

Real-time Detection and Quantitation of Genetically Modified Soy.

Surekha Karudapuram, Ph.D.¹, Babette Fahey, Ph.D.², and David Batey, Ph.D.¹

¹MJ Research, South San Francisco, CA ²MJ Research, Inc., Waltham, MA

Abstract

In this study, real-time quantitative PCR was used to detect and quantify genetically modified soy in soy-containing foods. Differentially labeled TaqMan® probes were used in a two-color assay to detect the Cauliflower Mosaic Virus (CaMV) 35S promoter sequence (a promoter often used to express transgenes) and an endogenous reference sequence. The amount of genetically modified soy was quantified by interpolation of a standard curve generated from a set of certified reference standards. The method presented here has several attractive features, including: it requires only small amounts of starting material; it is suitable for raw and processed foods; and it is adaptable for other genetically modified crops.

Objective

To develop a quick and accurate method to detect and quantify GMOs in food, using real-time quantitative PCR (qPCR) on the MJ Research® Chromo4™ Fluorescence Detection System

Introduction

GMO = Genetically Modified Organisms

- Many food crops have been genetically engineered to contain beneficial resistance to herbicides, disease, or insects.
- In some areas, laws require appropriate labeling of foods that contain GMOs.
- Accurate labeling of food requires accurate detection and quantification of GMOs.

Sequence detected: Cauliflower Mosaic Virus (CaMV) 35S promoter

- Is commonly used to express transgenes in genetically modified crops
- Does not require any viral trans-acting factors for activity
- Has little tissue specificity
- Has clearly defined sequence requirements for its activity

How qPCR Works

- Amplification is performed with fluorescent dye or probes present in the reaction mixture.
- Fluorescence intensity of dye or probe is proportional to the amount of product present.
- Cycle threshold [C(t)] is the point during the thermal cycling protocol at which a fluorescence signal rises above background levels.
- With greater starting concentration, threshold is reached earlier in the cycling protocol.
- qPCR allows the determination of the initial target concentration in a reaction by identifying the C(t) of a sample reaction and comparing it to the C(t) values of reactions with known starting concentrations.

Protocol

1. Isolate DNA from Reference Standards and Food Samples:

- Reference Standards:** dried soybean powder containing mass fractions of 0%–5% genetically modified soybean obtained from Sigma (Cat # 89305)

Food Samples: three types of soy burger, one soy dessert, one pancake mix and three brands of soy flour obtained from a local supermarket

- The DNeasy® Plant Mini Kit (Qiagen Cat# 69104)—DNA was isolated according to kit protocol.

- Determine DNA concentration by spectrophotometry; quality verified by agarose gel electrophoresis on a 1% TBE gel.

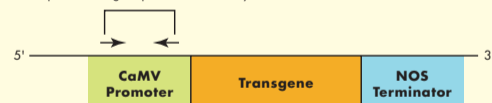
Average amount: 1–2µg of DNA from each sample preparation.

2. Amplify CaMV and soy lectin gene sequences

Soy lectin gene was used as an endogenous reference so that the amount of GM-soy detected could be normalized to the total amount of soy present in the food samples

- FAM™-labeled Taqman® probe detects GMO-specific CaMV 35S promoter
- VIC™-labeled Taqman probe detects soy lectin

Sequence being amplified in the assay



Reaction composition:

Component	Volume
DNA template	2.0µl
GMO Kit Mix*	17.5µl
AmpliTaq Gold®*	0.5µl
Total Volume	20.0µl

*TaqMan Soy GMO 35S detection kit (#4327692) and AmpliTaq Gold (#4311806) were from Applied Biosystems.

Cycling Conditions

1. 94°C, 9 min
 2. 96°C, 20 sec
 3. 60°C, 1 min
 4. 72°C, 30 sec
 5. Plate read
 6. Go to Step 2, 39 more times
 7. 10°C, Forever
- END

3. Generate standard curves (cycle threshold vs. log quantity DNA) using reference standards

4. Calculate $\Delta C(t) = C(t)_{GMO} - C(t)_{lectin}$

This allows normalization of the amount of GMO to the total amount of soy.

5. Generate standard curve: log percent GMO (in reference standards) vs. $\Delta C(t)$

The data were fitted to a line using the Microsoft® Excel Chart function *Add Trendline*. The function also returns the R² value, which indicates how well the linear equation fits the data.

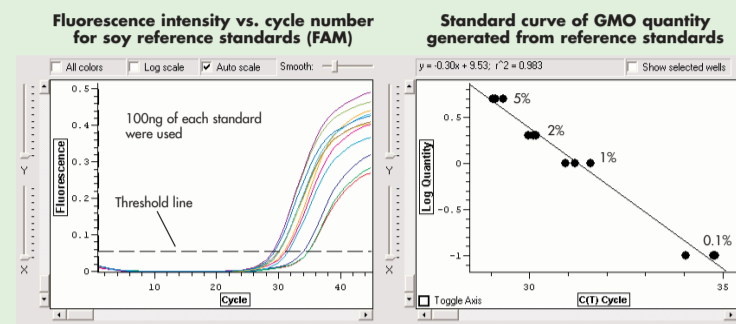
6. Determine GMO content of food samples

The Excel Trend function was used to interpolate the %GMO values of the food samples from the standard curve. The function returns the value log %GMO of the food sample. The %GMO soy content of the sample was then determined from the formula $10^{(\log \%GMO)}$.

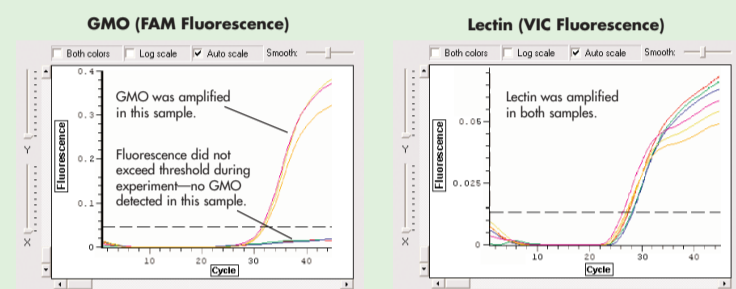
Results

Channel 1: CaMV 35S (FAM)

Channel 2: Soy lectin (VIC)



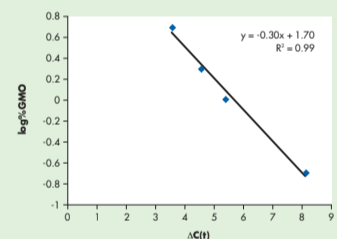
Amplification of GMO and Lectin in Food Samples, Indicated by Fluorescence Intensity



Triplicate reactions of representative food samples are shown.

Calculation of $\Delta C(t) = C(t)_{CaMV} - C(t)_{lectin}$

Standards/sample	C(t) _{CaMV} (Mean ± SD)	C(t) _{lectin} (Mean ± SD)	ΔC(t)
0.00	ND	25.06 ± 0.20	ND
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	26.25 ± 0.32	ND
Soy Flour 2	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



%GMO in food samples was calculated by interpolation against this curve, using the Excel function, "Trend."

GMO Quantity in Food Samples

- Determined by interpolation of the standard curve

Sample	ΔC(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	>5.0
Soy Burger 1	9.18	<0.1
SoyBurger 2	5.84	0.95
Soy Burger 3	4.80	1.30

Summary

Detection and quantification of genetically modified soy has been performed on the Chromo4 system. The protocol used:

- Can be used for detection and quantification of GM-soy in processed foods
- Can be completed in less than one day
- Requires small amounts of starting material (50–100 mgs)
- Is a one-tube assay
- Is sensitive enough to detect as low as 0.1% GM-soy in a food sample
- Can use pre-assembled kits designed for different GMO foods

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