
TRAINING COURSE ON

THE ANALYSIS OF FOOD AND FEED SAMPLES FOR THE PRESENCE OF GENETICALLY MODIFIED ORGANISMS

*The State General Laboratory
Nicosia, Cyprus*

June 6 - 10, 2005



State General Laboratory



Agricultural Research Institute

WORKPROGRAMME

1ST DAY – MONDAY, JUNE 6TH 2005

- 8.00 am Welcoming speech – opening of the course – **Dr. Costas Michael - Director SGL**
- 8:15 am Introduction to the course, presentation of the organizers and of the participants – **M. Querci - JRC**
- 8.30 am **Theory:** Introduction on the general procedures for GMO detection and course content - **M. Querci - JRC**

PREPARATION OF SAMPLES: DNA EXTRACTION

- 9.00 am Experimental: DNA extraction following the CTAB method (1st part)
- 9.30 am Coffee break
- 10.00 am **Theory:** gel electrophoresis for nucleic acids analysis
- Experimental: Preparation of agarose gels
- 11.00 pm Experimental: DNA extraction following the CTAB method (2nd part)
- 12:00 pm Lunch
- 1:00 pm Experimental: DNA extraction following the CTAB method (3rd part)
- 2:15 pm Experimental: sample loading
- 3:00 pm Coffee break
- 3:20 pm **Theory:** Sample preparation and DNA extraction - **N. Foti - JRC**
- 4:20 pm Experimental: interpretation of the gels

2ND DAY – TUESDAY, JUNE 7TH 2005

QUALITATIVE PCR

- 8.00 am **Theory:** Introduction to the Polymerase Chain Reaction and to the use of PCR for the detection of transgenic maize and soybean – **M. Querci - JRC**
- 8.30 am Experimental: PCR for maize (Bt-176) and soybean (Roundup Ready®)
- Plant specific: detection of the **zein** and **lectin** genes
- 9.15 am Coffee break
- 9.40 am Preparation of agarose gels
- 10.00 am **Theory:** Characteristics of Roundup Ready® soybean and Bt-176 maize and introduction to GMO specific nested PCR – **M. Querci - JRC**
- 11.30 pm Lunch
- 1.00 pm Sample loading (*zein* and *lectin* specific PCR)
- 1.30 pm **Theory:** General consideration on PCR lab set up. Lab implementation, Etc...- **M. Querci - JRC**
- 2.15 pm Interpretation of the gels (*zein* and *lectin* specific PCR)
- 3.00 pm Coffee break
- 3.15 pm Experimental: screening PCR: detection of the 35S promoter.
- 4.00 pm **Seminar:** Introduction to European legislation on GMOs – **M. Querci - JRC**

3RD DAY – WEDNESDAY, JUNE 8TH 2005

QUALITATIVE PCR

- 8.00 am Experimental: nested PCR for the specific detection of Bt-176 maize and Roundup Ready® soybean (1st PCR reaction)
- 9.00 am Preparation of agarose gels
- 9.30 am Coffee break
- 10.00 am **Seminar**: The JRC and its role in the European Community – Presentation of the different B&GMOs Unit activities – **M. Querci - JRC**
- 11.00 pm Sample loading (35S specific PCR)
- 12.00 pm Lunch
- 1.30 pm Experimental: nested PCR for the specific detection of Bt-176 maize and Roundup Ready® soybean (2nd PCR reaction)
- 2.00 pm Interpretation of the gels (35S specific PCR)
- 3:00 pm Experimental: preparation of agarose gels
- 3.30 pm Coffee break
- 3.45 pm Experimental: sample loading
- 4.00 pm **Theory**: Introduction to Real-Time PCR for GMO detection and quantification.
M. Mazzara - JRC
- 5.30 pm Experimental: interpretation of the gels (Bt-176 maize and Roundup Ready® soybean specific nested PCR)

4TH DAY – THURSDAY, JUNE 9, 2005

QUANTITATIVE REAL-TIME PCR

- 8.00 am **Experimental:** Preparation of samples for the Real-Time PCR for the specific detection of Roundup Ready® soybean using the LightCycler (Roche) and samples loading. **A Tello – SGL.**
- 9:15 am Coffee break
- 9:45 am ***Seminar:*** Real-Time PCR for GMO quantification using the LightCycler instrument . **M. Kiehne - Biotecon**
- 11.00 am **Experimental:** Interpretation of data obtained using the LightCycler
- 12.00 pm Lunch
- 1.00 pm ***Seminar:*** Method Validation and role of the Community Reference Laboratory - **M. Mazzara - JRC**
- 2:00 pm Experimental design, data analysis and interpretation – **N. Foti – JRC**
- 3:15 pm Coffee break
- 3:30 pm ***Seminar:*** Novel methodological approaches for GMO analysis – **M. Ermolli - JRC**

5TH DAY – FRIDAY, JUNE 10TH 2005

THE SEROLOGICAL APPROACH FOR GMO ANALYSIS

- 8.00 am **Theory:** Serological approach for the detection of GMOs. **M. Ermolli – JRC**
- 9.20 am Experimental: The use of lateral flow strips
- 10.00 am Coffee break
- 10.30 am **Seminar:** Sampling: basic principles – **M. Querci - JRC**
- 11.30 am **Seminar:** The Cyprus experience towards the establishment of a GMO testing facility – **M. Eleftheriadou – SGL - and I. Ioannides - ARI**
- 12.30 pm Lunch
- 1.30 pm **Round table:** Troubleshooting, data interpretation and practical experimental issues. Questions and answers session.
- 2.30 pm General discussion and conclusion of the course

Samples used during the course

During the course we will use different methods to detect the presence of Bt-176 maize and Roundup Ready® soybean in different materials. For this purpose, we will analyse mixtures of non-GM and GM maize (Bt-176) and soybean (Roundup Ready® soybean) respectively, at different concentrations. Two types of materials will be used:

- Certified Reference Materials (see User Manual Session 3)
- Different raw and processed materials distributed to the participants

Composition of raw and processed materials distributed to the participants and not included in the User Manual.

Mixed Flour 0% RR, Bt-176

The material was prepared at the State General Laboratory by mixing soya and maize flour from GMO proficiency testing Gemma Scheme, Rounds: S29B, test material 056 (Soya) and M1-A, test material 043 (maize).

Mixed Flour 2% RR, 2% Bt-176

The material was prepared at the State General Laboratory by mixing soya and maize flour from GMO proficiency testing Gemma Scheme, Rounds: S29A, test material 3 (Soya) and M1-B, test material 64 (maize).

Biscuit

This sample was prepared at the State General Laboratory by mixing together the following ingredients:

Biscuit	150g wheat flour, 100g sugar, 50g butter, 5g salt, 8g vanilla baking powder, 1 egg, 1g GM free maize flour, 1g CRM Bt-176 (2%) maize flour.
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This mix was then baked and blended in a thermomix mixer to ensure a homogeneous crumb was obtained.

Baby Quaker®

This sample was purchased from a supermarket in Aruba. Baby Quaker® is a pre-cooked cereal-based infant food produced by Quaker® S.A, Colombia and distributed by Supermercados La Favorita C.A., Ecuador. The ingredients as from label are: oats, rice flour, wheat flour, sugar, soybean flour, lecithin, Vitamin A, Vitamin E, Amylase Vitamin B12, Vitamin B6, Vitamin B2, Biotin.

The sample was analysed at the JRC Biotechnology and GMOs Unit and resulted positive for the presence of Roundup Ready soybean.

List of samples distributed during the course

Sample	% GMO (specific ingredient)		
	RR soybean	Bt-176 maize	
Mixed Flour (Bt 176, RR) ¹	0%	0%	See footnote and page 11
Soya Flour (RR) ²	~2%	-	See footnote
Maize Flour (Bt 176) ³	-	~2%	See footnote
Mixed Flour (Bt 176, RR)	2%	2%	See page 11
Baby Quaker (RRS)	~ 8 %	-	See page 11
Biscuit (Bt 176)	-	~1%	See page 11

¹ Mixture of GeMMA proficiency Schemes Rounds S29B and M1-A (Report S29, June-August 2004)

² GeMMA proficiency Scheme-GMO analysis Round S29A, test material 3 (Report S29, June-August 2004).

³ GeMMA proficiency Scheme-GMO analysis Round M1-B, test material 64 (Report M1, April-June 2004).

Expected results by PCR

Sample	zein	lectin	35S	nos	CryIA(b)	CTP/EPSPS
Mixed Flour ¹	+	+	-	-	-	-
Soya Flour (RR) ²	-	+	+	+	-	+
Maize Flour (Bt 176) ³	+	-	+	+	+	-
Mixed Flour (Bt 176, RR)	+	+	+	+	+	+
Baby Quaker (RRS)	-	+	+	+	-	+
Biscuits (Bt 176)	+	-	+	-	+	-