SIXTH FRAMEWORK
PROGRAMME

GM and non GM supply chains: Their CO-EXistence and TRAceability

Outcomes of Co-Extra

Reliability and costs of GMO detection

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The analytical procedure used for GM detection and quantification at the laboratory level is composed of different modules. Each of those modules can impact the accuracy of analytical result qualitatively and/or quantitatively. Within the CoExtra project we have investigated different aspects of the reliability of GMO detection. A system for quantification of GM presence in the samples with low DNA content was established, for example. Additionally, effort was put into improvements related to trade-off between reliability and cost of analysis.

Different DNA-extractions from highly refined materials (lecithin and oil) have been investigated in detail. The sample preparation steps as well as the DNA extraction and purification steps were optimized. Both types of samples benefited from a hexane extraction step followed by DNA extraction and purification. The methods have been optimized for maximum DNA recovery and low hands-on time. Standard operating procedures are available for analytical labs that would like to evaluate the procedure or for further validation studies. The procedure for DNA extraction from lecithin has already been in-house validated and the protocol successfully transferred to different labs. To improve quantification performance, the SIMQUANT approach was developed. The idea is to

perform a series of PCR reactions and quantify the target numbers in the sample using the distribution of positive/negative results and most-probable-number statistics. One of the advantages of SIMQUANT is also less sensitivity of qualitative PCR to inhibitors in reactions when compared to quantitative PCR. The SIMQUANT was additionally upgraded to multiplex a version, thus increasing its applicability.

The quality of extracted DNA is known to influence significantly the final result of GM detection and quantification. One approach to control this step is “matrix by matrix” validation of the DNA extraction method. For validation, the quality of DNA solutions should be controlled by testing the presence or not of statistically significant inhibitory effects. This is usually done by adding an exogenous DNA (other taxon genomic DNA than that tested or plasmid, provided they are inhibitor free) containing a specific PCR target into the DNA solutions under study at a concentration close to the limit of quantification or limit of sensitivity (for example, 50-100 copies) and then by amplifying the specific PCR target contained by the exogenous DNA. The most convenient and cheapest way is to use the DNA of other taxon as exogenous DNA. The problem in most routine detection labs is that the matrix is not well defined. Composition of feed and food samples can vary from supplier to supplier and even from batch to batch, making ‘matrix by matrix’ DNA extraction validation not feasible. Modular validation can then be performed, providing appropriate controls of PCR inhibition are applied with every sample.

In quantitative analysis, the target number quantification also introduces a bias. Two calculation methods can be used. The ΔΔCt method relies on both amplicons having similar efficiencies of amplification for accurate quantification. Therefore the bias of the method would only be acceptable if working with well established matrixes (e.g. with raw materials) if properly validated in combination with DNA extraction method or if the calibration standards are of the same matrix type.

Routine detection of genetically modified (GM) organisms is most often performed on Applied Biosystems machines (ABI7700 and ABI7900), using their prominent chemistry – TaqMan® and their Master-mix. With new developments in this area many different apparatuses and chemistries are available on the market that could potentially outperform the previous systems. Within the project the different apparatuses and performance of alternative chemistries was evaluated, thus extending options in the methodology used in routine practice to recent technological advances. For comparison of apparatuses a small ring trial was organized within which 8 different real-time PCR models were included which were available in the labs of different WP5 partners. Some machines were also replicated in different labs to evaluate interlab variability. Applying CRL validation method acceptance criteria (25% RSD_r, 50% Bias) results suggest that the type of machine used is not critical in GM quantification, at least for the methods examined here.

Similarly, the comparison of different available chemistries was organized to test those most widely used (MGB®, SYBR® Green and Molecular Beacons) in different laboratories and targeting different genes, while the more recently introduced were tested less extensively (Plexor, LNA, lux). The conclusion was that TaqMan®, MGB®, LNA are equal in performance characteristics and they can be used whenever they are better suited for the particular application, e.g. if there is special needs regarding specificity or target regions are problematic for design of longer TaqMan® probes. Molecular Beacons systems were more difficult to design to achieve a robust assay. SYBR® Green chemistry performed well, its drawback being slightly lower sensitivity when compared to probe based assays. The other primer only based system that performed well were Ampliflour and Plexor, while some of the more exotic systems performed significantly below specifications given by the manufacturer.