

Development of Real-time PCR kit for detection and quantification of genetically modified soybean in food and feeds

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Introduction

- Necessity of control of genetically modified plants spreading in the food market requires development of analytical methods for detection of genetically modified components in food
- In the food-processing industry soybean raw material, including genetically modified soybean, is widely used. Majority of created genetically modified soybean lines have 35S promoter of cauliflower mosaic virus, which may be used as a convenient target for screening and quantification
- We have developed Real-time PCR kit with TaqMan probes for detection and quantification of genetically modified soybean in food and feeds for Rotor Gene instrument (Corbett Research, Australia)

Materials and methods

- Our technique of DNA isolation, based on Guanidine chloride with proteinase treatment followed by silica-based sorbtion of DNA, allows to receive about 2,5 microgram of soybean DNA (measured by fluorimetry) from 20 mg of soybean flour
- Quantitation of relative amount of genetically modified soybean component was performed by regression analysis between Ct of 35S promoter and Ct of soybean genome target value difference and common logarithm of genetically modified soybean amount (in percent) in calibrators
- As the calibrators we use DNA samples derived from the certified reference material (CRM of Institute of Reference Materials and Measurements [IRMM], Belgium) containing 0,1%, 1% and 5% of Roundup Ready soybean flour in unmodified soybean flour

Analytical characteristics of kit were determined by testing:

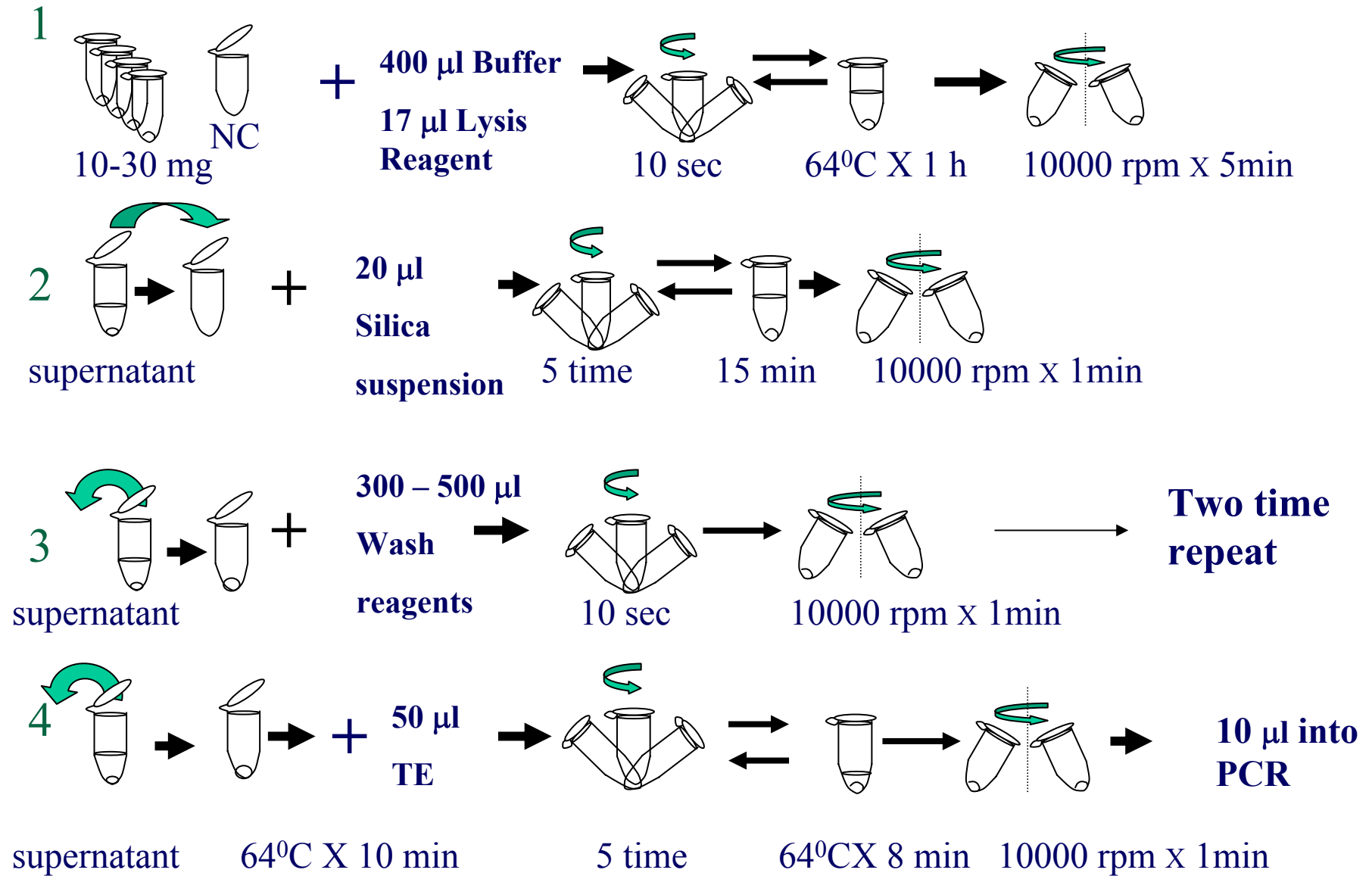
- the standards of certified reference material (CRM, IRMM, Belgium) containing 0,1%, 0,5%, 1%, 2%, 5% of Roundup Ready soybean flour in unmodified soybean flour
- 100% Roundup Ready soybean flour and unmodified soybean flour
- food and raw material samples with genetically modified soybean
- food and raw material samples with unmodified soybean

All food samples were previously tested with recommended by Lipp M. (1999) method using CTAB DNA isolation

Results

- The limit of detection measured by exhaustive dilution limit was 5 copies of 35S promoter as well as soybean genomic target in PCR reaction
- Food samples testing did not give false-negative or false-positive results compared with recommended by Lipp M. assay
- Dynamic range was determined from 0,05% to 50% of Roundup Ready soybean flour in unmodified soybean flour
- Evaluated limit of quantification was 0,1% of Roundup Ready soybean flour in unmodified soybean flour
- Relative amount of GM-soybean flour in unmodified soybean flour content was measured with coefficient of variation (after regression analysis) of 12% (for IRMM CRM 0,1% GM soya) and 17% (for IRMM CRM 1% and 5% GM soya)

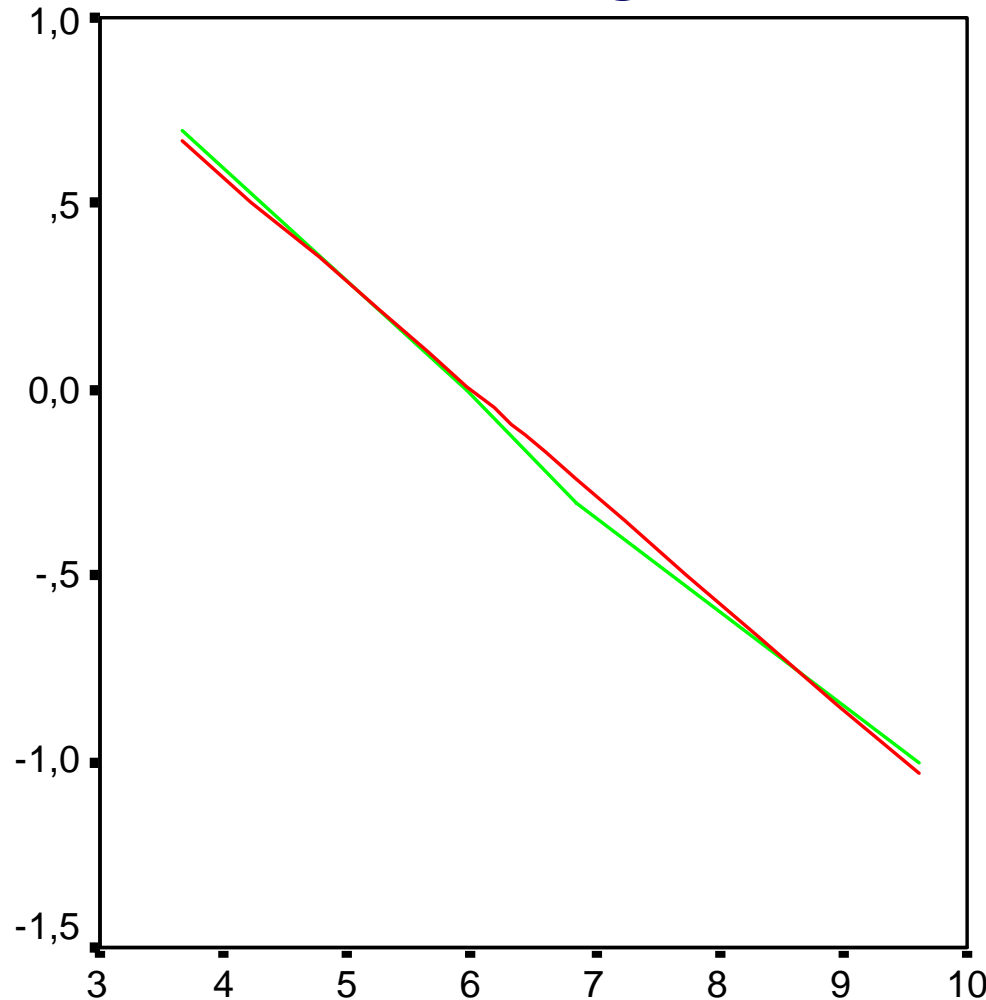
DNA isolation method



Functional dependence of Log % GMO and ΔCt

$\Delta Ct = Ct$ 35S target ampl. - Ct soybean genomic target ampl.

Log (%GMO) **Log % GMO = a - b (ΔCt) = 1,8 - 0,3 (ΔCt)**



| CRM % GMO | Log (%GMO) | ΔCt Mean |
|--------------|------------|---------------------|
| 0,1 | -1,0 | 9,61 |
| 1 | 0,0 | 5,97 |
| 5 | 0,7 | 3,67 |

— Observed
— Linear

ΔCt

$R^2 = 0,996$

Determination the Coefficient of variation

Treshold 35S (FAM) - 0,04 / Soybean target (JOE) - 0,02

| IRMM reference Standards (CRM) | GM soya % mean relative Concentration measured by regression analysis | CV % | N | 35S target copy per PCR reaction | Soya genomic target copy per PCR reaction |
|--------------------------------|---|-------|----|----------------------------------|---|
| 0,1 % | 0,1 | 11,95 | 8 | 20 | $1,4 \cdot 10^4$ |
| 0,5 % | 0,31 | 17,89 | 7 | 36 | $1,4 \cdot 10^4$ |
| 1,0 % | 1,0 | 16,7 | 13 | 140 | $1,3 \cdot 10^4$ |
| 2,0 % | 1,9 | 3,36 | 2 | 260 | $1,4 \cdot 10^4$ |
| 5,0 % | 4,8 | 16,86 | 13 | 640 | $1,4 \cdot 10^4$ |

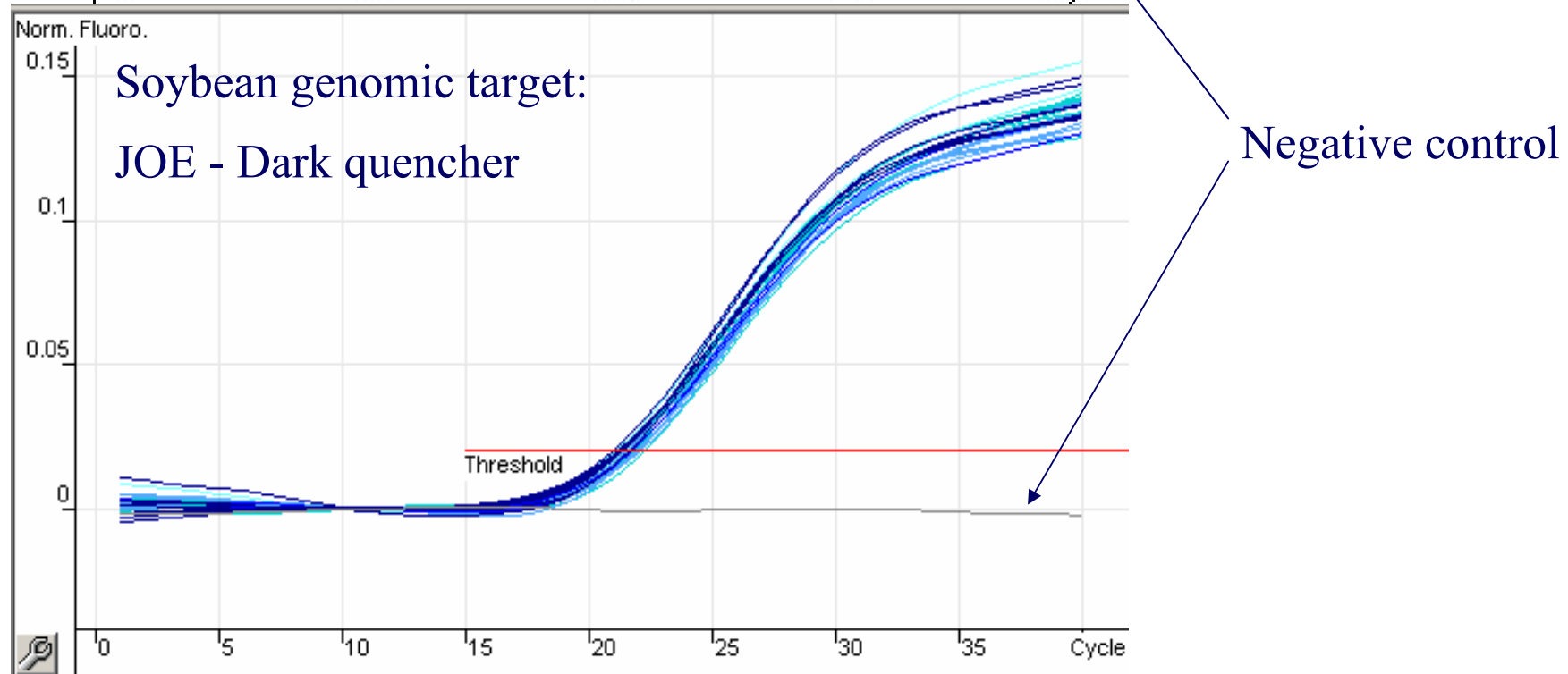
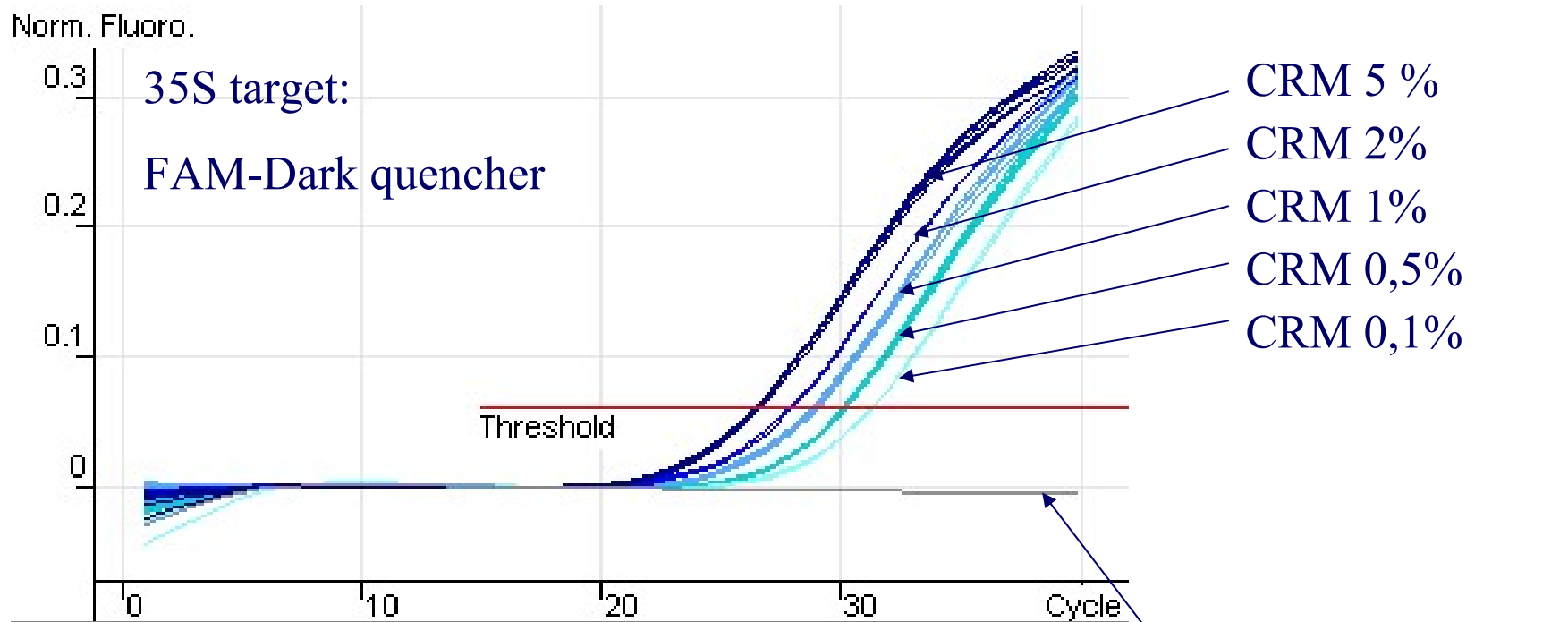


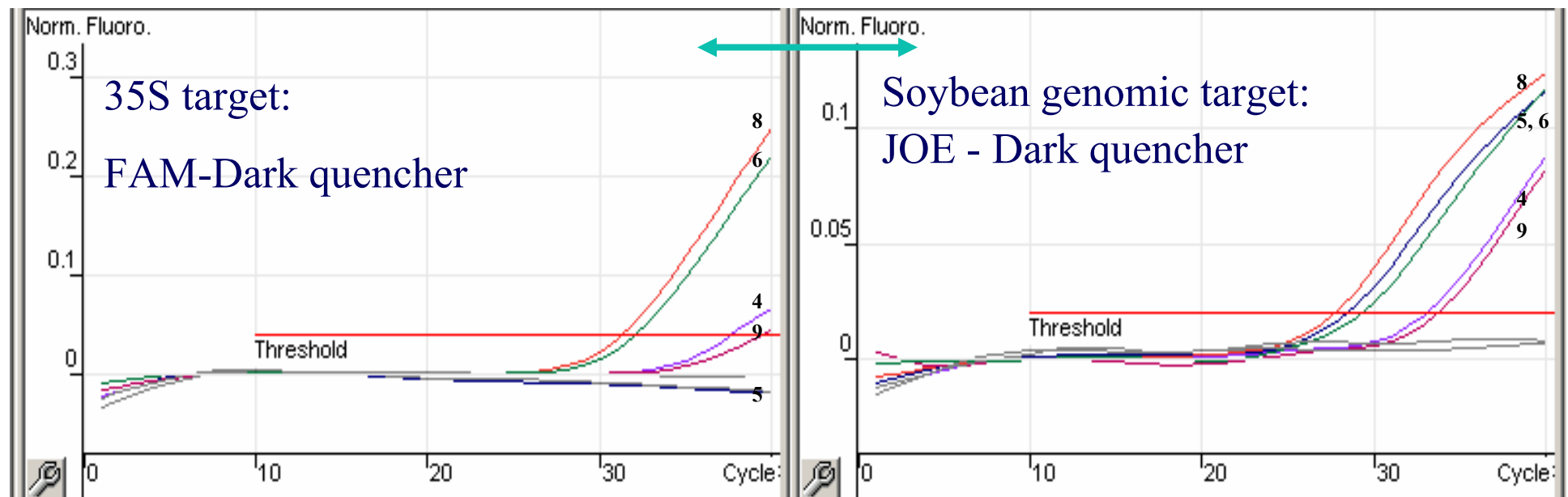
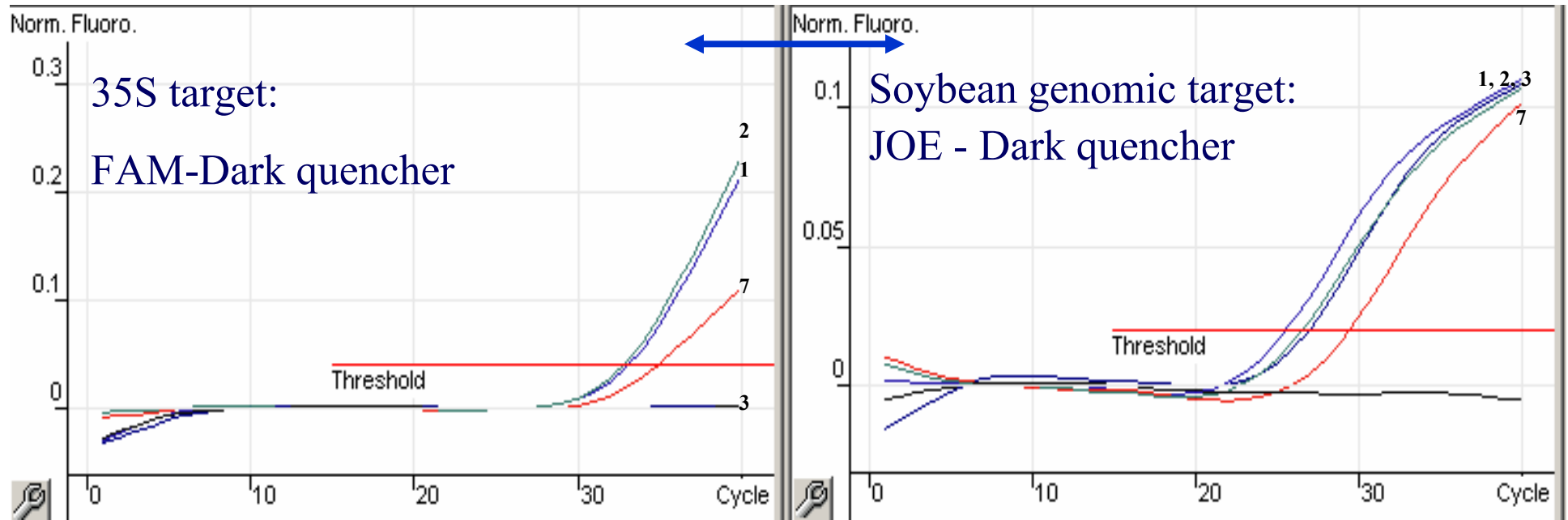
Table 15: Quantitation of Roundup Ready™-DNA with 35S screening real-time PCR, DNA extracted with DNeasy Plant Mini, determination in triplicate using 20 mg/extraction.

| CRM | Certified GMO Concentration [%] | Number of measurements [n] | Roundup Ready™ Concentration ¹ | |
|-------------|--|----------------------------------|--|---------|
| | | | [%] | std dev |
| IRMM-410S-0 | < 0.03 | 5 | (0.02) ² | (0.01) |
| IRMM-410S-1 | 0.10 ± 0.05 | 5 | 0.05 | 0.01 |
| IRMM-410S-2 | 0.50 ± 0.10 | 5 | 0.24 | 0.07 |
| IRMM-410S-3 | 1.00 ± 0.2 | 5 | 0.75 | 0.20 |
| IRMM-410S-4 | 2.0 ± 0.3 | 5 | 1.73 | 0.15 |
| IRMM-410S-5 | 5.0 ± 0.6 | 5 | 4.93 | 0.24 |

IRMM information reference materials

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel (BE), S. Trapmann et al,
Institute for Health and Consumer Protection (IHCP), Ispra (IT), G. Van den Eede et al.

Results of some food samples testing



Results of some food samples testing

| № | Food samples | GM soya % relative concentration measured by regression analysis | Ct of 35S promoter amplification | Ct of soybean genomic DNA target amplification |
|---|--------------|--|----------------------------------|--|
| 1 | Soya protein | 0,2 | 33,14 | 25,60 |
| 2 | Soya flour | 0,4 | 32,86 | 26,59 |
| 3 | Soya meat | 0 | 0 | 26,90 |
| 4 | Minced meat | 5,2 | 37,19 | 33,25 |
| 5 | Sausage | 0 | 0 | 28,57 |
| 6 | Ravioli | 18,2 | 31,80 | 29,30 |
| 7 | Sausage | 0,9 | 34,90 | 29,50 |
| 8 | Sausage | 10,6 | 31,01 | 27,90 |
| 9 | Ham | 1,7 | 39,0 | 33,80 |

Conclusions

- Kit includes our own set of reagents for DNA isolation, primers and TaqMan probes for 35S promoter and for genomic soybean DNA detection in one PCR tube. This approach allows to calculate the relative amount of genetically modified soybean DNA from all soybean DNA amount in sample
- Our technique of DNA isolation is more simple and rapid in comparison with traditionally used for plant CTAB method and may be used for any food, feeds and raw material
- It has been proved that our kit may be used on a wide spectrum of the foodstuff and feeds containing soybean material
- It is possible to detect the presence of genetically modified soybean in food even if its amount is 0,1 % from unmodified analogue