

# GMO Detection on the Opticon™ and Chromo4™ Systems

**Babette Fahey, Ph.D.**  
**MJ Research, Incorporated**



# Outline

- **Methods for GMO detection**
- One-color real-time qPCR assay (SGI) for GM-soy detection
- Two-color real-time qPCR assay (TaqMan<sup>®</sup>) for GM-soy detection

# What Are GMOs?

- Organisms whose genetic material has been modified
- GMO modifications typically confer a beneficial trait to the organism
  - Herbicide resistance
  - Insect resistance
  - Disease resistance
  - Increased nutritional value
- GM crops include soy, corn and cotton

# Why GMO Detection?

- Labeling of foods to define GMO composition and quantity
- Monitoring transgene spread between and within species

# Methods for GMO Detection

- ELISA
  - Rapid (strip testing)
  - Relatively cheap
  - Less sensitive than PCR\*
  - Protein may not be expressed in tissue being assayed
  
- PCR
  - Rapid
  - Highly sensitive
  - Native gene used as control
  - Both quantitative and qualitative
    - Qualitative - presence or absence of the GMO
    - Quantitative - amount of GMO

\*The polymerase chain reaction (PCR) is a process covered by patents owned by Hoffman-LaRoche, Inc. & F. Hoffmann-LaRoche Ltd. Users should obtain proper license to perform the reaction. Additional licensing information is presented at the end of this presentation.

# Detection of Roundup Ready<sup>®</sup> Soy Bean (1-Color Assay)

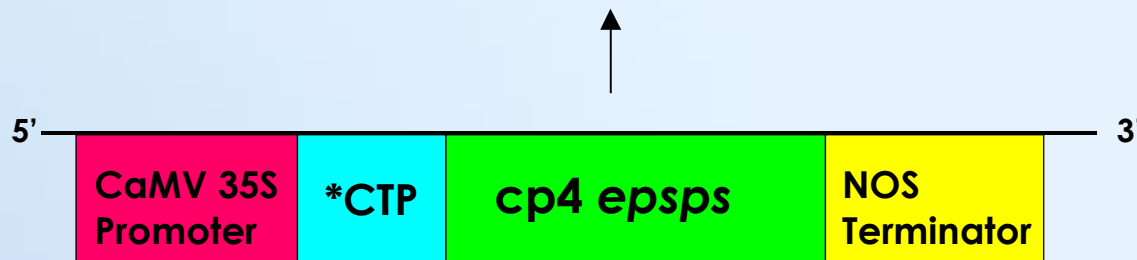


- What is Roundup Ready soy?
  - Soy bean engineered for resistance to the Roundup<sup>®</sup> herbicide (Monsanto Corporation)
- How does the Roundup<sup>®</sup> work?
  - Active ingredient in Roundup (Glyphosate) inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)
  - EPSPS is a critical enzyme required for the synthesis of aromatic amino acids
  - Aromatic amino acid deficiency is lethal

# Creating Roundup Ready Soy

- Soy beans are transformed with *epsps* enzyme from strain CP4 of *Agrobacterium* species
- Plants expressing CP4 EPSPS are resistant to Roundup

Sequence amplified in the real-time assay



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# Outline

- Methods for GMO detection
- One-color real-time qPCR assay (SGI) for GM-soy detection
- Two-color real-time qPCR assay (TaqMan) for GM-soy detection

# One-Color Real-Time qPCR Assay for GMO Detection

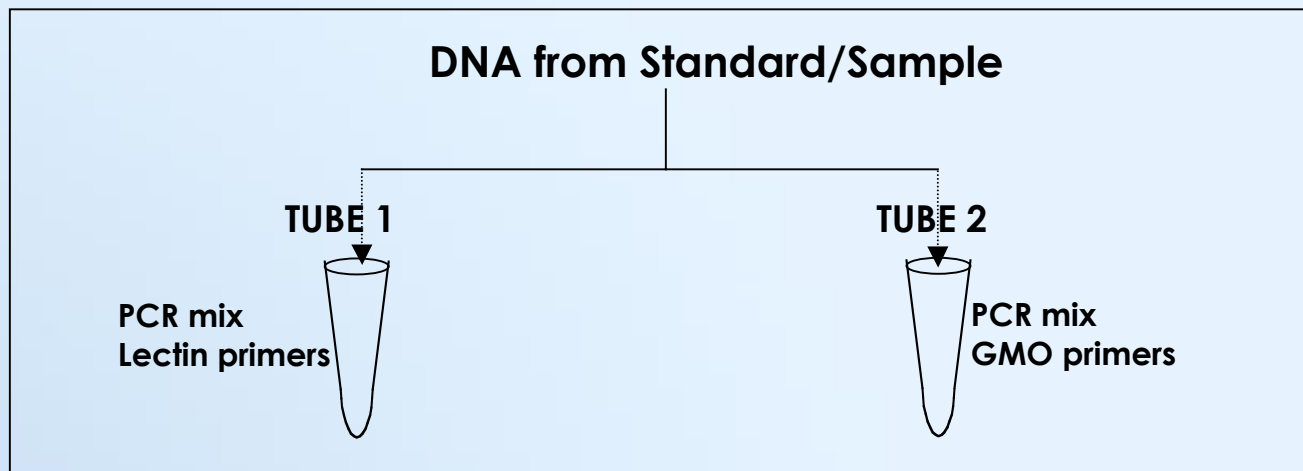


- Targets
  - CP4 *epsps*: GMO-specific Roundup resistance gene
  - Soy lectin: Endogenous reference to measure soy content

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# Templates Tested

- Reference standards - Institute for Reference Materials and Measurement. DNA from dried soy bean powder containing 0%, 0.1%, 0.5%, 1%, 2% and 5% GMO soy
- Experimental samples - DNA isolated from soy containing foodstuffs

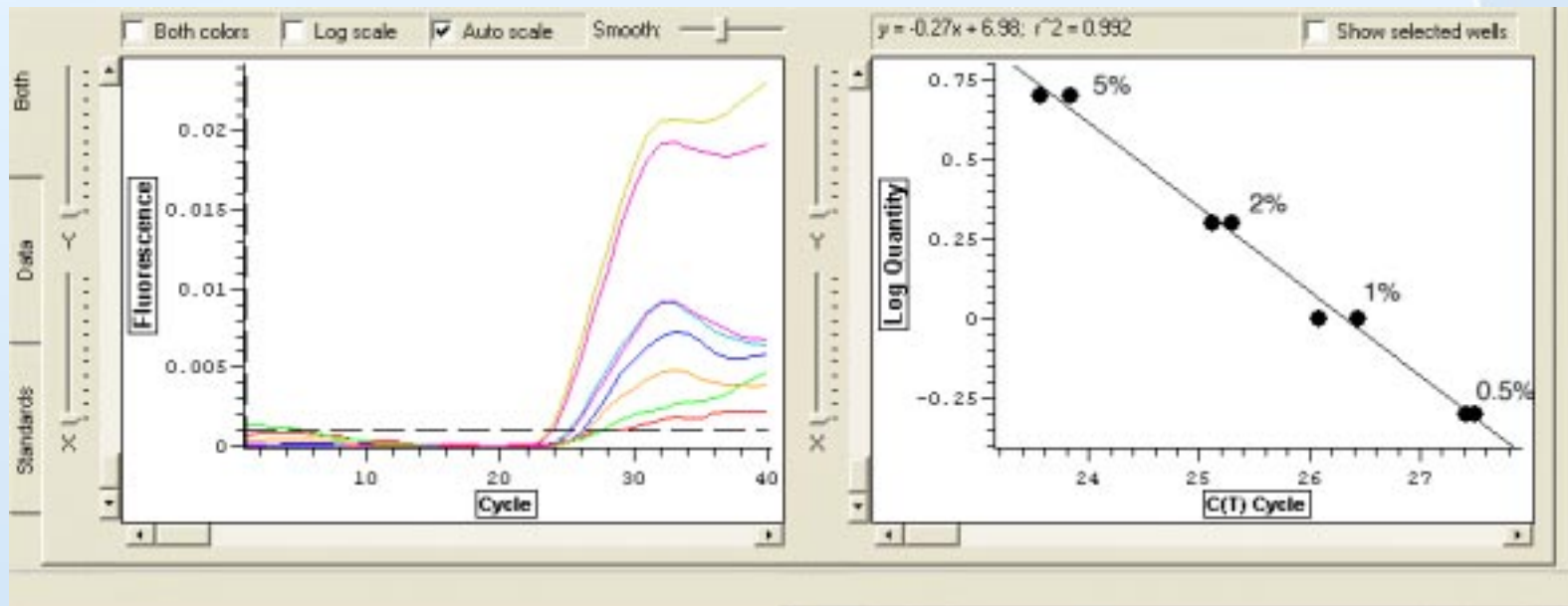


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# Data Generated

- Fluorescence curves
- Standard curve for reference standards
- $C(t)$  values for each reaction
- Melting curves of reaction products

# Standard Curve of Reference Standards



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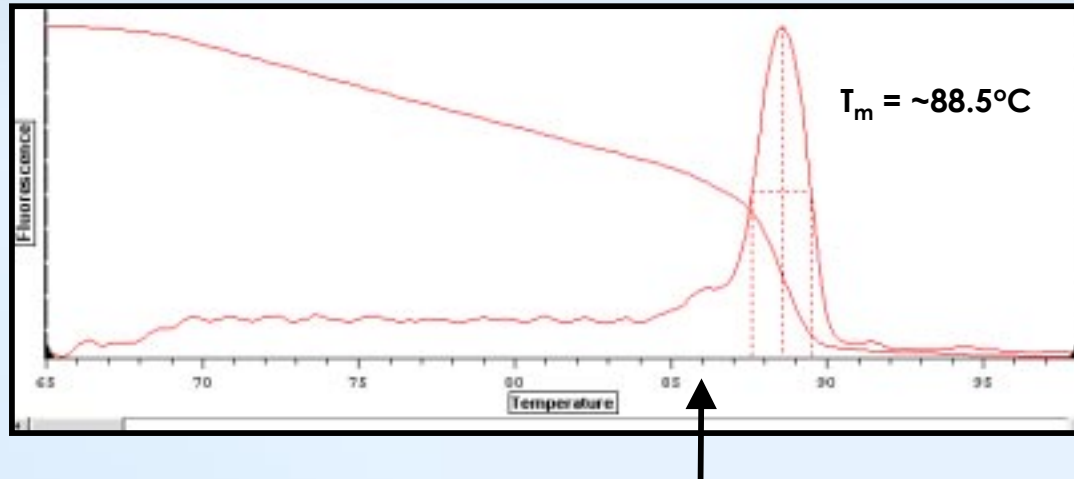
# C(t) Values of Reactions

Standards/ Sample	C(t) <i>epsps</i>	C(t) Lectin
0	ND	22.6
0.1	ND	22.6
0.5	28.2	22.5
1.0	27.2	22.5
2.0	26.2	22.8
5.0	24.6	22.7
Soy Dessert	24.6	22.4
Soy Flour	ND	22.2
Soy Burger	24.2	23.4

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# Melting Curve Analysis

Melting Curves – Lectin Amplicon



Melting Curve – epsps Amplicon

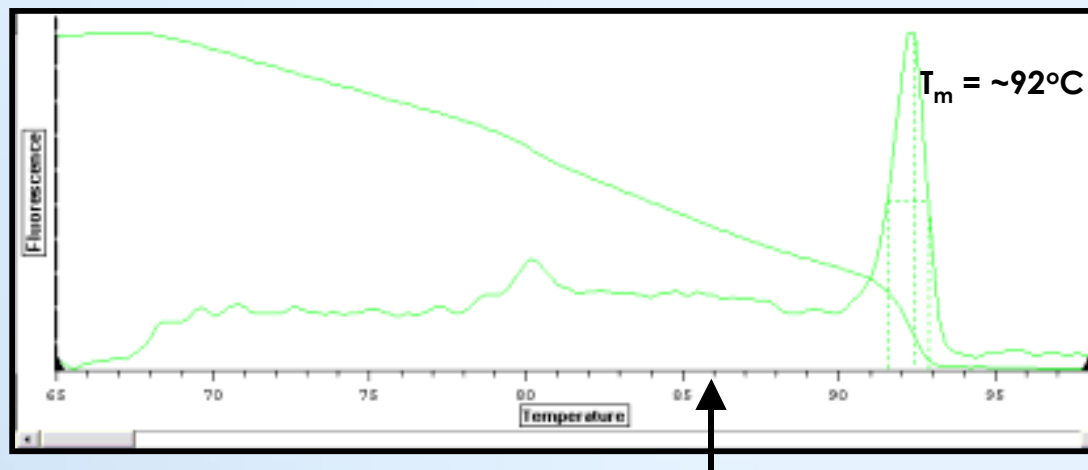


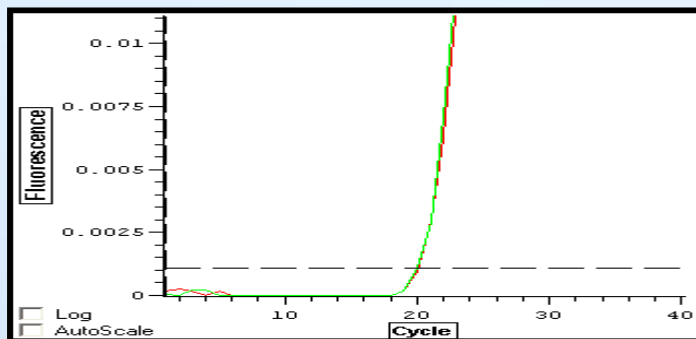
Plate read temperature

# Data Analysis

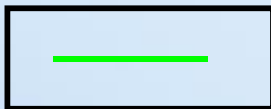
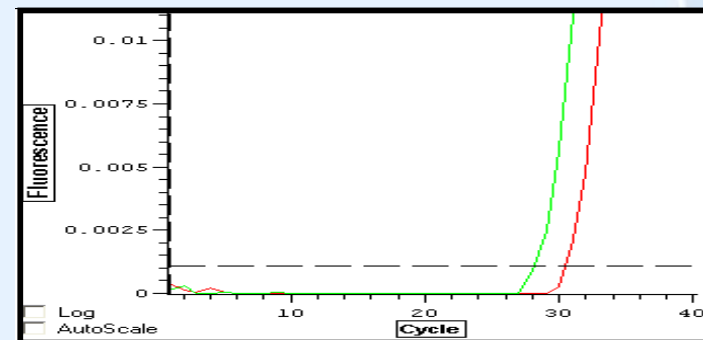
- $\Delta C(t)$  value =  $C(t)_{epsps} - C(t)_{lectin}$
- Standard curve for reference standards
- GMO content of unknown food samples

# Normalization to Lectin

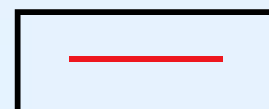
Lectin



*epsps*



Food Sample #1



Food Sample #2

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# Normalization to Lectin

1. Calculate  $\Delta C(t) = C(t)_{\text{epsps}} - C(t)_{\text{lectin}}$

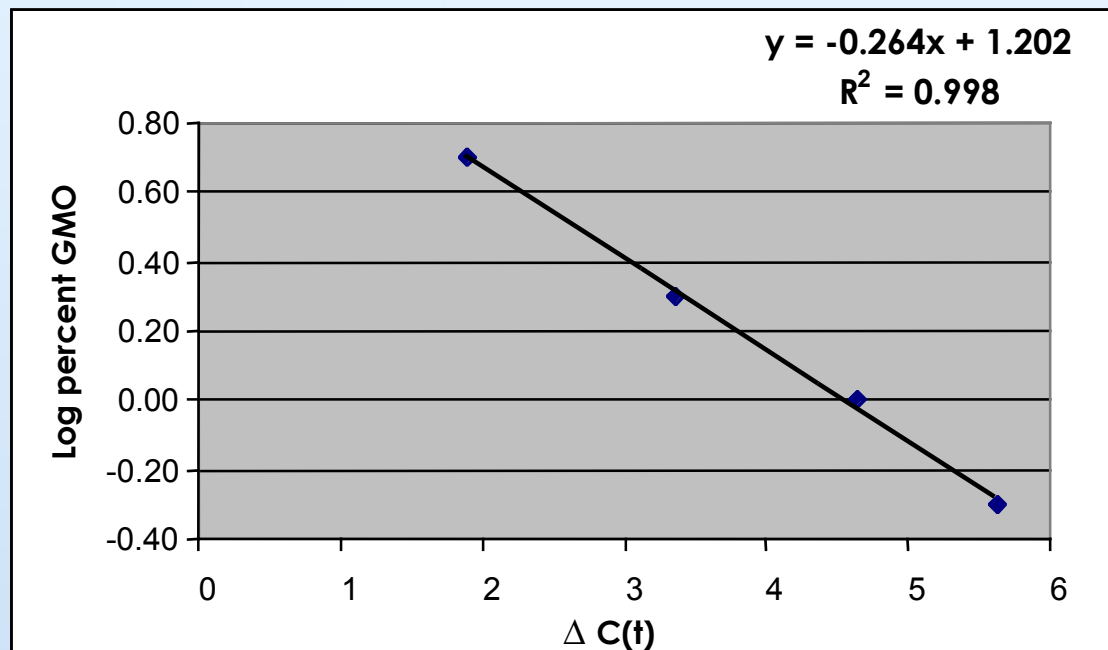
Standards/ Sample	C(t) epsps	C(t) Lectin	$\Delta C(t)$
0	ND	22.6	ND
0.1	ND	22.6	ND
0.5	28.2	22.5	5.6
1.0	27.2	22.5	4.7
2.0	26.2	22.8	3.4
5.0	24.6	22.7	1.9
Soy Dessert	24.6	22.4	2.1
Soy Flour	ND	22.2	ND
Soy Burger	24.2	23.4	0.7

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# Standard Curve for Reference Standards



2. Generate standard curve from the  $\Delta C(t)$  values of reference standards



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# Quantification of GMO Content in Food Samples



3. Determine %GMO of unknown from standard curve of  $\Delta C(t)$  values

Standards/ Sample	$\Delta C(t)$	%GMO
0	ND	—
0.1	ND	—
0.5	5.6	—
1.0	4.7	—
2.0	3.4	—
5.0	1.9	—
Soy Dessert	2.1	4.40
Soy Flour	ND	< 0.5
Soy Burger	0.7	> 5

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# One-Color Real-Time qPCR Assay for GMO Detection



## Summary

- Rapid protocol (<1 day)
- Usable for whole and processed foods
- Qualitative and quantitative
- Small amounts of starting material required
- Protocol may be adaptable to other GMO crops such as maize or rice

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# Outline

- Methods for GMO detection
- One-color real-time qPCR assay (SGI) for GM-soy detection
- Two-color real-time qPCR assay (TaqMan) for GM-soy detection

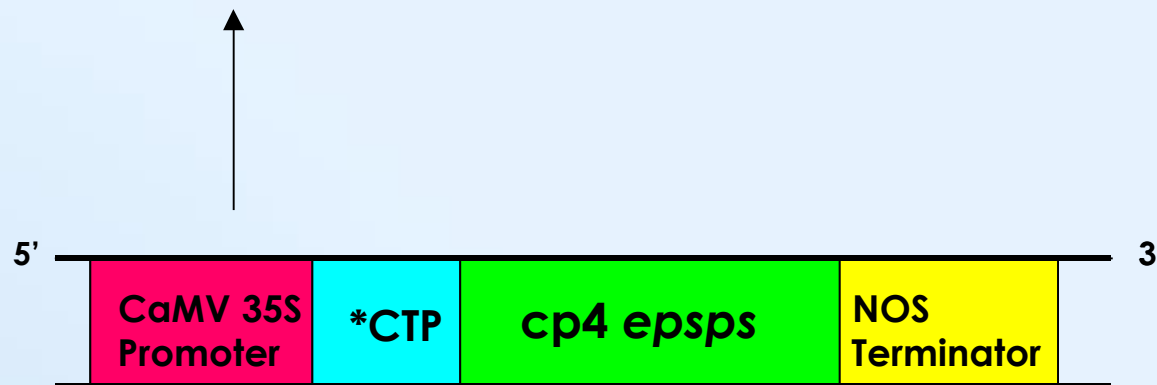
# GMO Detection Assay (2-Color)

- TaqMan chemistry
- Multiplexing (target and internal control in same reaction)
- Two-color assay for detection of GM-soy

# Detection of Roundup Ready Soy Bean (2-Color Assay)



Sequence amplified in the assay –  
Cauliflower Mosaic Virus Promoter



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# Two-Color Real-Time qPCR Assay for GMO Detection

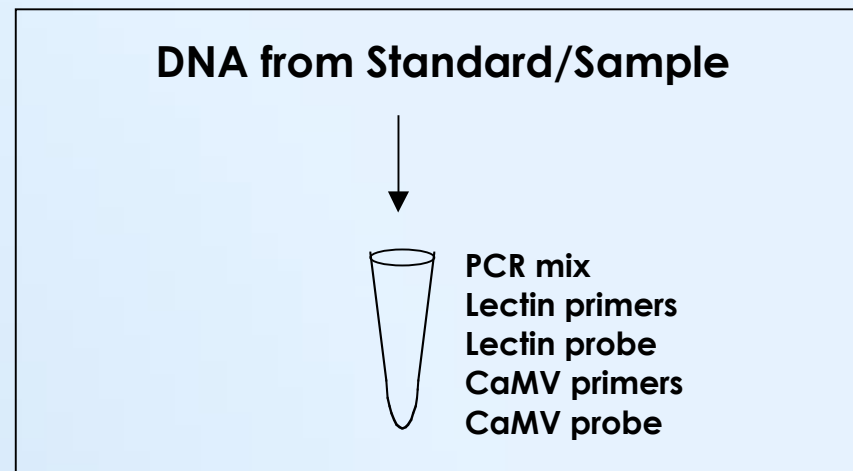


- Multiplex reactions with TaqMan chemistry
- Targets
  - CaMV 35S Promoter: GMO-specific amplicon
  - Soy Lectin: Endogenous reference to measure soy content

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# Templates Tested

- Reference standards - Institute for Reference Materials and Measurement. DNA from dried soy bean powder containing 0%, 0.1%, 0.5%, 1%, 2% and 5% GM-soy
- Experimental samples - DNA isolated from soy containing foodstuffs



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# Data Generated

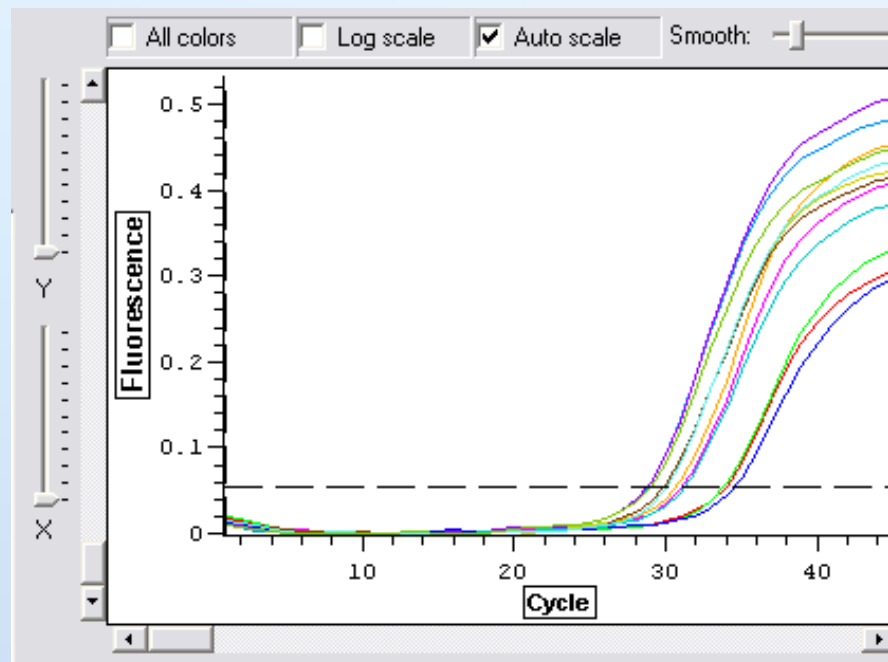
- Fluorescence curves
  - Channel 1: CaMV35S (FAM)
  - Channel 2: Soy lectin (VIC)
- Standard curve for reference standards
- C(t) values for each reaction

# Standard Curve for Reference Standards

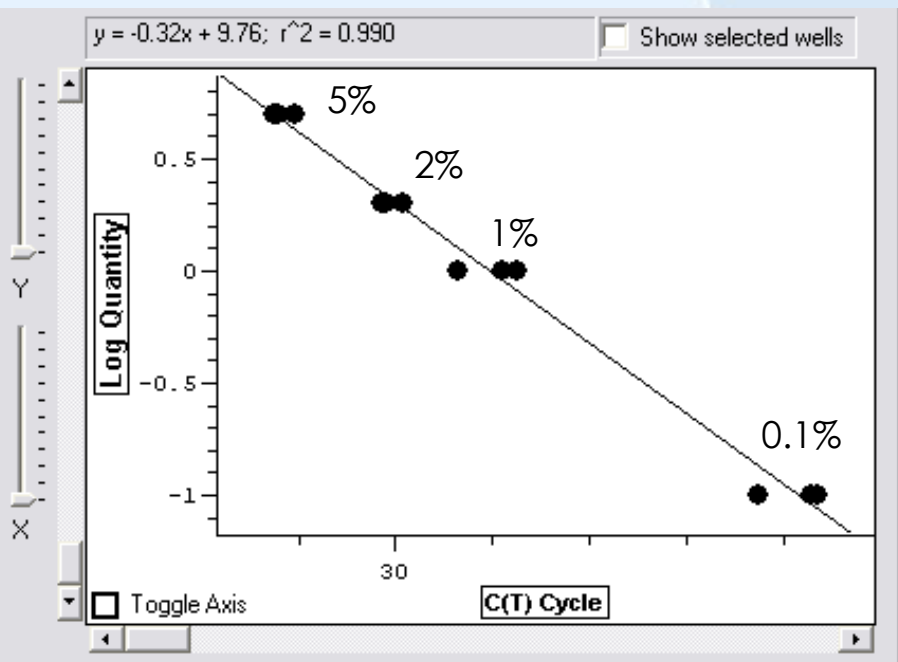


## Channel 1: CaMV35S (FAM)

Fluorescence Curves



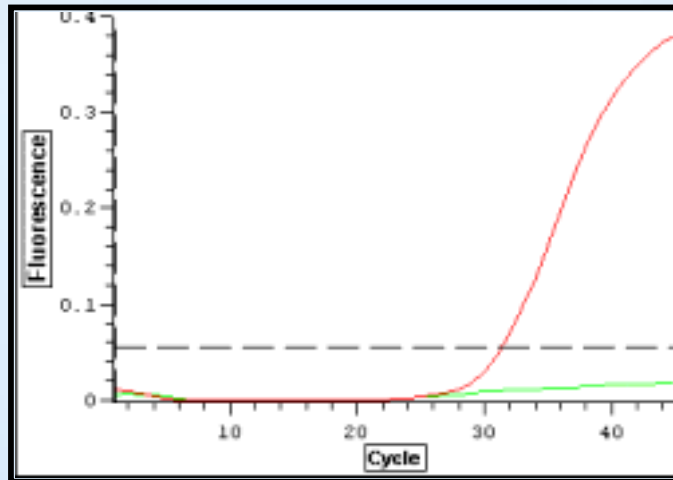
Standard Curve



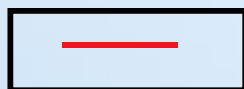
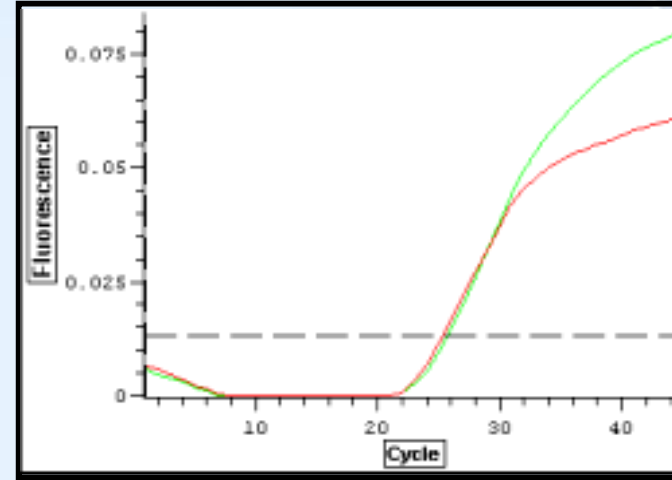
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# GMO Detection in Food Samples

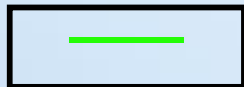
Channel 1:CaMV35S (FAM)



Channel 1:Soy Lectin (VIC)



Soy Burger



Soy Flour

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# C(t) Values of Reactions

Standards/sample	Average	SD	Average	SD
0.00	ND	N/A	25.06	0.20
0.10	34.09	0.37	25.96	0.15
1.00	30.82	0.30	25.43	0.41
2.00	31.01	0.11	26.45	0.08
5.00	28.78	0.14	25.21	0.08
Soy Flour 1	ND	ND	26.25	0.32
Soy Flour 2	ND	ND	27.96	0.41
Soy Flour 3	38.00	0.57	25.11	0.17
Pancake mix	37.26	0.32	26.94	0.06
Soy Dessert	27.40	0.22	26.25	0.32
Soy Burger 1	37.62	0.45	28.43	0.04
SoyBurger 2	31.54	0.03	25.70	0.05
Soy Burger 3	31.72	0.33	26.92	0.47

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# Data Analysis

- $\Delta C(t)$  value =  $C(t)_{epsps} - C(t)_{lectin}$
- Standard curve for reference standards
- GMO content of unknown food samples

# Normalization to Lectin

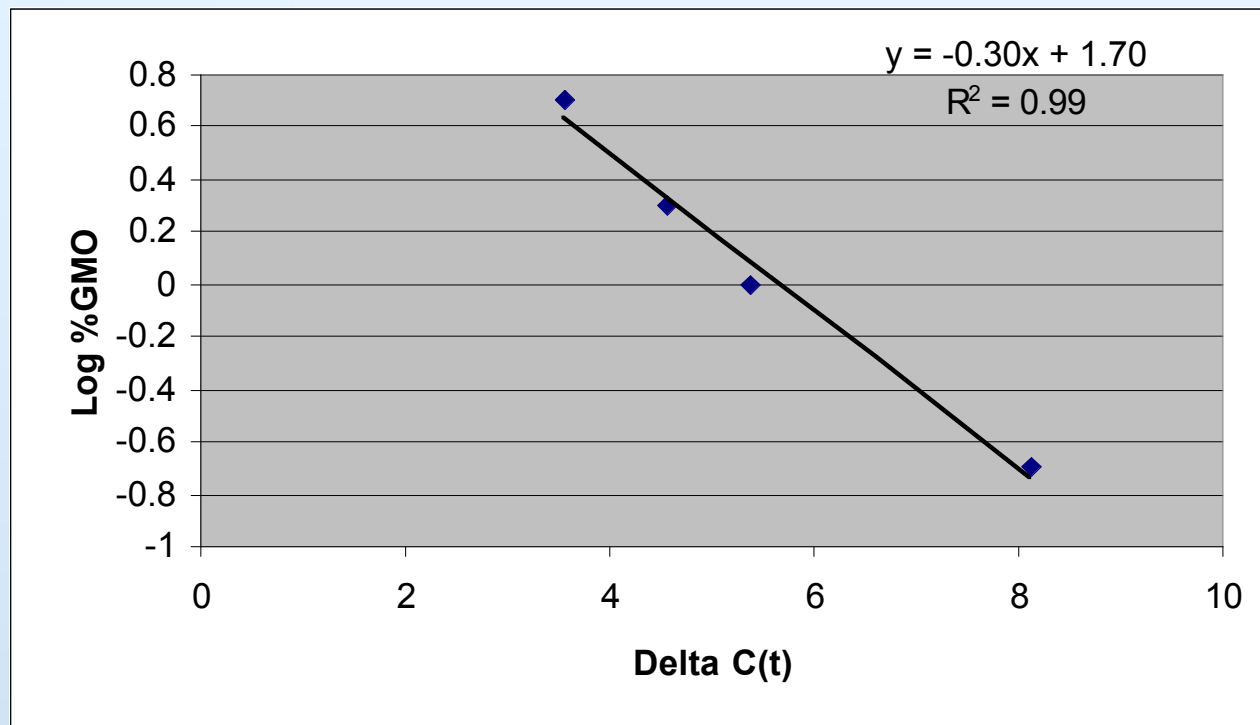
1. Calculate  $\Delta C(t) = C(t)_{CaMV} - C(t)_{lectin}$

Standards/sample	Average	SD	Average	SD	$\Delta C(t)$
0.00	ND	N/A	25.06	0.20	N/A
0.10	34.09	0.37	25.96	0.15	8.13
1.00	30.82	0.30	25.43	0.41	5.39
2.00	31.01	0.11	26.45	0.08	4.56
5.00	28.78	0.14	25.21	0.08	3.57
Soy Flour 1	ND	ND	26.25	0.32	ND
Soy Flour 2	ND	ND	27.96	0.41	ND
Soy Flour 3	38.00	0.57	25.11	0.17	12.88
Pancake mix	37.26	0.32	26.94	0.06	10.31
Soy Dessert	27.40	0.22	26.25	0.32	1.15
Soy Burger 1	37.62	0.45	28.43	0.04	9.18
SoyBurger 2	31.54	0.03	25.70	0.05	5.84
Soy Burger 3	31.72	0.33	26.92	0.47	4.80

# Standard Curve of Reference Standards



2. Generate standard curve from the  $\Delta C(t)$  values of reference standards



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# Quantification of GMO Content in Food Samples



3. Determine %GMO of unknown from standard curve of  $\Delta C(t)$  values

Sample	Delta C(t)	%GMO
Soy Flour 1	ND	ND
Soy Flour 2	ND	ND
Soy Flour 3	12.88	<0.1
Pancake mix	10.31	<0.1
Soy Dessert	1.15	>5
Soy Burger 1	9.18	<0.1
SoyBurger 2	5.84	0.95
Soy Burger 3	4.80	1.30

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# Two-Color Real-Time qPCR Assay for GMO Detection



## Summary

- One tube assay system
- Rapid protocol (<1 day)
- Usable for whole and processed foods
- Qualitative and quantitative
- Small amounts of starting material required
- Sensitive enough to detect 0.1% GMO content

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# Acknowledgements

**MJ Research, Incorporated**

**David Batey, Ph.D.**

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**MJ Bioworks, Incorporated**

**Chas André, Ph.D.**



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**TaqMan is a registered trademark of Roche Molecular Systems, Inc.**

**FAM, HEX, TAMRA and TET are trademarks, and VIC is a registered trademark of Applied Biosystems Corp.**

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The following table reflects MJ Research's understanding of the requirements set by Roche and its licensees for particular classes of users to purchase PCR licenses in the United States. This table is not endorsed by Roche, and all thermal cycler users are encouraged to contact Roche for further information.

**"Authorization" of thermal cyclers:** The Applied Biosystems division of Applied Biosystems Corporation holds the exclusive license from Roche to grant sublicenses to perform PCR in specific fields, such as research and forensics. Since 1992, Applied Biosystems has generally issued licenses in the form of a running royalty with sale of specific licensed enzymes, which carry a label stating that reactions must be run in an "authorized" thermal cycler.

MJ Research does not sell "authorized" thermal cyclers and believes that ABI's right to charge a thermal cycler "authorization" fee is probably legally unenforceable in the U.S. (this matter is under review by the U.S. Federal District Court). However, Applied Biosystems will sell such an "authorization" for any individual thermal cycler. It may be prudent in some circumstances to "authorize" an MJ cycler after purchase.

### Addresses for contacting Hoffmann-LaRoche:

In the United States:

Licensing Manager  
Roche Molecular Systems, Inc.  
1145 Atlantic Avenue  
Alameda CA 94501 USA  
(510) 814-2970 • Fax: (510) 814-2977

In other nations:

PCR Licensing Manager  
F. Hoffmann-La Roche Ltd.  
Building 222/350  
CH-4002 Basel, Switzerland  
41 61 687 3031 • Fax: 41 61 687 2113

Type of Use	PCR <sup>†</sup> for Human & Animal Diagnostics		Other PCR <sup>†</sup> Research, Forensics, etc.	Non-PCR <sup>†</sup> Cycle Sequencing, Prins, etc.*
	with Diagnostic Service License <sup>†</sup>	with Licensed Test Kits <sup>†</sup>		
Thermal Cycler License*	Usually none (see actual terms of license)	Usually none (see actual terms of license)	Thermal cycler "authorized" for PCR	None
Enzyme License*	Usually none (see actual terms of license)	Usually none (see actual terms of license)	Enzyme with PCR label license	None

\* This table refers to PCR licenses only; other processes, and particular types of enzyme or thermal cycler, may require separate license.

† For definitive information on where your application fits, please contact Roche.

‡ For information on PCR Diagnostic Product Licenses or PCR Diagnostic Service Licenses, please contact Roche.

**Note: Please do not construe the information on this page as legal advice—for more information, please contact your institution's legal department.**

N.B. For each column, both the indicated thermal cycler license and the indicated enzyme license are required.