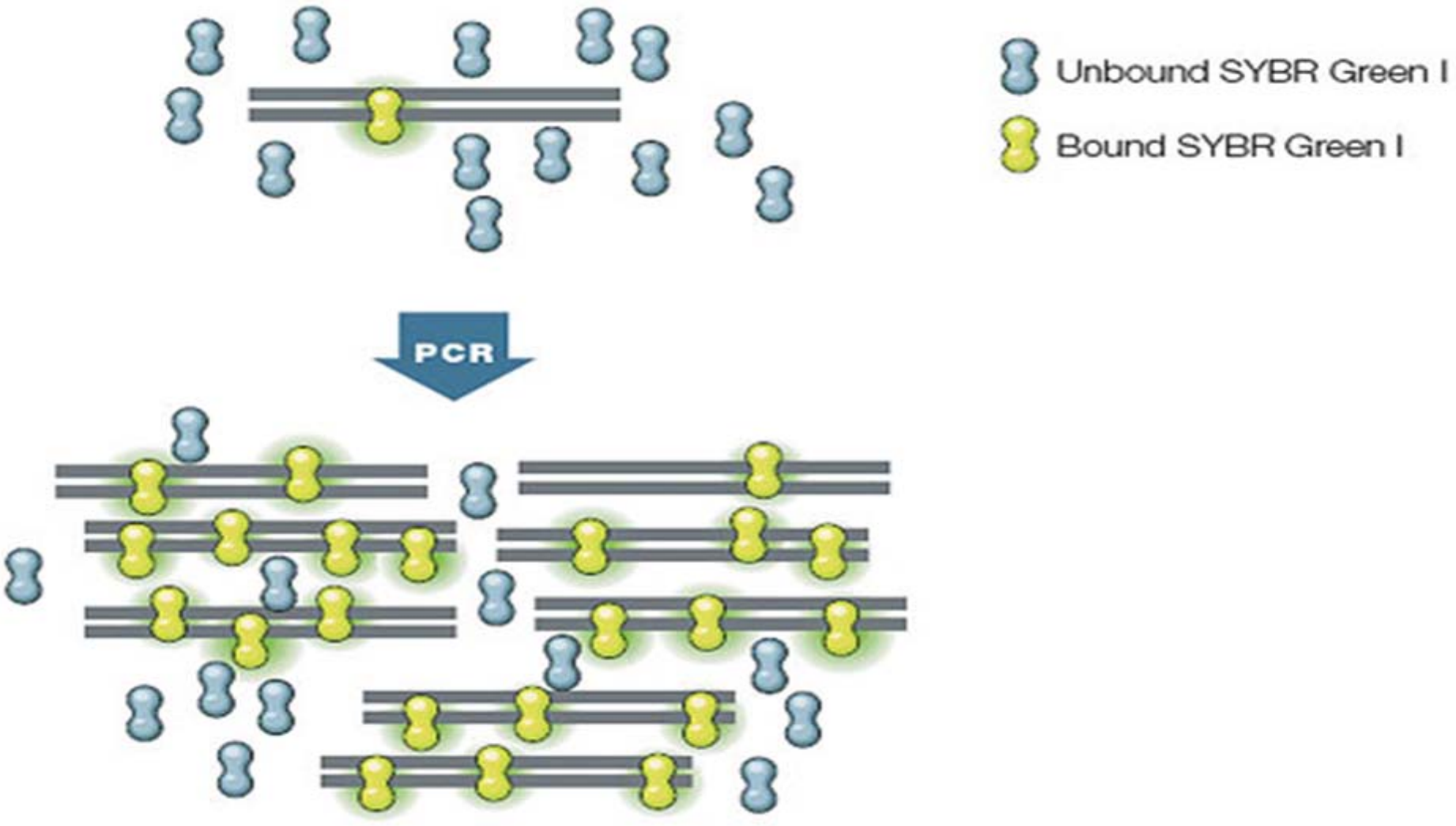
- **P-35S/T-Nos duplex** method developed by Waiblinger et al., 2008
  Cfr. QL-ELE-00-012 and QL-ELE-00-013

- **No suitable validated CryIAb method**

In GMOseek project, a broad-range CryIAb method has been developed by the CRA-W (Belgium) (currently for evaluation transferred to EURL)

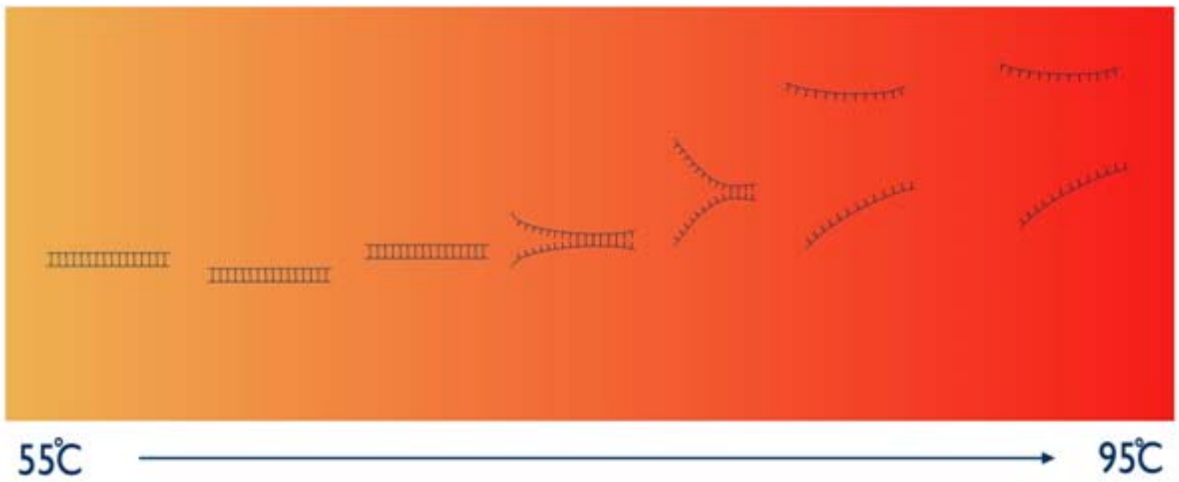
Communication by D. Zhang of a broad-range CryIAb method developed in China

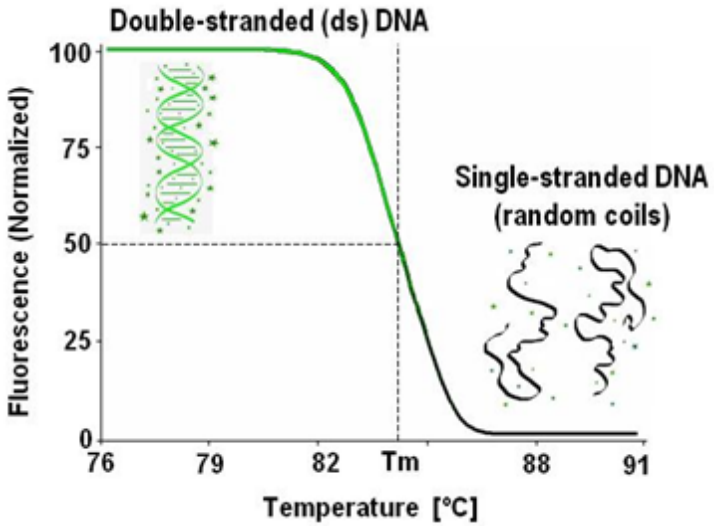
Fluorescent Dyes



Melting Analysis

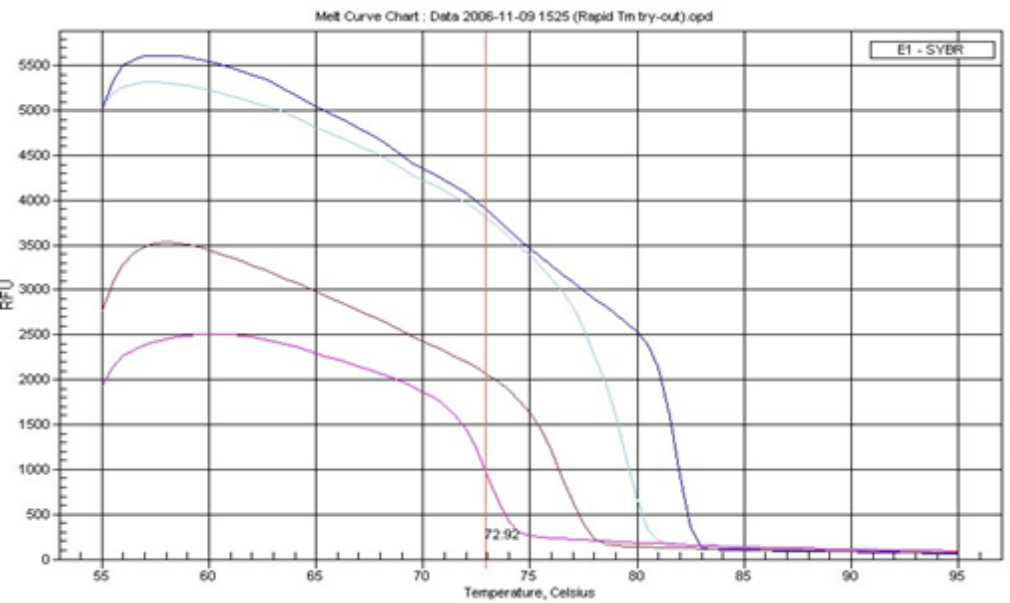
- Exploits the reversible nature of DNA-dye interaction
- The melting temperature (Tm) of each sequence is highly specific
- Allows detection of unspecific reaction products

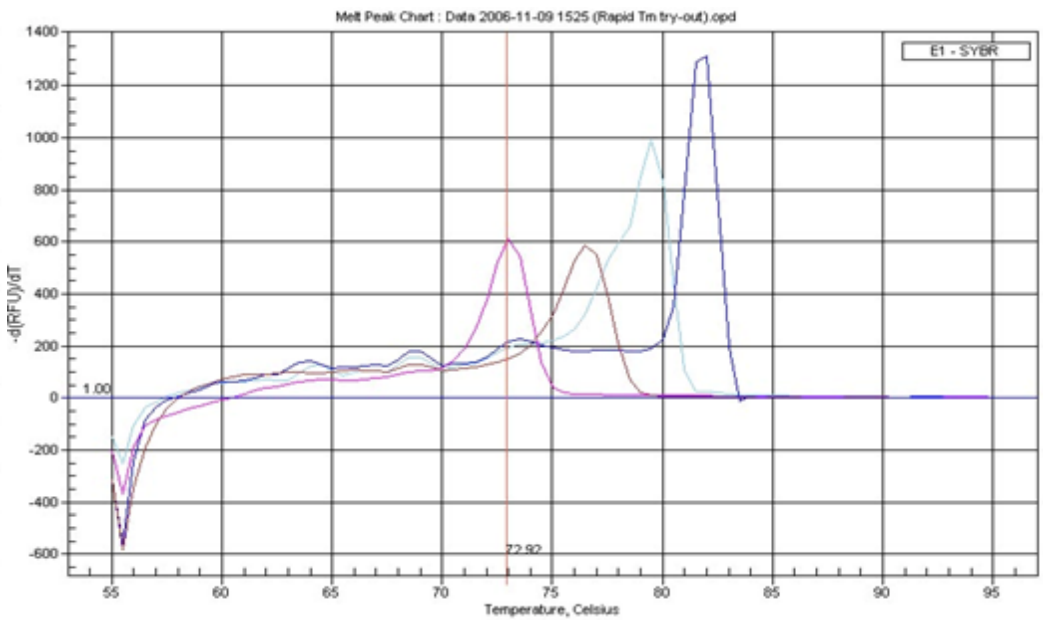




Melting Analysis

T-Nos ; P-35S ; CryIAb/Ac





Applications of Melting Analysis

- *A posteriori* specificity check
- Distinguish between different amplification products of the same primers
- Reaction optimization
  - Primer dimers
  - Non specific amplification

Publication of the SYBR®Green qPCR methods

Eur Food Res Technol (2010) 230:383–393
DOI 10.1007/s00217-009-1170-5

ORIGINAL PAPER

SYBR®Green qPCR screening methods for the presence of "35S promoter" and "NOS terminator" elements in food and feed products

Elodie Barbau-Piednoir · Antoon Lievens · Guillaume Mbongolo-Mbella · Nancy Roosens · Myriam Sneyers · Amaya Leunda-Casi · Marc Van den Bulcke

Eur Food Res Technol
DOI 10.1007/s00217-011-1605-7

ORIGINAL PAPER

Four new SYBR®Green qPCR screening methods for the detection of Roundup Ready®, LibertyLink®, and CryIAb traits in genetically modified products

Elodie Barbau-Piednoir · Antoon Lievens · Els Vandermassen · Etondoh-Guillaume Mbongolo-Mbella · Amaya Leunda Casi · Nancy Roosens · Myriam Sneyers · Marc Van den Bulcke

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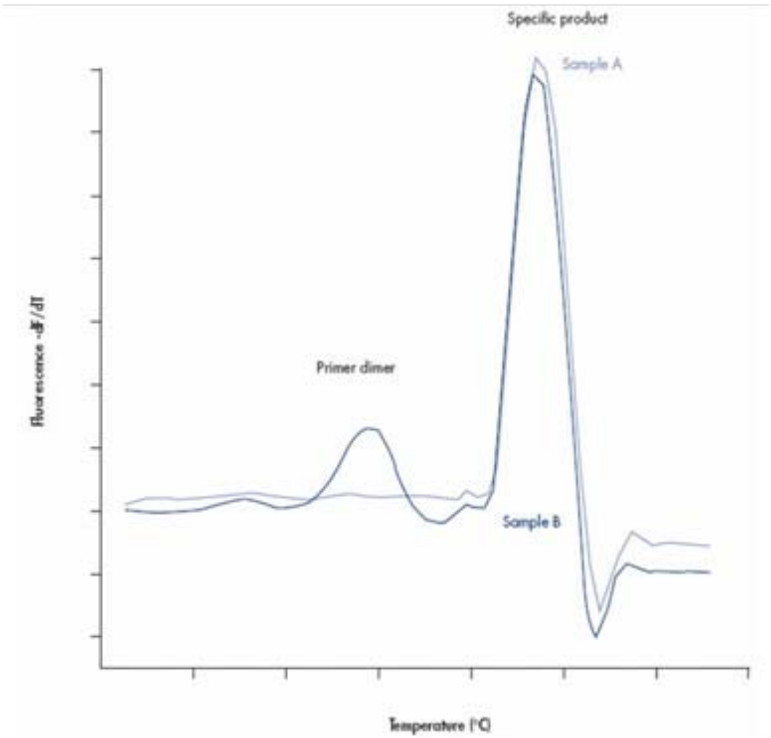
WETENSCHAPPELIJK INSTITUUT VOLKSGEZONDHEID
INSTITUT SCIENTIFIQUE DE SANTÉ PUBLIQUE

Applications of Melting Analysis

SYBR®Green method has ***only one homologous target*** (GMrice: P-35S & T-NOS)



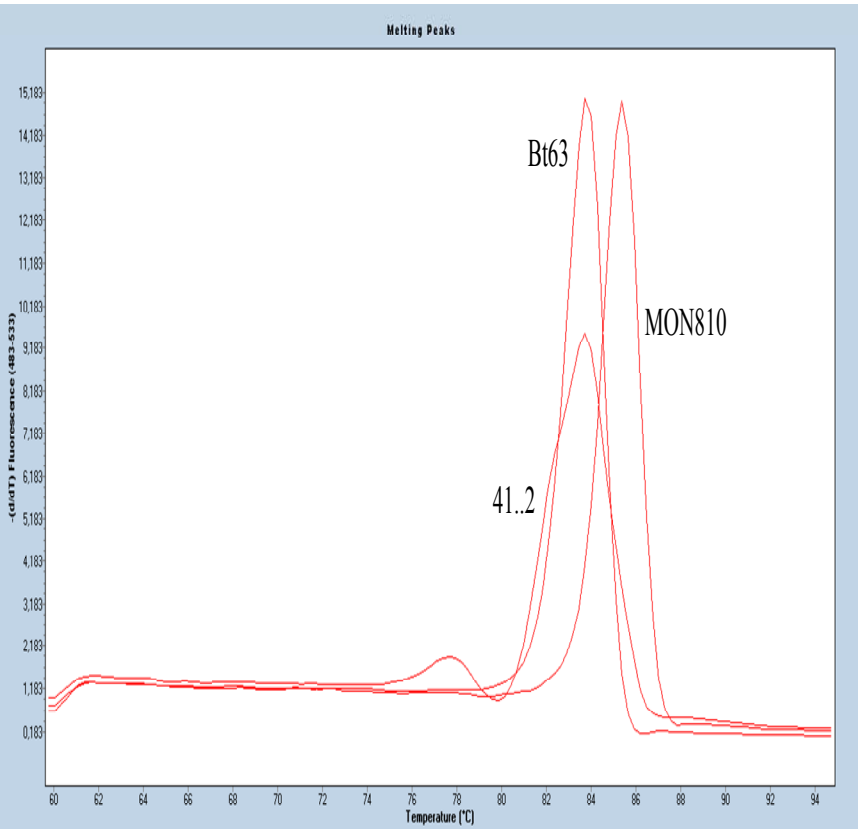
*How to set acceptance range e.g. for **P-35S***

In lab A:
Using as PC Bt11 maize : measured T_m = 75°C
and accepted T_m range for P-35S is 73.5 to 76.5°C

In lab B:
Using as PC Bt11 maize : measured Tm = 75.5°C
and accepted T_m range for P-35S is 74.0 to 77.0°C

Applications of Melting Analysis

SYBR®Green method has ***more then one homologous target*** (GMrice: CryIAb/Ac)



*How to set acceptance range e.g. for **CryIAb/Ac***

Using as PC Bt11 maize : measured T_m = 78,5°C
and accepted T_m range for Cry Bt11 is 77.0 to 80.0°C

Using as PC MON 810 maize : measured Tm = 80,5°C
and accepted Tm range for Cry MON 810 is 79.0 to 82.0°C

Thus: combined acceptance range for *CryIAb/Ac:* 77-82°C

SybrGreen methods: inhouse validated

- Positive and negative controls
- One unique Tm with clear dissociation peak
- Expected sequence of the amplicon
- No or almost no primer-dimer formation
- LOD and LOQ determined
- ...

Conclusions

- Both the duplex Taqman® and simplex SYBR®Green PCR methods for P-35S and T-Nos perform adequately to detect the corresponding targets in GM rice

- The SYBR®Green PCR method for CryIAb/Ac detects the corresponding targets in GM rice

Conclusions

- Fluo-dye methods are suitable for specific detection of targets
- 2 decision criteria: C_t (amount) and T_m (nature)
- T_m is *dominant* over C_t in the decision
- Fluo-dye methods are more suited for 'open' screening
- Fluo-dyes allow for post-amplification check within the PCR run

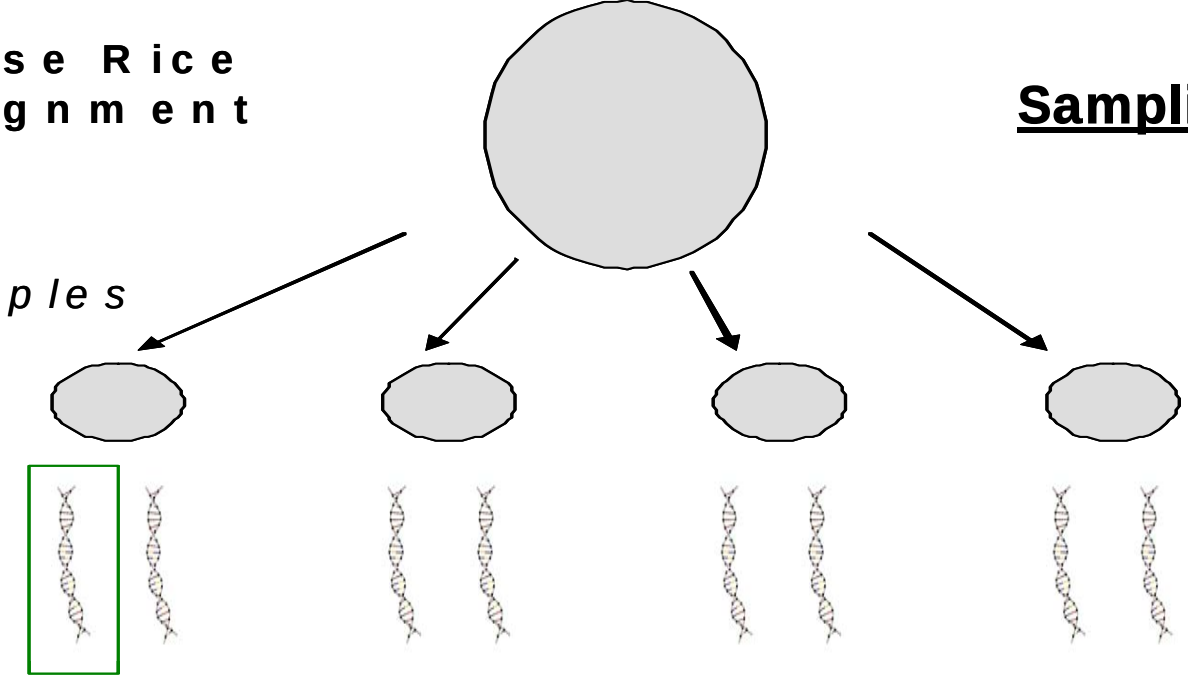
Wander off the beaten tracks

Increased workload and costs

- Sample preparation
- PCR strategy
- Interpretation of the results
- Non compliance based on screening and not on identification

Chinese Rice Consignment

Sampling scheme:



RT-PCR: PLD-rice P-35S T-nos CrylAb/Ac

Outcome of RT-PCR screening (3 examples)

Product A	Subsample 1	Subsample 2	Subsample 3	Subsample 4
PLD-rice	+/+	+/+	+/+	+/+
P-35S	-/-	-/-	-/-	-/-
T-nos	-/-	-/-	-/-	-/-
CrylAb/Ac	-/-	-/-	-/-	-/-

Outcome: No positive results — *Conclusion:* GM negative

Product B	Subsample 1	Subsample 2	Subsample 3	Subsample 4
PLD-rice	+/+	+/+	+/+	+/+
P-35S	-/-	-/-	-/-	-/-
T-nos	-/-	+/+	-/-	-/-
CrylAb/Ac	-/-	+/+	-/-	-/-

Outcome: Positive results — *Conclusion:* GM positive

Product C	Subsample 1	Subsample 2	Subsample 3	Subsample 4
PLD-rice	+/+	+/+	+/+	+/+
P-35S	-/-	-/-	-/-	-/-
T-nos	+/-	-/-	-/-	+/+
CrylAb/Ac	-/-	-/-	-/-	+/+

Outcome: Positive results — *Conclusion:* GM positive

Product D	Subsample 1	Subsample 2	Subsample 3	Subsample 4
PLD-rice	+/+	+/+	+/+	+/+
P-35S	-/-	-/-	-/-	-/-
T-nos	+/-	-/-	-/-	-/-
CrylAb/Ac	-/-	-/-	-/-	-/-

Outcome: Dissimilar result — *Conclusion:*
Retest: if " +" GM positive
if " - " GM negative

Collaborators:

EURL GMFF

Kluga L, Folloni S, Kagkli DM, Matetovici I, Jacchia S, Bogni A, Foti N, Savini C, Mazzara M, Van den Bulcke M, and Van den Eede G

Scientific Institute of Public Health

GMO detection Platform (Lievens A)

Thank you for your attention