

**Final report of workpackage 4:
“Development and testing of transformation-event specific primer-probe sets”**

Project title:

**“Reliable, standardised, specific, quantitative detection of
genetically modified food”**

Acronym: Qpcrgmofood



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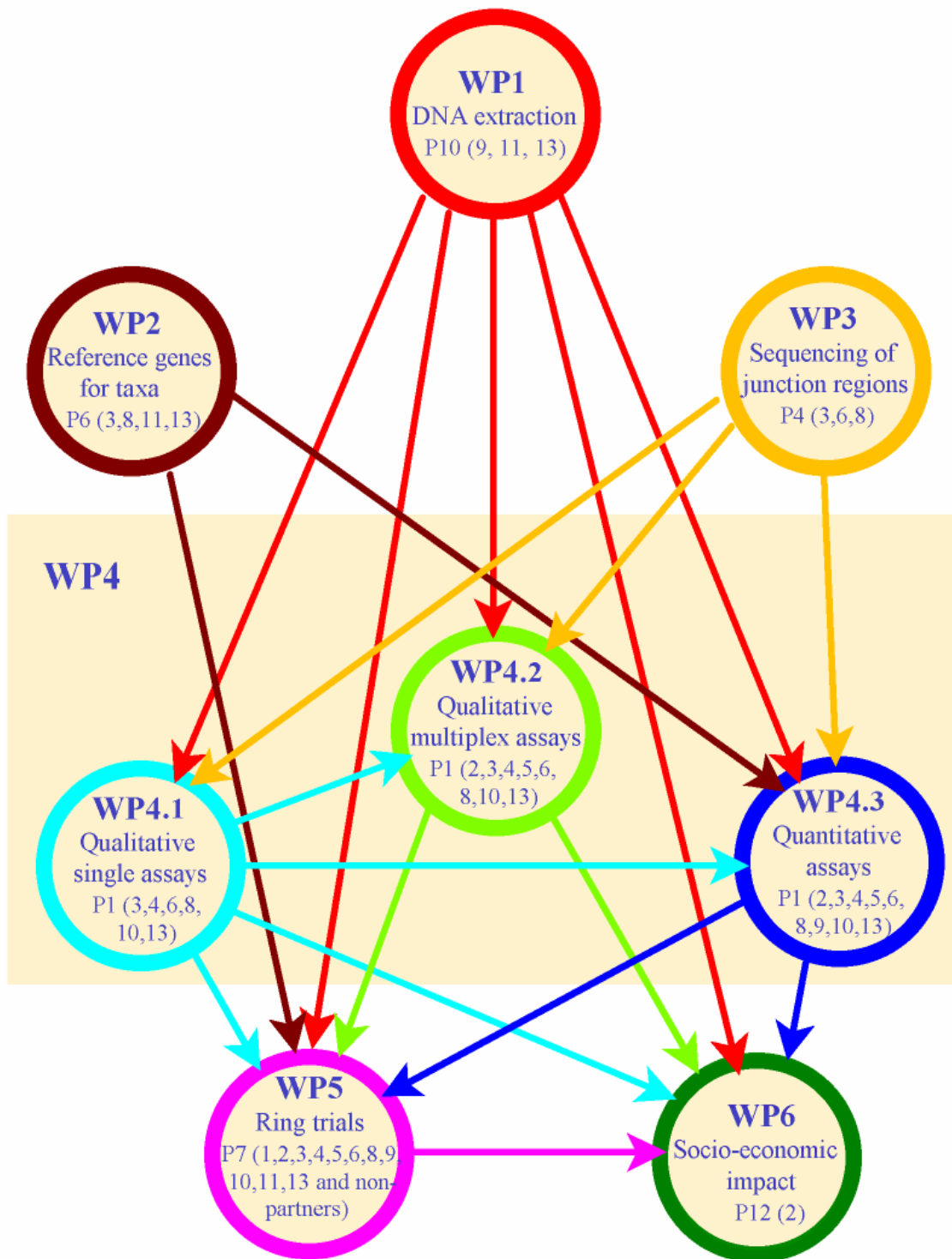
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DELIVERABLES NUMBER 4, 5 AND 6

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Outline of project structure, showing links between workpackages and partners

Summary:

Workpackage 4 of the project was responsible for development and testing of transformation-event specific primer-probe sets. The workpackage was subdivided into three topical items:

- WP4.1 Qualitative detection of GMO in single primer-probe assays
- WP4.2 Qualitative detection of GMO in multiplex primer-probe assays
- WP4.3 Quantitative detection assays for GMO

This structure was useful, although it did not reflect the exact and intricate nature of the research performed and results obtained. There was overlap between the subworkpackages, and sometimes it was also difficult to distinguish if work primarily belonged to WP4, WP2 “Identification and characterisation of suitable species-specific reference genes, and development of reference-gene specific primer-probe sets for qualitative and quantitative PCR amplification and detection”, or WP3 “Sequence characterisation of transformation events”. Furthermore, research focusing on detection and quantification limits, reviews of methods and challenges for method development, etc. only partially fit under these specific items, although clearly identified as relevant to the project (from the title: “Reliable, standardised, specific, quantitative...”).

The workpackage received its data primarily from WP2 and WP3, and the work was therefore delayed by difficulties faced in particular by WP3 due to unavailability of GM materials. A total of 11 out of 13 studied GMOs were sufficiently characterised in WP3, close to the 12 foreseen in the project proposal. All of these 11: GTS 40-3-2 (RoundupReady®) soybean, Bt11, Bt176 (Event 176), Mon810, T25, DBT418 (BTXtra), GA21 (RoundupReady) and CBH 351 (StarLink) maize (corn), and GT73 (RoundupReady), Ms8 and Rf3 rapeseed (canola) were included also in WP4, but for some the data were produced on the basis of contractual materials restricting dissemination of information to the project partners alone (including the European Commission). For each GMO from one to more than five methods were developed, exploiting mainly TaqMan® chemistry for real-time PCR, but also Scorpion™ and LightCycler™ primers/probes, and Amplifluor™ and SYBR® Green I formats.

The preferred mode of dissemination of results from the project was peer reviewed scientific papers and scientific conference contributions. At the time this report is written a total of 13 papers derived from WP4 had been published or accepted for publication in international peer-review journals or books, and 10 posters or oral presentations given at scientific conferences. Several additional papers are submitted, and yet more are expected within the next year or two.

Methods developed were optimised and successively forwarded to WP5 “Validation of techniques in ring-trials” for validation prior to eventual submission to CEN/ISO for standardisation. Priority was given to modularity, in the sense that a GMO specific PCR real-time PCR method should be combinable with any available validated reference gene specific method (from WP3 validated in WP5). However, to avoid potential conflict with previously accepted validation approaches, all methods were prevalidation and validation together with a specific reference gene method. A total of 10 methods for five GM maize (Mon810, Bt11, Bt176, T25 and GA21) and one GM soybean (GTS 40-3-2) were subjected to prevalidation, and some are presently under validation or will be subjected to collaborative trial validation soon. Multiplex methods were not subjected to prevalidation because no clear guidelines for validation of multiplex methods exist or were elaborated.

Overview of partners, workplan and deliverables of WP4

The following partners participated in the work performed in WP4:

- P1 National Veterinary Institute, Oslo, Norway (*project coordinator and WP task leader*)
- P2 Norwegian Food Research Institute, Aas, Norway
- P3 Institut National de la Recherche Agronomique, Versailles, France
- P4 Department of Plant Breeding, Center of Agricultural Research, Melle, Belgium
- P5 LGC Ltd., Teddington, U.K.
- P6 GeneScan GmbH, Freiburg, Germany
- P8 Tepral, Strasbourg, France
- P13 Consejo Superior de Investigaciones Cientificas, Barcelona, Spain

The workplan of WP4 in the project proposal and contract listed:

This workpackage consist of three sub-workpackages. They all have in common the development of specific PCR primers and detection probes. Because primers and probes may perform differently in different systems and assays, several alternative primers and probes targeted at detecting each GMO must be developed (e.g. Scorpion-probes, probes for PCR-ELISA, probes for real time PCR-systems: Taqman, LightCycler and ICycler). The partners will be equipped and work with different PCR and detection systems, and this should allow for development of well performing detection assays for all three sub-workpackages and a wide range of PCR and detection systems.

- *WP4.1. Qualitative detection of GMO in single primer-probe assays*
Transformation event specific primer-probe sets will be developed utilising DNA sequence data from WP3. The performance of the primer-probe sets must be evaluated in single assays, to identify optimal reaction conditions. The optimal reaction conditions for the primer-probe sets should allow for standardisation of reaction conditions across all primer-probe sets. If necessary some primer-probe sets will be re-designed to adjust to standard reaction conditions.
- *WP4.2. Qualitative detection of GMO in multiplex primer-probe assays*
Once primer-probe sets designed under WP4.1 perform well under standard reaction conditions, they will be combined in multiplex qualitative assays (dot-blotting, Taqman/Lightcycler) to test their performance against an array of DNA templates containing none, single and multiplex GMO reference materials.
- *WP4.3. Quantitative detection assays for GMO*
Once primer-probe sets designed under WP4.1 perform well, preferably but not necessarily under standard conditions, they will be combined with the relevant species specific reference gene primer-probe set developed under WP2 in order to optimise reaction conditions for quantitative PCR assays. Depending on the performance of the primer-probe sets, alternative primer-probe sets may have to be developed for the quantitative assays. Evaluation of performance will include testing on ultrapure DNA from reference material of the GMOs for which the tests are designed.

Overview of deliverables of WP4 listed in project proposal and contract:

<i>Number</i>	<i>Title of deliverable</i>	<i>Nature of deliverable</i>
4.	Development of transformation-event-specific qualitative detection assays for at least 12 GMOs.	Report (public)
5.	Development of multiplex transformation-event-specific qualitative detection assays including as many as possible of at least 12 GMOs	Report (public)
6.	Development of transformation-event-specific quantitative detection assays for at least 12 GMOs	Report (public)

WP4.1Objectives

Develop optimal and transformation-event-specific primer-probe sets for qualitative detection of at least 12 GMOs defined by their specific transformation-events.

Methodology and study material

- Primers and probes for qualitative assays will be designed on the basis of sequence data obtained under workpackages 2 and 3.
- The primers and probes will be optimised so as to have as similar melting temperatures and similar sized PCR products as possible.

WP4.2Objectives

Develop optimal and transformation-event-specific primer-probe sets for multiplex qualitative assays for detection of at least 12 GMOs defined by their specific transformation-events.

These assays will significantly reduce costs of quantitative analyses by reducing the number of quantitative analyses necessary to be performed.

Methodology and study material

- Primers and probes for qualitative assays will be designed on the basis of sequence data obtained under workpackages 2, 3 and 4.1.
- The primers and probes will be used to develop a solid phase based multiplex qualitative detection system. First primers will be optimised so as to have similar melting temperatures and giving similar sized PCR products. The primers will carry a fluorescent label or other suitable label for easy detection. The mixed PCR population will then be hybridised to solid- phase bound probe arrays. This facilitates easy and specific detection. Amplification efficiency of the different primer sets will be investigated to select for the best sets and assure that DNA from all GMOs in a mixture are amplified. This method is simple and will be «biochip ready», in that it easily could be incorporated into future developing microtechnology.

WP4.3Objectives

- Develop optimal and transformation-event-specific primer-probe sets for quantitative detection of at least 12 GMOs defined by their specific transformation-events.
- Develop reliable quantitative assays for detection of GMOs.
- Compare and evaluate fidelity/performance of three different quantitative assays.

Methodology and study material

- Primers and probes for quantitative assays will be designed on the basis of sequence data obtained under workpackages 2, 3, 4.1 and 4.2.
- Primers and probes will be used to establish three types of quantitative systems which will be evaluated by comparison, i.e. TaqMan, Lightcycler and competitive PCR. For the latter competitor plasmids will be developed.

Overview of methods developed per species, GMO and type of method:

Species	Event	Type of method	Reference¹
Soybean	GTS 40-3-2	Event-specific quantitation using real-time PCR (Multiplex) event-specific quantitation using LabChip™ technology	1, 2, 3 9
Maize (corn)	Bt11	Event-specific quantitation using real-time PCR ...in duplex with <i>Adh1</i> reference gene Event-specific detection by PCR (Multiplex) event-specific quantitation using DNA arrays (Multiplex) event-specific detection using SYBR® Green I (Multiplex) event-specific detection by PCR	6 <i>P3</i> <i>P6</i> 10 12 <i>P3</i> and <i>P13</i>
	Mon810	Event-specific quantitation using real-time PCR ...in duplex with <i>Adh1</i> reference gene Event-specific detection by PCR (Multiplex) event-specific quantitation using DNA arrays (Multiplex) event-specific detection using SYBR® Green I (Multiplex) event-specific detection by PCR	4, 8, <i>P4</i> and <i>P6</i> <i>P3</i> <i>P6</i> 10 12 <i>P3</i> and <i>P13</i>
	Bt176	Event-specific quantitation using real-time PCR ...in duplex with <i>Adh1</i> reference gene Event-specific detection by PCR (Multiplex) event-specific quantitation using DNA arrays (Multiplex) event-specific detection by PCR	<i>P8</i> <i>P3</i> <i>P6</i> 10 <i>P3</i>
	T25	Event-specific quantitation using real-time PCR ...in duplex with <i>Adh1</i> reference gene Event-specific detection by PCR (Multiplex) event-specific quantitation using DNA arrays (Multiplex) event-specific detection using SYBR® Green I (Multiplex) event-specific detection by PCR	<i>P3</i> <i>P3</i> <i>P6</i> 10 12 <i>P3</i> and <i>P13</i>
	DBT418	Event-specific detection by PCR (Multiplex) event-specific quantitation using DNA arrays	<i>P6</i> 10
	GA21	(Multiplex) event-specific quantitation using DNA arrays Event-specific quantitation using real-time PCR Event-specific detection by PCR (Multiplex) event-specific detection using SYBR® Green I	10 11, <i>P6</i> <i>P6</i> 12

	CBH 351	Event-specific detection using real-time PCR	5
		Event-specific detection by PCR	<i>P6</i>
		(Multiplex) event-specific quantitation using DNA arrays	10
Rapeseed (canola)	GT73	Event-specific quantitation using real-time PCR	<i>P4</i>
	Ms8	Event-specific quantitation using real-time PCR	<i>P4</i>
	Rf3	Event-specific quantitation using real-time PCR	<i>P4</i>

¹ Please refer to the original publications for details regarding the methods, since copyrights and other means of protection do not permit details to be published here. A number preceded by a P (both in italics) refer to a partner who has developed but not (yet) published the method. This may be due to confidentiality, need for further improvements of method or because a manuscript is not yet accepted for publication.

Overview of validation status for methods from WP4:

- Three methods for Mon810 maize have been prevalidated, and all performed well. One is presently being validated under the shared responsibility of the JRC and BfR in Germany.
- One method for Bt11 maize has been prevalidated and performed well. This method has also been prevalidated internally in Syngenta in comparison with a method developed by Syngenta, and the method provided by P1 performed better and will be validated in November.
- One method for Bt176 maize has been subject to prevalidation but may need some minor adjustments before being subjected to a large scale collaborative trial validation.
- One method for T25 maize has been subject to prevalidation. A systematic error in the calculated DNA concentrations was detected. This required some adjustments in the data processing. Both the simplex and duplex (with the *Adh1* reference gene) quantitative methods are presently prevalidated in collaboration with the notifier Bayer Crop Sciences, for compliance with the new EU regulations. On the basis of this, one method will be validated in a full collaborative trial in the near future.
- One method for GA21 maize has been subject to prevalidation and performed very well. Unfortunately this method had to compete with an event-specific method developed by Monsanto and submitted to the JRC under the requirements of Directive EC 2001/18, and is presently put on hold.
- Three methods for RoundupReady soybean have been subject to prevalidation. One for the LightCycler and two for the ABI. Unfortunately, the results indicate a reagents problem. The method validation is therefore presently put on hold.
- Validation of multiplex methods (duplex quantitative, multiplex qualitative and multiplex quantitative methods) will require very different schemes, and discussions have been initiated. Proposals include: 1) The use of a two-step procedure where the first step includes multiple and randomised combinations of concentrations, and the second step includes multiple replicates of selected combinations of concentrations. 2) Definition of performance ranges within which competitive effects may be ignorable. 3) Experimental design. This work is likely to continue in future research projects as well as under the interests and responsibilities of the European Network of GMO Laboratories (ENGL) and the JRC (as the Commission reference laboratory).

Other contributions within the responsibilities of WP4:

In the first months while awaiting input from WP3 a considerable part of the work in WP4 dealt with comparison of real-time PCR thermal cyclers to assess whether there were fundamental differences relevant to the objectives of the project. Although some potentially serious differences were indeed observed, it was not possible to identify reliably the source of these differences. We primarily compared the ABI Prism 7700 SDS and the Roche LightCycler. The observed repeatability was good (RSDr approx. 25%), but for the same DNA extracts from processed foods up to a 10-fold difference in the estimated quantity of GTS 40-3-2 was observed from one cycler to the other. Similar differences were observed in comparisons between commercial kits and self-designed assays, again with good repeatability. This led us into discussions about the needs for sample specific determination of the limits of detection and quantitation, for detailed knowledge about reagents, e.g. primer and probe specificity, amplicon sizes, reaction volumes, etc. This discussion still has relevance in relation to test reports and proficiency test results, e.g. the GeMMA scheme (organised by the CSL, UK).

Defining the limits of detection and quantitation was the basis for several discussions throughout the project duration. Much research and several publications include results from this work (3, 6 and 7, + several submitted or in preparation). This discussion was also brought to a working group of the European Committee for Normalisation (CEN), and to the ENGL. At present we believe that this has contributed considerably to the present state-of-the-art among experts throughout Europe and the rest of the world. It is linked with the regulatory framework, in particular with thresholds for labelling and acceptable contamination, and will be highly relevant for purchasers of analytical services.

Reviews of available methods, technologies, limits and applications of methods, challenges for the future and recent achievements were part of several publications (1, 7, 13, + several submitted or in preparation). Particularly important achievements include the event-specific real-time PCR methods developed within the present project (1, 2, 3, 4, 5, 6, 8, 11, + several in preparation) because these allow for identification of each transformation event, and for consistent quantitation on the basis of haploid genome equivalents of the GM target sequence relative to the species, a relationship that can be directly converted into a relative value in percent (see 7, 13, + several in preparation). Multiplex methods (10, + several submitted or in preparation) represent another important achievement, allowing for rapid screening and identification of the GMOs from which derived DNA is detected and should be quantified according to current EU legislation. Method limitations include in particular problems with distinction between stacked events (hybrid cross between two single events yielding an offspring containing the signature sequences of both parental events) and their corresponding parental events. If stacked events are considered as single events, then the contributed quantity to total GM quantity should be calculated on the basis of only one of the target sequences, not as the sum of the target sequences of the two parental events. At present EU legislation treats stacked events as new events relative to their parental events. However, no technology is presently available that allow for distinction between stacked events and their corresponding parents, and therefore the GM quantity will be overestimated in the eventual presence of DNA from stacked events. Hitherto, challenges of particular importance include access to relevant reference material for method development, validation and calibration in routine laboratories. Hopefully, future legislation will improve the situation within Europe. Considerable effort was allocated to working with plasmids instead of genomic DNA as an alternative source of reference materials for calibration and quantitation (1 and several in preparation). The results are promising, although there are still some concerns that for some situations there may be differences in the amplifiability of the target sequence with potential effect on quantitation.

Comparison of methods and alternative technologies was a priority from the earliest phases, as reflected in comparison of different technologies for quantitation of GTS 40-3-2 soybean (1, 2, 3, 9) and GA21 maize (12), and in development of alternative event-specific PCR methods for quantitation of GTS 40-3-2 soybean (1, 2, 3, published back-to-back), and Mon810 maize (4, 8 and an unpublished assay by P6). For some of these assays parallel prevalidation in WP5 contributed also to feed back information relevant to method optimisation. Observed differences relevant to choice of fluorescence chemistry for real-time PCR include price, dynamic range (number of logs of observable increase in fluorescence/amplification), restrictions on probe design, flexibility of detection systems on thermal cyclers (wave lengths that can be detected) and possibilities for combining different fluorophores in multiplex detection assays (e.g. to include PCR inhibition controls). Choice of thermal cycler is influence also by some of the above, as well as by the flexibility of the system including number of reactions that can be performed in parallel, reaction volumes, run-time and access to validated methods. Within the present project major focus was on TaqMan chemistry, the *de facto* standard in quantitative real-time PCR, because this allows

for a high degree of flexibility, the dynamic range of the chemistry is very good, there are well characterised criteria for probe design, different fluorophores can be combined, the methods can be applied on practically all commercial real-time thermal cyclers, and the access to validated methods and comparable thermal cyclers in various laboratories is good. It is also clear that ABI Prism 7700 and 7900 SDS are presently the most widely distributed real-time thermal cyclers. However, this should not be interpreted as concluding that other alternatives are inferior to TaqMan chemistry and ABI cyclers. As a matter of fact, price may suggest that other alternatives should be explored. Moreover, as new technologies emerge, these may allow for the use of new fluorophores and measurements at other wavelengths. In particular in relation to multiplexing, this may lead to new preferences regarding chemistries and choice of thermal cyclers.

The number of simultaneous wavelengths detectable by a quantitative real-time PCR thermocycler constitutes an upper technical limit of the number of simultaneous detections. There has apparently been some progress in technological development in this context from the ABI 7700 apparatus to the recent iCycler thermal cycler. It is thus in the interest of scientists in several fields concerned with PCR based detection (GMOs, pathogens, allergens producing plants...) to prepare and support such a technological jump.

PCR technology is affected by numerous factors generally interacting. The workload and costs associated with determination of specific needs for particular reagents and optimal concentrations are often limiting. An alternative that has proven very useful to decrease the number of interactions to be examined and in parallel to increase the robustness of the results is to determine the specific combinations to be tested using a statistical approach such as experimental design plans. Partner 3 has focused particularly on these aspects and worked closely with a statistician for this purpose. This experience showed that that optimal conditions of PCR are to be defined according the purpose of the detection method, i.e. detection either at low levels (e.g. for adventitious presence) or high levels of target DNA. This means that the optimised conditions, and thus probably the value of the performance criteria, are not the same for the entire dynamic range of a PCR test (e.g. 0-100% of GMO). As yet no systematic approach for the optimisation of multiplex-PCR has been published. Facing the number of numerous combinations (GM plants, reference genes, screening / identification, controls, IPC...) which might be of interest, development of a model was identified as one of the major target achievements of the project: the simultaneous detection of a GM and a corresponding reference gene system.

Different constraints were taken into account for the determination of the primers to be used for a multiplex PCR: 1) all primers pairs should have a common annealing temperature, 2) the interference between primer pairs should be minimized, e.g. by theoretical evaluation of all primers in pairwise combinations using software like Primer express and Oligo 6, 3) amplified products must have distinct sizes, in order to be separable on electrophoresis gel or capillary electrophoresis, 4) it should be possible to design specific TaqMan probes for the amplicons, and 5) the size of the amplicon should not restrict its fitness for purpose in analyses of processed foods and therefore the length of amplicon should be no more than 200 bp. Even though the approach made possible considerable progress, it was still not possible to include all the desired targets in a single "conventional" multiplex assay.

Because of the steadily growing number of GMOs to be detected, multiplexing is likely to be one of the major challenges for future method development and validation. The main problem is to find efficient ways to rapidly develop robust methods, at least for qualitative tests, but also for quantitative real-time PCR in the absence of technology allowing for use of multiple distinctly labelled real-time PCR probes.

The experience of the partners is that the classical molecular rules of primers design (no strong homology between primers, no primer-dimer reactions, GC content, ΔG profiles, etc.)

are insufficient. Consequently, using the approaches described above, the development of reliable and robust duplex quantitative real-time PCR systems must be seen as an important achievement.

An alternative approach was developed by partner 2, which required a very different way of optimisation. This approach build on initial synthesis of a fairly representative population of amplification products by PCR with few cycles, followed by removal of all interfering reagents including non-amplified DNA, before performing a second amplification reaction generating labelled products for hybridisation to capture probe arrays and analysis of the resulting signals to obtain (semi-)quantitative relative measurements of the GMO content. This approach is published, and a patent application is presently processed.

Scientific publications from WP4 (peer reviewed journals, books) :

1. Taverniers, I., Windels, P., Van Bockstaele, E. & De Loose, M. (2001). Use of cloned DNA fragments for event-specific quantification of genetically modified organisms in pure and mixed food products. *Eur. Food Res. Technol.* 213: 417-424.
2. Terry, C. & Harris, N. (2001). Event-specific detection of Roundup Ready Soya using two different real time PCR detection chemistries. *Eur. Food Res. Technol.* 213: 425-431.
3. Berdal, K.G. & Holst-Jensen, A. (2001). Roundup Ready® soybean event-specific real-time quantitative PCR assay and estimation of the practical detection and quantification limits in GMO analyses. *Eur. Food Res. Technol.* 213: 432-438.
4. Holck, A., Vaitilingom, M., Didierjean, L. & Rudi, K. (2002). 5'-Nuclease PCR for quantitative event-specific detection of the genetically modified Mon810 MaisGard maize. *Eur Food Res Technol.* 214: 449-454.
5. Windels, P., Bertrand, S., Depicker, A., Moens, W., Van Bockstaele, E. & De Loose, M. (2003). Qualitative and event-specific PCR real-time detection methods for StarLink™ maize. *Eur. Food Res. Technol.* 216: 259-263.
6. Rønning, S.B., Berdal, K.G. & Holst-Jensen, A. (2003). Event specific real-time quantitative PCR for genetically modified Bt11 maize (*Zea mays*). *Eur. Food Res. Technol.* 216: 347-354.
7. Holst-Jensen, A., Rønning, S.B., Løvseth, A. & Berdal, K.G. (2003). PCR technology for screening and quantification of genetically modified organisms (GMOs). *Anal. Bioanal. Chem.* 375: 985-993.
8. Hernandez, M., Pla, M., Esteve, T., Prat, S., Puigdomenech, P. & Ferrando, A. (2003). A specific real-time quantitative PCR system for event MON810 in Maize YieldGard® based on the 3'-transgene integration sequence. *Transgenic Res.* 12: 179-189.
9. Burns, M., Shanahan, D., Valdivia, H. & Harris, N. (2003). Quantitative event-specific multiplex PCR detection of RoundupReady soya using LabChip™ technology. *Eur. Food Res. Technol.* 216: 428-433.
10. Rudi, K., Rud, I., Holck, A. 2003. Multiplex quantitative DNA array based PCR (MQDA-PCR) for detecting transgenic maize in food and feed. *Nucleic Acids Res.* 31, 11 e62 (DOI: 10.1093/nar/gng061).
11. Hernández, M.; Esteve, T., Prat, S. and Pla, M. 2003. Development of real-time PCR systems based on SYBR® Green I, Amplifluor™ and TaqMan® technologies for specific quantitative detection of the transgenic maize event GA21. *Journal of Cereal Science.* (in press).
12. Hernández, M., Rodríguez-Lázaro, D; Esteve, T., Prat, S. & Pla, M. 2003. Development of Melting Temperature (T_m)-based SYBR® Green I PCR methods for multiplex GMO detection. *Analytical Biochemistry.* (in press).
13. Holst-Jensen, A. 2003. Advanced DNA based detection techniques for genetically modified food. Chapter 27. *In: Food authenticity and traceability.* M. Lees (ed.), Woodhead publishing, UK, ISBN 1 85573 526 1.

Oral and poster presentations from WP4 at scientific conferences, etc.:

1. Terry, C. & Harris, N. (2000). Multiplex detection of GM food ingredients. Poster presented at the Food Safety in Europe Conference, London 19th-20th October 2000.
2. Berdal, K., Løvseth, A., Holck, A. and Holst-Jensen, A. (2000) Quantitative analysis of GMO foods in Norway: Methods and results. Poster presented at the European Commission and International Life Sciences Institute (ILSI) workshop on Method development in relation to regulatory requirements for the detection of GMOs in the food chain, Brussels, Belgium, 11th -13th December 2000.
3. Berdal, K. & Holst-Jensen, A. (2001). Specific detection and quantitation of genetically modified RoundupReady soybean. Poster presented at the conference “The Biotech society – where are we going?” organised by the Norwegian Research Council, Oslo, June 5th 2001.
4. Holst-Jensen, A. (2001). Development of standardised detection and quantitation methods for genetically modified foods. Poster presented at the conference “The Biotech society – where are we going?” organised by the Norwegian Research Council, Oslo, June 5th 2001.
5. Holck, A., Rud I. & Rudi, K. (2002). Multiplex quantitative DNA array based PCR quantitation of genetically modified maize in foods. Poster presented at the 38th contact meeting of the Norwegian Biochemical Society, Røros, January 17-20th 2002.
6. Rudi, K. (2002). Dangerous bacteria and foreign genes in food - DNA based methods for detection. Oral presentation at Modern Biotechnology, Norwegian Agricultural University, Ås, Norway, March 5 2002.
7. Holst-Jensen, A. (2002). PCR technology for screening and quantification of GMs. Oral presentation at Euroanalysis 12, Dortmund, Germany, September 9-13th 2002.
8. Holst-Jensen, A. (2002). Detection, identification and quantification of GMOs and derived products: *present and future challenges*. Oral presentation at the ENGL stakeholders meeting, Brussels, December 3rd 2002.
9. Rudi, K., Rud, I. & Holck, A. (2003). Quantitative multiplex detection of genetically modified maize by universal tail competitive PCR in combination with array hybridisation. Poster presented at the 39th Norwegian Biochemical Society meeting, Geilo, Norway, January 23-26, 2003.
10. Berdal, K.G. & Holst-Jensen, A. (2003). Detection, traceability, and quality assurance, oral presentation at the ENTRANSFOOD concluding conference, Rome 29-30 May, 2003.